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GUIDELINES
ON HEPATITIS B AND C TESTING

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GUIDELINES

ANNEXES

WHO guidelines on hepatitis B and C testing

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ANNEX 1: The Global Hepatitis Health Sector Strategy – Global targets

TARGET AREA	BASELINE 2015	2020 TARGETS	2030 TARGETS
Impact targets			
Incidence: New cases of chronic viral hepatitis B and C infections	Between 6 and 10 million infections are reduced to 0.9 million infections by 2030 (95% decline in hepatitis B virus infections, 80% decline in hepatitis C virus infections)	30% reduction (equivalent to 1% prevalence of HBsAg[1] among children)	90% reduction (equivalent to 0.1% prevalence of HBsAg among children)
Mortality: Viral hepatitis B and C deaths	1.4 million deaths reduced to less than 500 000 by 2030 (65% for both viral hepatitis B and C)	10% reduction	65% reduction
Service coverage targets			
Hepatitis B virus vaccination: childhood vaccine coverage (third dose coverage)	82%[2] in infants	90%	90%
Prevention of hepatitis B virus mother-to-child transmission: hepatitis B virus birth-dose vaccination coverage or other approach to prevent mother-to-child transmission	38%	50%	90%
Blood safety	39 countries do not routinely test all blood donations for transfusion-transmissible infections 89% of donations screened in a qualityassured manner[3]	All countries have haemovigilance systems in place to identify and quantify viral hepatitis transfusion transmission rates	Reduce rates of transmission by 99% compared with 2020.
Safe injections: percentage of injections administered with safetyengineered devices in and out of health facilities	5%	50%	90%
Harm reduction: number of sterile needles and syringes provided per person who injects drugs per year	20	200	300
Viral hepatitis B and C diagnosis	<5% of chronic hepatitis infections diagnosed	50%	90%

Viral hepatitis B and C treatment	<1% receiving treatment	5 million people receiving hepatitis B virus treatment 3 million people received hepatitis C virus treatment	80% of eligible persons with chronic hepatitis B virus infection treated 80% of eligible persons with chronic hepatitis C virus infection treated

[1]The abbreviation “HBsAg” refers to hepatitis B virus surface antigen.
[2] WHO/UNICEF coverage estimates 2013 revision, July 2014, see: http://apps.who.int/immunization_monitoring/globalsummary/timeseries/tswucoveragebcg.html (accessed 1 November 2015).
[3] Global Database on Blood Safety, Summary Report 2011, see: http://www.who.int/bloodsafety/global_database/GDBS_Summary_Report_2011.pdf?ua=1(accessed 1 November 2015).

ANNEX 2: Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection – Summary of recommendations

WHO recommendations for the prevention, care and treatment of persons with chronic hepatitis B infection

Non-invasive assessment of liver disease stage at baseline and during follow up:

APRI (aspartate aminotransferase [AST]-to-platelet ratio index) is recommended as the preferred non-invasive test (NIT) to assess for the presence of cirrhosis (APRI score >2 in adults) in resource-limited settings. Transient elastography (e.g. FibroScan) or FibroTest may be the preferred NITs in settings where they are available and cost is not a major constraint.

(Conditional recommendation, low quality of evidence)

Who to treat: As a priority, all adults, adolescents and children with CHB and clinical evidence of compensated or decompensated cirrhosis (or cirrhosis based on APRI score >2 in adults) should be treated, regardless of ALT levels, HBeAg status or HBV DNA levels.

(Strong recommendation, moderate quality of evidence)

Who to treat: Treatment is recommended for adults with CHB who do not have clinical evidence of cirrhosis (or based on APRI score ⩽2 in adults), but are aged more than 30 years (in particular), and have persistently abnormal ALT levels and evidence of high- level HBV replication (HBV DNA >20 000 IU/mL), regardless of HBeAg status.

(Strong recommendation, moderate quality of evidence)

Who to treat: Where HBV DNA testing is not available: Treatment may be considered based on persistently abnormal ALT levels alone, regardless of HBeAg status.

(Conditional recommendation, low quality of evidence)

HBV/HIV coinfected persons: In HBV/HIV-coinfected individuals, a Tenofovir based ART regimen should be initiated in all HIV-infected persons, regardless of stage of liver disease or CD4 count. (Strong recommendation, low quality of evidence) UPDATED RECOMMENDATION 2015 ARV GUIDELINES

Who not to treat but continue to monitor: Antiviral therapy is not recommended and can be deferred in persons without clinical evidence of cirrhosis (or based on APRI score ⩽2 in adults), and with persistently normal ALT levels and low levels of HBV DNA replication (HBV DNA <2000 IU/mL), regardless of HBeAg status or age.

(Strong recommendation, low quality of evidence)

Who not to treat but continue to monitor:

Where HBV DNA testing is not available: Treatment can be deferred in HBeAg-positive persons aged

30 years or less and persistently normal ALT levels.

(Conditional recommendation, low quality of evidence)

Who not to treat but continue to monitor: Continued monitoring is necessary in all persons with CHB, but in particular those who do not currently meet the above-recommended criteria for who to treat or not treat, to determine if antiviral therapy may be indicated in the future to prevent progressive liver disease. These include:

persons without cirrhosis aged 30 years or less, with HBV DNA levels >20 000 IU/mL but persistently normal ALT levels;
HBeAg-negative persons without cirrhosis aged 30 years or less, with HBV DNA levels that fluctuate between 2000 and 20 000 IU/mL, or who have intermittently abnormal ALT levels;
Where HBV DNA testing is not available: Persons without cirrhosis aged 30 years or less, with persistently normal ALT levels, regardless of HBeAg status.

Who not to treat but continue to monitor: It is recommended that the following be monitored at least annually:

(Strong recommendation, moderate quality of evidence)

ALT level (and AST for APRI), HBsAg, HBeAg, and HBV DNA levels (where HBV DNA testing is available)
Non-invasive tests (APRI score or FibroScan) to assess for the presence of cirrhosis, in those without cirrhosis at baseline;
If on treatment, adherence should be monitored regularly and at each visit.

More frequent monitoring: In persons who do not yet meet the criteria for antiviral therapy: More frequent monitoring for disease progression may be indicated in: persons who have intermittently abnormal ALT levels or HBV DNA levels that fluctuate between 2000 IU/mL and 20 000 IU/mL (where HBV DNA testing is available), and in HIV-coinfected persons.

(Conditional recommendation, low quality of evidence)

More frequent monitoring: In persons on treatment or following treatment discontinuation: More frequent on-treatment monitoring (at least every 3 months for the first year) is indicated in: persons with more advanced disease (compensated or decompensated cirrhosis); during the first year of treatment to assess treatment response and adherence; where treatment adherence is a concern; in HIV-coinfected persons; and in persons after discontinuation of treatment.

(Conditional recommendation, very low quality of evidence)

Monitoring for tenofovir and entecavir toxicity: Measurement of baseline renal function and assessment of baseline risk for renal dysfunction should be considered in all persons prior to initiation of antiviral therapy.

Monitoring for tenofovir and entecavir toxicity: Renal function should be monitored annually in persons on long-term tenofovir or entecavir therapy, and growth monitored carefully in children.

(Conditional recommendation, very low quality of evidence)

Monitoring for hepatocellular carcinoma: Routine surveillance for HCC with abdominal ultrasound and alpha-fetoprotein testing every six months is recommended for:

persons with cirrhosis, regardless of age or other risk factors (Strong recommendation, low quality of evidence)
persons with a family history of HCC (Strong recommendation, low quality of evidence)
persons aged over 40 years (lower age may apply according to regional incidence of HCC), without clinical evidence of cirrhosis (or based on APRI score $\leqslant$2), and with HBV DNA level >2000 IU/mL (where HBV DNA testing is available). (Conditional recommendation, low quality of evidence)

Source: WHO, Guidelines for the screening, care and treatment of persons with chronic hepatitis B infection. 2015

ANNEX 3: Guidelines for the screening, care and treatment of persons with chronic hepatitis C infection – Summary of recommendations

Existing and updated WHO recommendations (from 2016 guidelines)

Screening to identify persons with HCV infection: It is recommended that HCV serology testing be offered to individuals who are part of a population with high HCV prevalence or who have a history of HCV risk exposure/behaviour.

(Strong recommendation, moderate quality of evidence)

When to confirm the diagnosis of chronic HCV infection: It is suggested that nucleic acid testing (NAT) for the detection of HCV ribonucleic acid (RNA) be performed directly following a positive HCV serological test to establish the diagnosis of chronic HCV infection, in addition to NAT for HCV RNA as part of the assessment for starting treatment for HCV infection.

(Conditional recommendation, very low quality of evidence)

Screening for alcohol use and counselling to reduce moderate and high levels of alcohol intake: An alcohol intake assessment is recommended for all persons with HCV infection followed by the offer of a behavioural alcohol reduction intervention for persons with moderate-to-high alcohol intake.

(Strong recommendation, moderate quality of evidence)

Assessing degree of liver fibrosis and cirrhosis: In resource-limited settings, it is suggested that the aminotransferase/platelet ratio index (APRI) or FIB4 tests be used for the assessment of hepatic fibrosis rather than other noninvasive tests that require more resources such as elastography or Fibrotest.

(Conditional recommendation, low quality of evidence)

Assessing for HCV treatment: All adults and children with chronic HCV infection, including people who inject drugs, should be assessed for antiviral treatment.

(Strong recommendation, moderate quality of evidence)

Treatment with direct-acting antiviral agents: it is recommended that DAA regimens be used for the treatment of persons with hepatitis C infection rather than regimens with pegylated interferon and ribavirin.

(Strong recommendation, moderate quality of evidence) **UPDATED in 2016**

Subgroup considerations: for patients with HCV genotype 3 infection with cirrhosis and patients with genotypes 5 and 6 infection with and without cirrhosis, an interferon-based regimen: sofosbuvir/pegylated interferon and ribavirin is still recommended as an alternative treatment option. **UPDATED in 2016**

Removal of recommendation for treatment with telaprevir or boceprevir: the use of boceprevir- or telaprevir-containing regimens is no longer recommended for the treatment of persons with hepatitis C infection.

(Strong recommendation, moderate quality of evidence) **UPDATED in 2016**

Source: WHO, Guidelines for the screening, care and treatment of persons with chronic hepatitis C infection. Updated version April 2016.

Summary of recommended preferred regimens for hepatitis C with treatment durations*

Persons without cirrhosis

	Daclatasvir/sofosbuvir	Ledipasvir/sofosbuvir	Sofosbuvir/ribavirin
Genotype 1	12 weeks	12 weeks[a]	
Genotype 2			12 weeks
Genotype 3	12 weeks		24 weeks
Genotype 4	12 weeks	12 weeks	
Genotype 5		12 weeks	
Genotype 6		12 weeks	

Persons with cirrhosis

	Daclatasvir/sofosbuvir	Daclatasvir/sofosbuvir/ribavirin	Ledipasvir/sofosbuvir	Ledipasvir/sofosbuvir/ribavirin	Sofosbuvir/ribavirin
Genotype 1	24 weeks	12 weeks	24 weeks	12 weeks[b]	
Genotype 2					16 weeks
Genotype 3		24 weeks			
Genotype 4	24 weeks	12 weeks	24 weeks	12 weeks[b]	
Genotype 5			24 weeks	12 weeks[b]	
Genotype 6			24 weeks	12 weeks[b]	

* Treatment durations are adapted from the 2015 guidelines of the American Association for the Study of Liver Diseases (AASLD) and European Association for the Study of the Liver (EASL).

a Treatment may be shortened to 8 weeks in treatment-naive persons without cirrhosis if their baseline HCV RNA level is below 6 million (6.8 log) IU/mL.

The duration of treatment should be shortened with caution.

b If platelet count <75 x 10^3/µL, then 24 weeks' treatment with ribavirin should be given

ANNEX 4. PICO questions and decision-making tables

4.1 **Who to test HBV**- What is the impact, cost and cost-effectiveness of different HBV testing approaches and scenarios?

4.2 **Who to test HCV**- What is the impact, cost and cost-effectiveness of different HCV testing approached and scenarios?

4.3 **How to test HBV - PICO 1-** HBsAg testing: Among persons identified for hepatitis B testing, what is the diagnostic accuracy of available assays for detecting HBsAg (RDT, EIA)?

4.4 **How to test HCV - PICO 2**- To ascertain exposure to HCV through anti-HCV testing: Among individuals identified for hepatitis C testing, what is the diagnostic accuracy of available assays for detecting anti-HCV (RDT, EIA)?

4.5 **How to test HBV - PICO 3**- Testing strategy to diagnose chronic HBV infection through detection of HBsAg: Among individuals identified for hepatitis B testing, what is the best strategy (diagnostic accuracy and other outcomes) for detection of HBsAg? (One assay versus two-assay strategy)

4.6 **How to test HCV - PICO 4**- Testing strategy to ascertain exposure to HCV: Among persons identified for hepatitis C testing, what is the best testing strategy (diagnostic accuracy and other outcomes) for detection of HCV? (One assay versus two-assay strategy)

4.7 **How to test – HCV - PICO 5a and 6-** Testing strategy for diagnosis of HCV active infection: Among individuals with confirmed exposure to HCV (HCV Ab positive), what is the best testing strategy (diagnostic accuracy and other outcomes); comparing HCV core antigen versus NAT for HCV RNA to diagnose active HCV infection? **PICO 6-** Testing strategy for diagnosis of HCV active infection (quantitative or qualitative NAT): Among individuals with confirmed exposure to HCV (HCV Ab positive), what is the diagnostic test accuracy of qualitative NAT methods versus quantitative NAT methods to diagnose active HCV infection?

4.8 **Dried blood spots - PICO 7-** Dried blood spots as sample collection method for serology/NAT for HBV/HCV: Among persons identified for (1) hepatitis B, or (2) hepatitis C testing, what is the diagnostic accuracy and impact of detecting HBsAg/HCV Ab or NAT from DBS samples versus venous samples?

4.9 **Treatment monitoring HCV - PICO 9-** Monitoring for treatment response using HCV Ag testing in individuals with confirmed active HCV infection: Among individuals receiving antiviral treatment for HCV, what is the diagnostic accuracy of HCV core antigen versus NAT for HCV RNA qualitative detection (and/or) quantification to confirm successful treatment response with viral clearance?

4.10 **Interventions to linkage to care -** to optimize uptake of hepatitis testing and linkage to care across the viral hepatitis treatment cascade

4.1. WHO to test HBV?

Decision-making tables – PICO 1

What is the impact, cost, and cost–effectiveness of different HBV testing approaches and scenarios?

Population:

1. *Risk-based screening in different high-risk populations:* Injecting drug users (IDUs), men who have sex with men (MSM), immigrants, recipients of blood transfusion and blood products, sex workers, and health-care workers (HCW), HIV-infected persons
2. *General population* (excluding blood donors) or selected subpopulations of general population (women during pregnancy, those with raised alanine aminotransferase [ALT], Infants, schoolchildren and adolescents)
3. *Other approaches:* Birth cohort screening (based on different age cut-offs, born between 1945–1960 or 1965 or 1970).
4. *One off screening* vs repeat screening every five years

Intervention: Testing strategies for HBV in different populations (risk based and general population and birth cohort); and at different prevalence thresholds

Comparator: No testing or current practice or comparison of different testing strategies

Outcomes: Benefits, harms and costs, and cost–effectiveness with different screening strategies for different target populations

Individual patient outcomes: No. of cases detected, overall mortality, liver-related mortality, cirrhosis, end-stage liver disease, rate of hospitalizations, serious adverse events, quality of life

Prevention: New infections (mother to child, horizontal [IDUs needle sharing and sexual; and sexual, esp MSM])

Cost–effectiveness: Cost and incremental cost per case diagnosed; cost and incremental cost per case screened and treated; cost and incremental cost per life saved; cost and incremental cost per infections averted; quality-adjusted life years (QALYs) gained

Background:

Epidemiology:

Chronic hepatitis B (CHB) – defined as persistence of hepatitis B surface antigen (HBsAg) for six months or more – is a major public health problem. Worldwide, there are an estimated 250 million chronically infected persons, particularly in low- and middle-income countries (LMICs). Universal hepatitis B immunization programmes that target infants, with the first dose at birth, have been highly effective in reducing the incidence and prevalence of hepatitis B in many endemic countries. However, these programmes will not have an impact on HBV-related deaths until several decades after their introduction.

The major complications of CHB are cirrhosis and hepatocellular carcinoma (HCC). Between 20% and 30% of those who become chronically infected will develop these complications, and an estimated 650 000 people will die annually due to CHB.

The risk of developing chronic HBV infection decreases with age at infection, from about 90% when infected perinatally up to 6 months of age to 20–60% between the ages of 6 months and 5 years. Of those who acquire HBV as children 25% will develop primary liver cancer or cirrhosis as adults.

Routes of transmission worldwide: In sub-Saharan Africa and east Asia, transmission predominantly occurs in infants and children by th eperinatal and horizontal routes (i.e. resulting from close contact that is not parenteral, perinatal, or sexual in nature) whereas in more industrialized countries, rates of new infection and acute disease are highest among young adults and transmission predominantly occurs via Injecting drug use and other high-risk behaviours. Worldwide, the majority of infections are acquired at birth or in early childhood.

Low rates of diagnosis: The majority of people are unaware of their HBV infection, and therefore often present with advanced disease. At present, there is a massive burden of undiagnosed and untreated hepatitis B and C, with 40–85% of infected persons undiagnosed, but varies greatly by setting. By contrast, the estimated awareness of status among people living with HIV (PLHIV) is within 40%–60% range for two thirds of countries, but varies significantly (CHAI, UNAIDS Info).

- Based on still limited studies, overall <15% of the estimated 180 million who are chronically infected with HCV are aware of their diagnosis, based on data from higher-income setting – United States, Europe and China.
- And from a survey in the US, a similar proportion of those with chronic HBV infection are aware of their diagnosis.
- The proportion in low-income settings is even higher, with only a tiny fraction diagnosed and aware.

Reasons for low uptake of testing are multifactorial, and include lack of awareness at all levels, lack of clear guidelines, competing health-care priorities, limited health-care budgets and political will.

This leads to many people remaining undiagnosed until the later stages of the disease, when prognosis is poor.

In addition to the very low access to and uptake of testing, there is also further attrition in the care cascade with very poor linkage to care and therefore treatment, among those who test positive.

Hepatitis B and C testing and diagnosis are at the core of entry to both the prevention and treatment cascade.

- Testing is required to *identify those with are positive*, linking them with care, counselling them on measures to reduce transmission to others then assessing who needs treatment, initiating treatment, achieving treatment response (sustained virological response [SVR] for hepatitis C) or long-term viral suppression for HBV and retaining in care for HBV.
- Hepatitis testing is also needed to *identify those who are negative*, to provide hepatitis B vaccination, and the opportunity to implement individual or facility-level prevention measures, counsel to reduce risk behaviours, or institute facility-level prevention measures on measures to acquisition.

There are three key approaches to screening:

1. **Population- or community-based screening (including antenatal).** This means that all members of the population have access to the screening programme under consideration. It may also include home-based testing (house to house); campaigns (e.g. HTC plus – malaria, safe water, noncommunicable diseases e.g. diabetes and hypertension); outreach (mobile) in general and key populations; workplaces and schools; and health-care facility0based screening.
2. **Health-care facilities.** Testing could also be offered in special dedicated clinics, e.g. HIV, STI clinics. Screening at health-care facilities may include primary care settings, inpatient and outpatient settings, and may involve screening on the basis of clinical presentation or focus on only those with abnormal liver function tests, abnormal ultrasound scan, family history of liver disease or other clinical suspicion of liver function test.
3. **Targeted risk factor-based screening.** This refers to screening of specific groups including key populations, who are generally at higher risk of being infected than the general population. This includes people who inject drugs (PWID), people in prisons and other closed settings, migrant populations, some indigenous populations, MSM and sex workers, but may also include health-care workers. People attending services providing care and treatment for viral hepatitis or HIV can be encouraged to bring their partners to be tested.
4. **Birth cohort screening** for HBV and HCV.

Existing guidelines: what are countries doing?

1. Most countries have based their list of high-risk groups as defined by the Centers for Disease Control and Prevention (CDC), and are largely based on known modes of transmission. Generally they include recommendations for three main screening approaches: (Apata, MMWR Morb Mortal Wkly Rep. 2014;63:613–19; Weinbaum, Hepatology. 2009;49:S35–S44; Han, Vaccine.

2013;31 Suppl 9:J36–J42)

- Population-based screening that includes antenatal clinic screening
- Screen those with high-risk behaviours, exposures and other conditions
  - Family members and household contacts of hepatitis B patients
  - MSM
  - PWID
  - HIV-positive patients
  - Patients on immunosuppression or chemotherapy
  - Persons with liver disease of unclear etiology
  - Health-care workers.
- Birth cohort for HCV screening in US and Japan.

2. At present, there is no universally accepted recommended screening programme. There is widespread testing of blood donors (but not necessarily universal), and widespread antenatal screening and infant vaccination in Asia. In addition, there is a risk factor-based testing in high-risk groups in Asia (PWID, liver disease, renal dialysis) and use of a birth cohort approach in the US and Japan.

Survey of guidelines (Surjo De)

Evidence: systematic reviews of prevalence of HbsAg

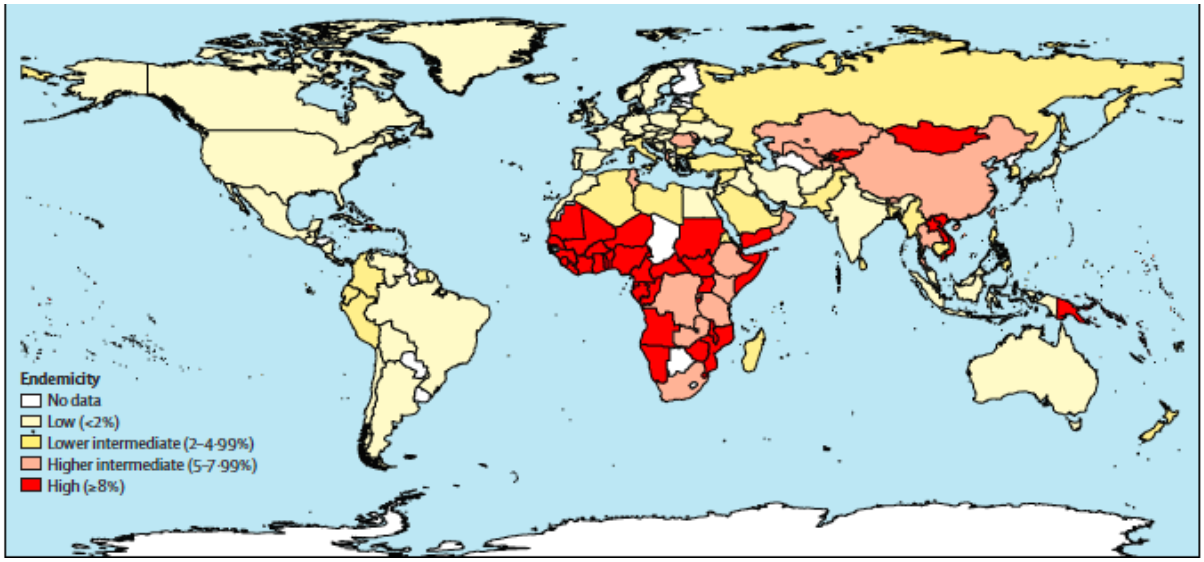
1. General population: systematic review (Ott, Lancet 2015)

161 countries included.

High endemicity (>5%): Most countries in Africa were of higher–intermediate endemicity (HBsAg prevalence 5–7·99%), or highly endemic for HBV (HBsAg prevalence ≥8%. The Western Pacific Region was also a high–intermediate endemicity region (5–7.99%), especially in the Pacific Island States such as the Solomon Islands.

Intermediate endemicity (2–5%): The Eastern Mediterranean Region was of lower–intermediate endemicity (2·00–4·99%), but Djibouti, Somalia and Sudan showed a higher prevalence of HBsAg than other countries in the region such as Iran.

Low endemicity: Countries in the Americas, such as Mexico, Guatemala, and the USA had mostly low endemicity levels (HBsAg prevalence <2%), ranging from 0.01% (95% CI 0.01–0.01) in the UK to 10.32% (8.56–12.38) in Kyrgyzstan. Overall, the South-East Asia Region had low endemicity levels but on country level, an HBsAg prevalence below 2% was only noted in India, Indonesia and Nepal.



Summary of prevalence across risk groups

General Population (Schweitzer et al. 2015)	PWID (Nelson et al. 2011)	MSM (Hope, et al. 2014 data is for European countries outside of the EU)	Sex Workers (Hope, et al. 2014 data is for European countries outside of the EU)	Migrants & Refugees (Rossi et al. 2012; Hanhe et al. 2013; Nilgun, 2011))	Prisoners	Pregnant Women (Hanhe et al, 2013; Nilgun, 2011))	HIV-infected persons (general population) (Easterbrook et al. 2015)	Healthcare Workers
East Asia and Pacific 5-7.99% South Asia Region 2-4% Central and Eastern Europe and Central Asia Region 2-4% North Africa and Middle East Region 2-4% Sub-Saharan Africa Region 5-7.99% Latin America	Southeast Asia and East Asia 2.9 - 19.5% South Asia 5.8 - 17.3% Central Asia 7.9% Eastern Europe 0.5-21.3% North Africa and Middle East region 0.0 - 18.5% Central Africa 3.8 - 9.0% Andean Latin America 2.3	Albania 18% Azerbaijan 4% Croatia 0.90% Georgia 10% Serbia (incl. Kosovo) 8.70% Turkey 3.60% Ukraine 9.80%	Azerbaijan 3.3% Bosnia 1.4% Serbia 18.3% Turkey 2.4%5 Ukraine 9.1%	East Asia and Pacific 11.3% South Asia Region 4.6% Central and Eastern Europe and Central Asia Region 5.8% North Africa and Middle East Region 2% Sub-Saharan Africa Region 10.3% Latin America and Caribbean Region 1.7% Europe 1.0-		Europe 0.1(Spain)-4.4% (Slovakia) Middle East 1% (Qatar) 2.9% (Lebanon)	East Africa 5-11% West, Central Africa 5-15% Latin America 0.6-2% South East Asia 1-2% Eastern Mediterranean 10%	Tanzania tertiary hospital 5.6-7% (Mueller et al. 2015) Uganda tertiary hospital 8.1% (Ziraba et al. 2010).

2. PWID: Systematic review ((*Nelson et al. 2011***))**

PWID are a key population who are at particularly high risk of HBV, HCV and HIV infection. In many high-income countries and some developing countries, ongoing HCV transmission is driven mainly by PWID populations. A review of global prevalence data from 77 countries estimated that exposure to HCV (anti-HCV positive) among PWID is estimated to be between 60% and 80% in 25 countries, and over 80% in 12 countries. Similarly, of 59 countries where data were available, prevalence of HBsAg

among PWID ranged from 5% to 10% in 21 countries and over 10% in 10 countries.
PWID data are global by 20 Global Burden of Disease regions
Southeast Asia and East Asia: 2.9–19.5%
South Asia: 5.8–17.3%
Central Asia: 7.9%
Eastern Europe: 0.5–21.3%
Central Africa: 3.8–9.0%
Andean Latin America: 2.3–8.6%

3. MSMs and sex workers: systematic review ((Hope et al. 2014)))

MSM can acquire HBV and HCV sexually. In many populations, there are higher rates of HBV infection among MSM, requiring targeted HBV screening and vaccination. MSM who are HIV positive are at significantly higher risk of acquiring HCV infection than HIV-negative MSM.

Sex workers are a key population who are at high risk of acquiring HBV and HCV infection. Multiple factors may contribute to this vulnerability, including unsafe working conditions, barriers to negotiating consistent condom use, and difficulties accessing health-care services.

MSM (12 countries), sex workers (5 countries)

Country	MSM	Sex workers
Albania	18%	
Azerbaijan	4%	3.3%
Croatia	0.9%	1.4% (Bosnia)
Georgia	10%	11.1%
Serbia (incl. Kosova)	8.7%	18.3%
Turkey	3.6%	2.4%
Ukraine	9.8%	9.1%

4. Migrants and refugees: systematic review (Rossi et al. 2012)
sub-Saharan Africa Region: 10.3%
East Asia and Pacific: 11.3%
Central and Eastern Europe and Central Asia Region: 5.8%

5. HIV-infected persons: systematic review (Easterbrook et al. 2015)
HIV/HBV (483 estimates from 75/193 (39%) countries

	Gen pop	PWID	MSM	Hetero	Pregnant
	Mid-point co-infection prevalence (Interquartile range) **Number of studies**				
East Africa	8% (6-11) **10**		9% **1**	6.5%(5-10) **10**	4% (2-5) 2
West, Central Africa	11% (6-15) **11**		22% **1**	12% (8-20.5) **32**	9% (0-13) **3**
South Africa			6.5% **1**	7% (5-20) **7**	5% (3-6) **2**
Latin America	1% (0.6-2) **3**	27% **1**	9% (6-11) **5**	3% (2-7) **4**	0.5% (0.5-1.8) **3**
North America		7% **1**	5% (5-6) **2**	17% **1**	
South East Asia	2% (1-2) **2**	18% (10-20) **10**	15% (10-19) **6**	9% (0-15) **10**	
Eastern Europe and CAR					
Europe		4% (3-7) **3**	5% (4-6) **9**	7% (2-11) **2**	
East Med	10% **1**	8% (4-44) **6**			
East Asia		9.5% (2.5-37) **4**	12% (10-13.5) **4**	5% (4-6%) **4**	
Western Pacific			4% (3-5) **6**		

HBsAg prevalence based on a total of 170 estimates in HIV-infected persons, based on population type (general population, PWID, MSM, heterosexual, and pregnant women) and by eleven geographical regions.

1. First, reflecting the epidemiology of HBV in Africa whereby the majority of HBV infections are acquired perinatally or in childhood, the prevalence among key populations of HIV-infected PWID and MSM is not substantially higher than the background rate in the general population or among heterosexuals, especially in Africa,
2. Only in the South-East Asia Region is there a higher prevalence in among PWID and MSM.

6. Prisoners

The prevalence of HBV in prisons is often significantly higher than in the general population. Globally, the prevalence of HIV, STIs, hepatitis B and C and tuberculosis in prison populations is estimated to be two to ten times higher than in the general population, and in some settings, 50 times higher. People in prisons and closed settings may be at particular risk for HBV, HCV and HIV infection for a number of reasons. Most commonly, this is due to sharing of needles and syringes and other injecting equipment; often because prevention hardware such as clean needles and syringes are not accessible to prisoners.

7. Indigenous populations: In some settings, indigenous populations are also disproportionately affected by viral hepatitis infection, along with a number of other health problems. Contributing factors to these disparities may include higher rates of injecting risk behaviours among indigenous people who inject drugs and higher rates of incarceration.

Epidemic scenarios

The broad categories of “generalized” and “concentrated” epidemics are not necessarily helpful in determining how best to prioritize hepatitis testing services. But some general principles apply.

Epidemic Scenarios			
	Definition	Disaggregation	Country Example
Generalised	High >8%		West Africa
	High Intermediate (5-7%)		China, Vietnam
	Low Intermediate (2-4%)		Turkey, Taiwan
	Low (<2%)		Brazil, Ukraine
	<1%		United Kingdom, France
Mixed	Generalised population prevalence, low intermediate (and potentially high intermediate), with a sizeable risk population	High	Greece
		High intermediate	Tanzania, Myanmar
		Low intermediate generalised	Iran, India
Concentrated	Generalised population prevalence <2% with high risk groups	Low (<2%) with risk groups	Italy, Russian Federation
		Low (<2 %) without risk group	
		PWID with >8% prevalence	Estonia, Bulgaria
		PWID with < 8% prevalence	Western Europe
		Countries with migrant populations with prevalence >5%	Canada, Netherlands

Recommendations

Guiding principles for hepatitis B and C testing:

1. Promotion of health equity and human rights in national hepatitis B and C testing so that: expanded testing and access is fair and equitable; priority for testing is on diagnosing the undiagnosed; identifying those in greatest need of treatment and those with ongoing risk of infection; and that testing is voluntary and care is provided in a supportive environment free of stigma and discrimination.

   This is critical as many of the affected population are those who are systematically excluded from access to testing, treatment and care, such as sex workers, injection drug users, men who have sex with men, and prisoners.
2. All persons who test positive for hepatitis B and C (in addition to HIV) should have access to and be linked to hepatitis care and treatment services.
3. Testing of key populations should be undertaken where possible in conjunction with other risk or harm-reduction services.

DRAFT recommendation(s): Existing recommendations on prisons, for sex workers and PWID on HBV vaccination

1. Prisons should have a comprehensive hepatitis programme, including the provision of free hepatitis B vaccination for all prisoners, free hepatitis A vaccination to those at risk, and other interventions to prevent, diagnose and treat hepatitis B and C equivalent to those available in the community (including condom, needle and syringe programmes and drug dependence treatment as needed).
2. Include sex workers as targets of catch-up hepatitis B immunization strategies in settings where infant immunization has not reached full coverage (*Source*: WHO, 2012).[36]

3. It is suggested to offer people who inject drugs the rapid hepatitis B vaccination regimen.

Summary and quality of evidence (*see SR_Who to screen_HBV modelling report* for references)

Summary of evidence base for different screening approaches

The evidence base for these different screening approaches remains very limited, and largely relies on observational data and modelling.

- There are descriptive data showing that targeted testing and community-based screening programme approaches can increase uptake of testing and detection of cases, but very limited data to show impact on patient important outcomes (Pollack, Health Aff (Millwood) 2011;30:1974–83; Bryce BD, Yartel AK. Am J Prev Med 2014;47:23341). Community-based: BFreeNYC screening program (~9000 people screened, 6 cases HCC + 22 end-stage liver failure diagnosed and managed)
- Lack of evidence and uncertainty as to whether risk-based targeted screening is reaching targeted populations.

Cost–effectiveness evidence summary: overview of report – summary of existing studies on cost–effectiveness of screening and treatment for HBV, with an analytic summary of key considerations.

- 32 studies all from high-income countries in settings with low HBV prevalence. No data on cost or cost–effectiveness of screening for HBV in LMICs was identified. Eight published studies, and one unpublished study (PROLIFICA screening study in Gambia) met inclusion criteria.
- Two studies evaluated HBV screening in the general population and seven studies in "high-risk" groups (all but one concerned screening in migrant or refugee populations). There was one previously published study in the USA and one forthcoming study in the Gambia, looking at the cost–effectiveness of offering screening and treatment to the general population.
- The studies used different methods of screening the "high-risk groups"

☐ High
☐ Moderate
☐ Low
☐ Very low

including, in the clinical setting (Wong, Rein), community outreach methods (Rein) and overseas screening (Jezwa). Various outcome measures were used, including cost per quality-adjusted life year (QALY) gained, cost per LY saved and cost per case screened. Many of the models were simulated using hypothetical cohorts.

- Overall, data show that offering screening to the general population with subsequent antiviral treatment strategy is cost–effective in HICs (Eckman), as well as LICs (Nayagam), even down to a population prevalence as low as 0.3% and 2%, respectively, in these studies.
- **PROLIFICA study of HBV community-based screening in Gambia:** the feasibility of large-scale screening and treatment in sub-Saharan Africa (SSA) has been demonstrated by the ongoing PROLIFICA (Prevention of Liver Fibrosis and Liver Cancer in Africa) study in West Africa (Lemoine et al., forthcoming). This implementation study has screened nearly 10 000 adults for HBsAg at the community level in the Gambia and Senegal using an active outreach method. This is followed by full clinical assessment of those found to be HBsAg positive and antiviral treatment if meeting eligibility criteria. A cost–effectiveness analysis of this community-based screen and treat strategy in the Gambia (Nayagam et al., forthcoming), compared to status quo, revealed an incremental cost–effectiveness ratio (ICER) of $705/LY gained (other outcome measures also calculated: $476/QALY gained or $575/DALY averted). The authors acknowledge that willingness to pay (WTP) thresholds levels, and their use, are highly debated in LMICs. However, it can be regarded as cost-effective if using the WHO WTP threshold of three times the country's GDP per capita to define a cost–effective intervention (3 times GDP per capita = $1460 in the Gambia). This is the only cost–effectiveness study of screening and treatment we have found in LMIC settings. Furthermore, it is furnished with real-life cost and effectiveness data from a large-scale screening and treatment intervention programme. Furthermore, screening also has benefits that extend beyond the person screened to also others, for example, prevention-of-mother to child transmission.

Conclusions: The data on the cost–effectiveness of screening for HBV is lacking, especially in LMICs. Difficult to draw conclusions regarding the best screening strategy, in terms of who to screen and where to screen, based on cost–effectiveness alone. Currently, there is not enough literature to make strong recommendations for screening based on cost–effectiveness arguments alone.

Relatively low screening costs, highly effective and relatively low-

cost antiviral therapy at generic price and a fraction of HBsAg-positive persons requiring antiviral therapy should help drive the cost–effectiveness of a test-and-treat strategy. However, this has to be balanced against long-term treatment and the fact that a high proportion with CHB will survive without treatment.

Limitations of comparing models/generalizability of results: WHO recommendations are primarily aimed for use in LMICs. All models were from HICs (except PROLIFICA); making generalizations of results from cost–effectiveness analyses between countries or regions with such differing health-care structures, costs, patient behaviours, disease prevalence profiles and willingness-to-pay thresholds can be misleading.

Key determinants of testing approach for countries (from cost–effectiveness review):

HBsAg prevalence:

HBsAg prevalence had a relatively small influence on cost–effectiveness in most of the studies. General population screening was found to remain cost–effective (i.e. ICER below the respective WTH threshold) down to HBsAg prevalence of 0.3% in the USA (Eckman) and 2% in the Gambia (PROLIFICA).

Costs:

Cost components that need to be considered in economic evaluations of screening and treatment for HBV include costs of screening, diagnostics, monitoring and drugs. This should involve both the cost of consumables, as well as other costs, including human resource costs (which are included to various extents between different studies). A key driver of cost–effectiveness of a screen-and-treat strategy reported in some studies is the cost of antiviral drug (Rossi, Hutton, PROLIFICA). Screening costs varied between the studies, and were only found to be drivers of cost–effectiveness in the Wong and PROLIFICA studies.

Linkage to care and adherence:

Adherence to treatment and linkage to care were reported as key drivers of cost–effectiveness in several studies (Rossi, Veld). In the PROLIFICA study, variation in treatment adherence was also a key driver of cost-effectiveness.

Uptake of screening is not reported to be a key driver of ICER in the studies; however, this does not imply that high participation levels in screening is not important, as when considering health impact alone, increasing uptake

is key. The implication of this result is that it is likely to be worthwhile performing screening and treatment, even if participation in screening is assumed to be low. This could be because screening costs are low, relative to the costs and health benefits of treatment for those who are infected. **Distribution of patients between different disease states** The proportion of people who would benefit from treatment in a population will guide cost–effectiveness, but by how much is difficult to quantify based on current evidence, and needs further research.	
Risks/benefits **Community-based testing (outreach, mobile or venue-based)** **Benefits** • Leads to earlier diagnosis and access to treatment before development of cirrhosis • Worldwide, the majority of infections are acquired at birth or in early childhood, and there is therefore generalized high prevalence throughout population, which requires population-based testing approaches. • Highly acceptable with index partner testing, home-based and mobile outreach for HIV • Generally good uptake • Way of accessing missing populations, such as men, key populations and young women who are not pregnant • Community-based testing is a critical approach for reaching people from key populations and vulnerable populations who are unlikely to go to a facility, particularly those who are asymptomatic. **Risks** • May lead to lower-than-expected positivity rates with home-based testing, testing within campaigns, key population outreach and testing of index partners. • Suboptimal linkage to care is highly variable and may be problematic. • Unit costs may be higher, but may be cost–effective. **Provider-initiated testing and counselling (PITC) in health-care facilities** **Benefits** • 89/117 low- or middle-income countries recommend HIV PITC in all patient encounters	☐ Benefits clearly outweigh harms ☐ Benefits and harms are balanced ☐ Potential harms clearly outweigh potential benefits *Are the desirable anticipated effects large?* ☐ No ☐ Probably ☐ Uncertain ☐ Yes ☐ Varies

- High HIV PITC acceptance in antenatal care (ANC) and TB settings
- Introduction of PITC increased paediatric HIV testing
- Many clinical settings in generalized epidemic settings not offering hepatitis testing – e.g. STI clinics, primary care, and so many missed opportunities for HBV diagnosis in health-care facilities.

Key and other populations targeted testing

Benefits

- Key populations are disproportionately affected by hepatitis in all regions.
- Key populations are less likely to have received HBV vaccination and offer of HBV testing will facilitate higher rates of completion of vaccination.

Partner testing

Benefits

- Participating in couples and partner HBV testing has a number of benefits. These include adoption of prevention strategies by the couple (for example, condom use, safe injecting practices) and promotion of linkage to and retention in appropriate health-care services.
- Also applies to opportunistically offering HBV testing and vaccination to family members and other close household contacts of people diagnosed with CHB re access to vaccination and care.
- Couples and partner testing helps more people know their HBV and/or HCV status, particularly men, who in generalized epidemic settings may be less likely to test than women.
- Partners: <5% of people currently HIV test with their partners and similar low rates for HBV. Note: HIV serodiscordance is common (half to two thirds of HIV-positive adults with a co-habiting relationship have an HIV-negative partner
- Offering **partner testing** for persons with HBV and HCV – highest possible yield. Although risk of infection may be low, a negative test in the partner provides reassurance and the opportunity to provide counselling on reducing future risk including vaccination.

Risks

- People may be reluctant to admit risk behaviours, or may be unaware they are at risk, and so a screening approach that relies on history may miss a substantial proportion of cases.

Acceptability, values and preferences **PITC** • High HIV PITC acceptance in ANC and TB settings **Partner testing** • Offering partner testing for persons with HBV and HCV – highest possible yield • Need to overcome reluctance to provide partner testing/index partner testing **Community-based testing** • Community-based testing services would need to be made available in settings acceptable and convenient to people from key populations and vulnerable populations. • Services need to be convenient and available, through flexible opening hours and/or walk-in or same-day appointments. • Involving affected populations, including adolescents in design, delivery and evaluation of testing services is necessary to ensure that these programmes address their need. • Need to address concerns that older relatives, neighbours or family friends will see them attending viral hepatitis/HIV services, including testing services.	☐ No major variability ☐ Major variability Is the option acceptable to key stakeholders? ☐ No ☐ Probably ☐ Uncertain ☐ Yes ☐ Varies
Equity, ethics and human right implications *Will recommendation raise questions around equity?* • As for all testing services, programmes for key populations need to emphasize WHO's "5 Cs" – particularly consent, confidentiality and connection to comprehensive prevention, care and treatment. • The use of community-based and hepatitis B and C rapid testing can increase the likelihood of some key populations, such as prisoners, receiving their results. • Testing in certain populations, such as in prisons may increase the chances of stigmatization. *Are there ethical implications to this recommendation?* • No major concerns.	☐ Less equitable ☐ More equitable
Resource use and financial implications	

Resource use (see parameter matrix for sample HIV testing programme costs) Estimating the costs associated with a given hepatitis testing approach can be challenging. Costs for similar hepatitis testing may differ significantly between countries and by programme type within a country. Differences in programme costs may be due to general cost differences between countries, in what specific services are provided (referral to clinic for those testing hepatitis-positive vs enhanced linkage support), cadre of staff employed (nurses vs community health workers), the ease of reaching different populations, the capacity of the health system, and the level of HIV testing coverage. **Standardized approach to costing of hepatitis testing:** A common approach to estimating costs involves identifying and estimating costs incurred by the health-care provider within the following broad categories: • personnel (for example, health-care providers at facilities, counsellors, other paid programme staff, volunteers); • recurrent costs (for example, HIV test kits and commodities, printed materials, office supplies); • capital expenses, often amortized over their useful life and discounted annually at 3% (for example, office space, transportation, equipment); **Materials:** • Cost of testing kits, buffer/reagents • Cost of sterile lancets, pipettes, gloves, sharps-bins or other method of disposal of used-kits • Cost of automated reading machine, if applicable • Quality-control reagents, if applicable (some kits are supplied with positive and negative controls) **Training and supervision:** • Cost of training testing providers and appropriate assessment, validation and revalidation of their skills • From included studies, excellent robust specificity of all tests is reassuring in terms of ensuring cost–effective initiation of algorithms for further investigation and treatment. • If being utilized at the point of care, it will be the responsibility of the testing provider to record and report the result appropriately. **Other:**	*Are the resources required small?* ☐ No ☐ Probably ☐ Uncertain ☐ Yes ☐ Varies

- Creation of a database into which results obtained by POC can be recorded
- Linkage to care, e.g. antenatal clinics.

Possible test procurement cost:

Test	Cost (US$) per test	Source
RDT	0.3–0.95 (procurement cost)	WHO database
EIA	0.4–2.8 (procurement cost)	WHO database

Costs

In the PROLIFICA study, despite an active community-based screening campaign, screening costs were low ($7.43 per person offered screening) and the intervention remained cost–effective even if there was a 3-fold increase in screening costs. The Rein study in USA reported costs per person screened between $40 and $280, with the higher costs representing the more active outreach strategies.

Feasibility and constraints to implementation

Are any major barriers expected for the implementation of this recommendation?

The feasibility of large-scale screening and treatment in sub-Saharan Africa (SSA) has been demonstrated by the ongoing PROLIFICA (Prevention of liver fibrosis and liver cancer in Africa) study in West Africa (Lemoine et al., forthcoming). This implementation study has screened nearly 10 000 adults for HBsAg at the community level in the Gambia and Senegal using an active outreach method. This is followed by full clinical assessment of those found to HBsAg positive and antiviral treatment if meeting eligibility criteria.

A cost–effectiveness analysis of this community-based screen and treat strategy in the Gambia (Nayagam et al., forthcoming), compared to status quo, revealed an ICER of $705/LY gained (other outcome measures also calculated: $476/QALY gained or $575/disability-adjusted life year [DALY] averted). They authors acknowledge that WTP thresholds levels, and their use, are highly debated in LMICs. However, it can be regarded as cost–effective if using the WHO WTP threshold of three times the country's GDP per ca–ita to define a cost-effective intervention (3 times GDP per capita = $1460 in the Gambia). This is the only cost–effectiveness study of screening and treatment we have found in LMIC settings.

Couples and partners

Is the option feasible to implement?

☐ No
☐ Probably
☐ Uncertain
☐ Yes
☐ Varies

HIV testing for couples and partners has been conducted in various settings, including ANC and community-based TB services, through ART services and during premarital health visits. Couples and partner HIV testing for the partners of women attending ANC, in particular, is a focus in the 21 priority eMTCT countries. These countries are all highly endemic for HBV, and this provides a unique opportunity to integrate concurrent HBV testing for partners of women with CHB, or chronic HCV infection if risk factors are present.	

Relevance to different settings/populations

Will this recommendation be most relevant for particular settings (e.g. endemicity)?

Adolescents

In high HBV-prevalence settings there are two groups of adolescents (that is, people 10–19 years of age) who may need access to HBV testing: (1) undiagnosed adolescents who were exposed perinatally or in early childhood and; (2) adolescents who acquire HBV sexually (through early sex, sex with multiple partners or sex with a person with CHB), or through injecting drug use. Perinatally infected adolescents urgently need to be diagnosed so that they can be linked to HBV monitoring and care and start antiviral treatment if and when this is clinically indicated. In many highly endemic HBV settings, there are a significant number of undiagnosed perinatally infected adolescents. Perinatally exposed adolescents who do not have evidence of CHB need to be vaccinated if this has not yet been done. In many countries, adolescents and young adults may have missed out on HBV vaccination depending on the timing of introduction of universal infant vaccination.

Children

Universal HBV immunization, including a vaccine birth dose within 24 hours after birth, is key to preventing MTCT of HBV, but many countries have not been able to implement this crucial intervention, due to economic and logistic constraints.

Most infants whose mothers have been diagnosed with HBV or HCV should be followed-up and routinely offered EID, and those diagnosed with either with should be regularly monitored for signs of liver disease so that treatment can be offered when necessary. However, some infants are lost to follow-up, so additional pediatric case finding is important. This can be achieved through the routine offer of PITC in health facilities, particularly in high prevalence settings, and also through testing the family members of index cases where appropriate.

HBV testing services for infants should be implemented with the aim of identifying as many HBV-infected infants as early as possible. Although a conservative approach to treatment is usually indicated, children born to HBV-infected mothers should be screened early so that monitoring for progression of liver disease can be organized and so that testing and vaccination of household contacts can be carried out.

In high-prevalence settings: HBV and HCV testing of mothers and infants should be routinely

available through a variety of services – child health services, immunization clinics, under-5 clinics, malnutrition services, well-child services and services for hospitalized and all sick children, TB clinics, and services for orphans and vulnerable children.

Testing the family members of index cases

Gaps in HBV testing and in documenting the HBV status of children of HBV-positive parents constitute significant missed opportunities. These gaps can be closed by following up the families of cases identified in ANC or facilities offering HBV testing. In all settings all children with an HBV-positive parent or close household contact should be tested for HBV as a priority.

Rationale for recommendation:

Strength of recommendation

Implementation considerations

- As for all testing services, programmes for key populations need to emphasize WHO's "5 Cs" – particularly consent, confidentiality and connection to comprehensive prevention, care and treatment.
- Need to overcome reluctance to provide partner testing/index partner testing
- Make use of lay providers/peer testing for outreach especially among key populations
- Viral hepatitis testing for key populations needs to be delivered alongside other key primary prevention interventions.
- Accessibility and coverage of testing would need to be high to have an impact on the prevalence of HBV among PWID and other key populations. Offering DBS testing for HCV to PWID attending drug treatment programmes increased uptake of testing services.

Research gaps

- Further research and large scale-implementation studies should be performed to evaluate this further in other high-endemic, low-income settings.
- What proportion of HBV- or HCV-positive cases will be missed by a testing policy based on screening for at risk behaviours and exposures?
- Evaluation of different testing approaches in terms of cost, impact and cost–effectiveness and evaluation of key drivers in a range of different settings.

4.2. Who to test HCV

Decision-making tables – PICO 2

What is the impact, cost and cost–effectiveness of different HCV testing approaches and scenarios?

Topic for analysis: who to screen?

Population:

1. Risk-based screening in different high-risk populations

Injecting drug users (IDUs), men who have sex with men (MSM), immigrants, recipients of blood transfusion and blood products, sex workers, and health-care workers (HCW), HIV-infected persons

2. General population (excluding blood donors) or selected subpopulations of general population (women during pregnancy, those with raised alanine aminotransferase (ALT), infants, schoolchildren and adolescents)

3. Other approaches: Birth cohort screening (based on different age cut-offs; born between 1945–1960 or 1965 or 1970)

4. One-off screening vs repeat screening every five years.

Intervention: Testing strategies for HBV in different populations (risk-based and general population and birth cohort); and at different prevalence thresholds

Comparator: No testing or current practice or comparison of different testing strategies

Outcomes: Benefits, harms and costs, and cost–effectiveness with different screening strategies for different target populations

Individual patient outcomes: Number of cases detected, overall mortality, liver-related mortality, cirrhosis, end-stage liver disease, rate of hospitalizations, serious adverse events, quality of life

Prevention: New infections (mother to child, horizontal (IDUs needle sharing and sexual; and sexual, especially MSM)

Cost–effectiveness: Cost and incremental cost per case diagnosed; cost and incremental cost per case screened and treated; cost and incremental cost per life saved; cost and incremental cost per infections averted; quality-adjusted life-years (QALYs) gained

Background:

Hepatitis C virus (HCV) is a global public health burden and major cause of morbidity and mortality including liver failure and hepatocellular carcinoma. Current global HCV seroprevalence is estimated to be 2.8%, or >185 million infected individuals worldwide.

Routes of transmission: In many countries, HBV, HCV and HIV transmission occurs predominantly in high-risk key populations, often via common routes of transmission. Key populations include people who inject drugs (PWID), people in prisons and other closed settings, some mobile populations, some indigenous populations, MSM and sex workers. PWID are a key population who are at particularly high risk of HCV infection. In many high-income countries and some developing countries, ongoing HCV transmission is driven mainly by PWID populations.

The advent of high-efficacy, low duration therapy, however, generates prioritization for testing for HCV infection, linking infected patients to care, and curing HCV before patients begin to experience the consequences of cirrhosis and end-stage liver disease.

Low rates of diagnosis and linkage to care: The majority of people are unaware of their HCV infection, and therefore often present with advanced disease. Based on still limited studies, overall <15% of the estimated 180 million who are chronically infected with HCV are aware of their diagnosis, based on data from higher-income settings – United States, Europe and China.

In addition to the very low access to and uptake of testing, there is also further attrition on the care cascade with very poor linkage to care and therefore treatment, among those who test positive.

Hepatitis B and C testing and diagnosis are at the core of entry to both the prevention and treatment cascade. Testing is required to *identify those with are positive*, linking them with care, counselling them on measures to reduce transmission to others then assessing who needs treatment, initiating treatment, achieving treatment response (sustained virological response [SVR] for hepatitis C). Hepatitis testing is also needed to *identify those who are negative*, to provide hepatitis B vaccination, and the opportunity to implement individual or facility level prevention measures counsel to reduce risk behaviours.

There are three key approaches to HCV screening

1. Targeted risk factor-based screening. This refers to screening of specific groups including key populations, who are generally at higher risk of being infected than the general population. This includes PWID, people in prisons and other closed settings, migrant populations, some indigenous populations, MSM and sex workers, but may also include health-care workers. People attending services providing care and treatment for viral hepatitis or HIV can be encouraged to bring their partners to be tested.

   This involves screening those with high-risk behaviours, exposures and other conditions.

Most countries have based their list of high-risk groups as defined by the Centers for Disease Control and Prevention (CDC), and are largely based on known modes of transmission.

i. Family members and household contacts of hepatitis B patients
ii. MSM
iii. PWID
iv. HIV-positive patients
v. Patients on immunosuppression or chemotherapy
vi. Persons with liver disease of unclear etiology
vii. Health-care workers.

2. **Birth cohort screening** for HBV and HCV
3. **Population or community-based screening (including antenatal).** Routine general population screening: i.e. testing among the general population without attempt to identify high-risk behaviours or characteristics ("routine testing"). This means that all members of the population have access to the screening programme under consideration. It may also include home-based testing (house to house); campaigns (e.g. HTC plus – malaria, safe water, non-communicable diseases (diabetes and hypertension); outreach (mobile) in general and key populations; workplaces and schools; and health-care facility-based screening.
4. **Health-care facilities.** Testing could also be offered in special dedicated clinics, e.g. HIV, STI clinics. Screening at health-care facilities may include primary care settings, inpatient and outpatient settings, and may involve screening on the basis of clinical presentation or focus on only those with abnormal liver function tests, abnormal ultrasound scan, family history of liver disease or other clinical suspicion of liver function test.

   Persons in whom there is clinical suspicion of viral hepatitis: even when risk factors for HBV and/or HCV are not present, screening is indicated wherever there is clinical suspicion of viral hepatitis infection. This may occur, for example, where there is existing liver disease, including liver cirrhosis or hepatocellular carcinoma, or where there is unexplained liver disease including abnormal liver function tests.

Existing guidelines: what are countries doing?

The main approach to HCV testing is a targeted risk factor-based testing for those with high-risk behaviours, exposures and other conditions, e.g. (PWID, liver disease, renal dialysis) and use of a birth cohort approach in the US and Japan. At present, no country guidelines recommend routine testing for all individuals regardless of demographics or specific behavioural risk.

1. Screen those with high-risk behaviours, exposures and other conditions. Most countries have based their list of high-risk groups as defined by CDC, and are largely based on known modes of transmission.

   i. Family members and household contacts of hepatitis B patients
   ii. MSM
   iii. PWID

iv. HIV-positive patients
v. Patients on immunosuppression or chemotherapy
vi. Persons with liver disease of unclear etiology
vii. Health-care workers.

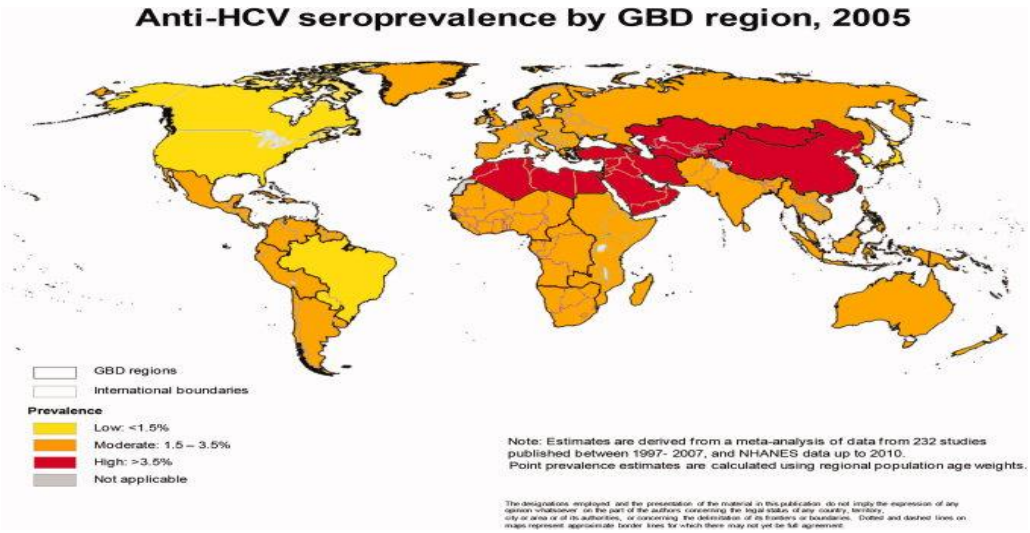
Survey of guidelines (Surjo De)

Global prevalence of hepatitis C virus

General population (Hanafiah et al. 2013)	PWID (Nelson et al. 2011)	MSM (Hope, et al. 2014 – data is for European countries outside of the EU)	Sex workers (Hope, et al. 2014 – data is for European countries outside of the EU)	Migrants and refugees (Hanhe et al. 2013)	Prisoners (Larney et al. 2013)	Pregnant women (Hanhe et al., 2013)	HIV-infected persons	Health-care workers
Southeast Asia 2% East Asia 3.7% Oceana 2.6% South Asia 3.4% Central Asia 3.8% Central Europe 2.4% Eastern Europe 2.9% North Africa and Middle East region 3.6% Central Africa 2.3% East Africa 2% Southern Africa 2.1% West Africa 2.8% Andean Latin America 2% Central Latin America 1.6% Southern Latin America 1.6% Tropical Latin America 1.2% Caribbean 2.1% Asia Pacific High income 1.4% Australasia 2.7% Western Europe 2.4%	Southeast Asia and East Asia 41–89.8% South Asia 36.0– 87.3% Central Asia 51.7–61.3 % Eastern Europe 22.6–90.5% North Africa and Middle East region 28.–67.6% West Africa 22.2 - 97.3% Andean Latin America 9.8 – 97.4% Australasia 51.9-54.6 % Western Europe 20.7 – 86.2%	Azerbaijan 14% Bosnia 12% Croatia 3% Georgia 16% Kazakhstan 4.20% Kyrgyzstan 1.20% Republic of Moldova 11% Russian Federation 2.30% Ukraine 20%	Azerbaijan 9.30% Bosnia and Herzegovina 4.30% Croatia 4% Kazakhstan 11% Kyrgyzstan 3.9–28% Republic of Moldova 13% Russian Federation 14–40% Serbia 3.30% Tajikistan 6.30% Turkey 2. 40% Ukraine 32% Uzbekistan 11–12.8%	Europe 0–23.4% (Hungary)	SSA 7–26% Western Europe 26–34% Eastern Europe 14–31% Latin America 8–19% Australasia 28–43% North America 24–34% South Asia 4–11% Middle East and North Africa 1–5% East and SE Asia 13–38% Central Asia 32–43% Extrapolated global 23–29%	Europe 0 (Slovakia) –1.7% (Italy)		United States Hospital workers 1% (Alter, 1997) Egypt National Liver Institute HCWs 16.8% (Adelwahab et al. 2013)

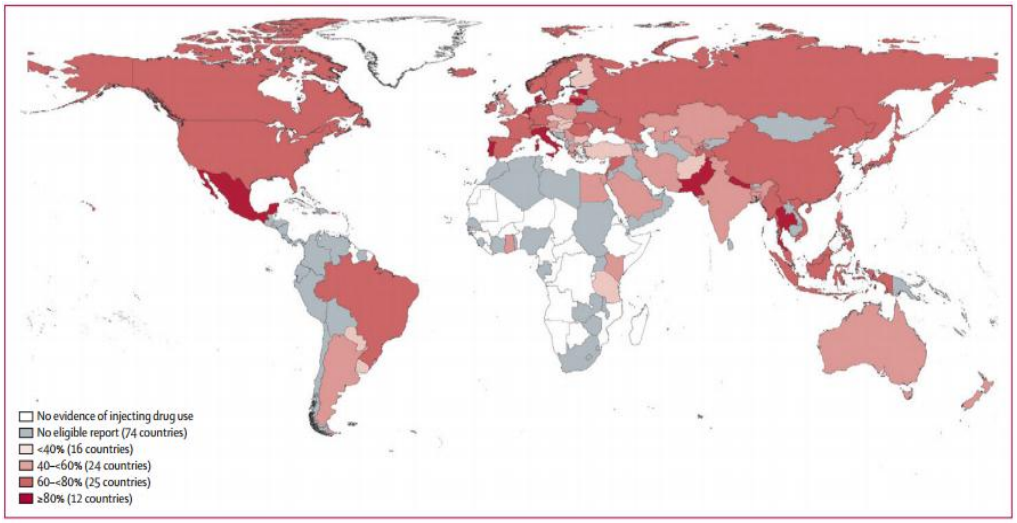
North America High Income 1.3%								

Global epidemiology of hepatitis C virus infection: new estimates of age ‐ specific antibody to HCV seroprevalence (Hanafiah, 2013)



Prevalence of hepatitis C antibodies in injecting drug users

A review of global prevalence data from 77 countries estimated that exposure to HCV (anti-HCV positive) among PWID is estimated to be between 60% and 80% in 25 countries and over 80% in 12 countries.



MSM

MSM can acquire HBV and HCV sexually. MSM who are HIV positive are at significantly higher risk of

acquiring HCV infection than HIV negative MSM. Incidence rates of sexually acquired HCV among MSM have been rising in several industrialized countries since 2000, and outbreaks have been described in some less industrialized nations.

Systematic review of HCV prevalence in HIV-infected persons (Platt et al. 2015).

Coinfection estimates were identified for 78 of the 194 countries (40%). There were 760 HIV/HCV coinfection prevalence estimates. Findings suggest that globally HCV/HIV coinfection is 1.9% (IQR = 0·4–6·6%) among general population samples, 7% (IQR = 2·6–11·1%) among people living with HIV (PLHIV) who are pregnant or where heterosexual transmission is reported, 6.2% (IQR = 3·3–13·5%) among men who have sex with men (MSM) and 83% (IQR = 55·2–94·1%) among people who inject drugs (PWID). Odds of HCV infection are 11 times higher in the presence of HIV infection, but varied by risk group. The global estimate of HCV coinfections among PLHIV is 3.2 million (IQR = 1.4–4.3 million) of whom 1.2 million (IQR = 0.9–1.4 million) are PWID

	Mid-point co-infection prevalence (Interquartile range) **Number of studies**				
	Gen pop	**PWID**	**MSM**	**Hetero**	**Pregnant**
East Africa	1.3% (0-4.9) **5**	71 % (42-99) **2**	20%(1-38) **2**	4% (3-9) **10**	0.6% (0.1-5) **3**
Cental and West Africa	5 % (2-12) **9**		8% **1**	8% (4-12.4) **19**	10.1 (5-16) **4**
South Africa			2% **1**	0.5% (0-1) **3**	
Latin America	7% (0.8-16.1) **3**	82% (52-88) **4**	4% (0-16) **6**	11% (8-15) **2**	10% (5-18) **4**
North America		84 (41-89) **25**	13 (8-15) **16**	12 (9-25) **9**	4% **1**
South East Asia	5% (3-29) **7**	90 (86-97) **18**	6% (5-8) **5**	5% (1.5-7) **5**	
Eastern Europe and Central Asia		82% (68-95) **8**			
Europe	6% (0.3-30) **3**	82% (53-91) **41**	8% (4-17) **40**	11% (4-23) **11**	3% **1**
East Med	1% **1**	81% (74-89) **7**			
East Asia		96% (80-98) **15**	4% (2-9) **3**	51% (6-89) **7**	
Western Pacific			9% (7-10) **4**		

We compared the prevalence of HCV among 105 samples of HIV-positive and -negative population groups (general population, PWID, MSM, sex workers, prison inmates and high-risk populations). This is summarized in Figure 4 and in the online table. Overall, there was a 12-fold (95% CI 11·2–11·8) increased odds for HCV positivity across all population groups among HIV-positive compared to HIV-negative persons. Odds of HCV were highest among HIV-positive prison inmates (OR=16·5 95% CI 15·9–17·1) and other high-risk populations (OR = 11·7 95% CI 11·0–12·4), PWID (OR = 5·1 95% CI 4·7–5·5), followed by MSM (OR = 3·8 95% CI 3·1–4·5) and general population samples (OR = 3·7 95% CI 3·3–4·3) and sex workers (OR = 2·5 95% CI 2·0–3·2).

Epidemic scenarios hepatitis C virus

Generally, HCV epidemics around the world are heterogeneous and represent mixtures of three core

epidemic components:

1. *Infection related to high-risk behaviours:* In essentially every geographical region, the highest prevalence of HCV infection is among persons who use injection drugs (PWID). The prevalence of injection drug use differs between countries and regions, but within those who do inject drugs, HCV prevalence is nearly universally high. Commercial sex workers and prisoners also have increased prevalence (presumably related to both drug use and perhaps sexual transmission) as do men who have sex with men, especially those who are HIV infected. In many cohorts of PWID in North America, Europe and Asia, HCV prevalence ranges from 30% to 75%.
2. *Infection related to past generalized exposures that have since been identified and removed:* this epidemic pattern, in which there is a high prevalence of HCV within a given age group, is commonly referred to as a "birth cohort epidemic." While typically identified as being the infection pattern in North America and Europe, many nations have some element of birth cohort epidemics with their unique HCV epidemiology (Table 1). Birth cohort epidemics reflect an HCV exposure source that was once present and to which a large portion of the population was exposed, but that has since been identified and removed. For example, before it was identified and sequenced, HCV infected the blood supply of many countries in all regions of the world. When the blood supply began to be screened for the presence of HCV, the exposure was removed. As a result, the incidence of HCV fell dramatically among the general population, but there remains a burden of prevalent, chronic HCV among patients who were alive and likely to get a blood transfusion during the time that HCV existed in the blood supply.
3. *Generalized population epidemic:* This pattern is related to a widespread exposure, often iatrogenic, that results in high prevalence (8–10%) across essentially all age groups. Note that the primary difference between a "birth cohort" pattern and a generalized pattern of infection is the duration of time that the generalized exposure existed and whether it has been removed or mitigated. An example of a generalized exposure is the common use of reusable hypodermic syringes and needles in medical settings without adequate sterilization between uses.

Few epidemics fall into one of the above three categories. Rather, most are mixed, and represent some combination of all components (Table).

Epidemic scenarios			1.1.2
	Definition	**Disaggregation**	**Country example**
Generalized	High (>5%)	With birth cohort	Egypt, Pakistan
		Without birth cohort	1.1.3
	High intermediate (3–5%)	With birth cohort	Congo, Ukraine
		Without birth cohort	1.1.4
	Low intermediate (2–3%)	With birth cohort	Cote d'Ivoire, Thailand
		Without birth cohort	1.1.5

Mixed	Generalized population prevalence, low, moderate or high with a sizeable risk population (PWID)	High generalized	Uzbekistan
		High intermediate generalized	Taiwan
		Low intermediate generalized	Syria, Hong Kong
		Low (1–2%) with PWID	Mexico, Switzerland
		Low (1–2 %) without PWID	The Gambia, Singapore
		To check (UNDOC % of population)	1.1.6
Concentrated	Generalized population prevalence <1% with high-risk groups		United Kingdom, Turkey

Extra risk classification	**Country example**
Unsafe blood transfusions prior to 1990	Brazil, Portugal

Draft recommendation(s):

Existing recommendations (*Source:* WHO, 2014[9])

1. **Risk-based:** It is recommended that HCV serology testing be offered to individuals who are part of a population with high HCV seroprevalence or who have a history of HCV risk exposure/behaviour. These include:

- Persons who inject drugs (PWID)
- Persons with HIV infection (HIV-positive men who have sex with men)
- Prisoners and persons previously incarcerated
- Persons who have had tattoos, body piercing or scarification procedures where infection control is not guaranteed
- Children born to mothers infected with HCV
- Close contacts of persons infected with HCV
- Persons who have used intranasal drugs
- Persons from a country with intermediate or high prevalence (2% or greater) of hepatitis C
- Persons who have received medical or dental interventions in health-care settings where infection control is not guaranteed
- Persons who have received blood transfusions prior to the time when HCV serologic testing of blood donors was initiated or in countries where HCV serologic testing of blood donations is not routinely performed.

2. **General population and birth cohort:** It is recommended that in settings with a high HCV seroprevalence (>8%) in the general population, testing be offered, especially at least once, to persons born between 1945 and 1955.

Existing WHO recommendations

HIV testing services should be routinely offered to all key populations in the community, closed settings such as prisons, and clinical settings.

PWID: WHO recommends delivery of a comprehensive package of nine evidence-based interventions for HIV prevention, treatment and care for PWID, all of which are also directly relevant to prevention, treatment and care for HBV and HCV, and one of which is specific to viral hepatitis testing (vaccination, diagnosis and treatment of viral hepatitis, see Box).

The nine interventions in the comprehensive package for HIV prevention, treatment and care for people who inject drugs

1. Needle and syringe programmes
2. Opioid substitution therapy and other drug dependence treatment
3. HIV testing and counselling
4. Antiretroviral therapy
5. Prevention and treatment of sexually transmitted infections
6. Condom programmes for people who inject drugs and their sexual partners
7. Targeted information, education and communication for people who inject drugs and their sexual partners
8. Vaccination, diagnosis and treatment of viral hepatitis
9. Prevention, diagnosis and treatment of tuberculosis.

Source: WHO, UNODC, UNAIDS,

Sex workers

WHO outlines a comprehensive set of interventions and approaches, both to promote enabling environments and to provide prevention, testing, care and treatment, in relation to HIV and STI programming for sex workers (see Box). These recommendations are directly relevant to the response to viral hepatitis among sex worker populations. Essential interventions include enabling sex workers to access and consistently use condoms, access prevention and care, treatment and support services, diagnosis and treatment of important comorbid conditions such as for TB (particularly in HIV endemic settings, incarcerated persons, PWID and sex workers living in exposed to poor cramped working and living conditions), other STIs, and access to harm reduction services for sex workers who inject drugs.

Importantly, as sex workers are a key population highly affected by the HBV epidemic, particularly in settings where vaccine coverage is suboptimal, and at high risk for HCV if they inject drugs, they must have access to hepatitis testing services (HepTS), repeat viral hepatitis testing, and partner and family testing wherever appropriate. The offer of HIV testing services (HTS) should be offered alongside HepTS, and HepTS can be integrated into HTS wherever possible. Delivery of HepTS should be informed by recommendations for the delivery of HTS. A variety of settings may be

appropriate in which to implement testing services, including health-care settings as well as community settings, and via multiple different approaches. Outreach peer-led testing is likely to be particularly effective and acceptable in many sex worker populations.

Good practice recommendations

1. All countries should work toward decriminalization of sex work and elimination of the unjust application of non-criminal laws and regulations against sex workers.
2. Governments should establish antidiscrimination and other rights-respecting laws to protect against discrimination and violence and other violations of rights faced by sex workers in order to realize their human rights and reduce their vulnerability to HIV infection and the impact of AIDS. Antidiscrimination laws and regulations should guarantee sex workers' right to health and financial services.
3. Health services should be made available, accessible and acceptable to sex workers based on the principles of avoidance of stigma, non-discrimination and the right to health.
4. Violence against sex workers is a risk factor for HIV and must be prevented and addressed in partnership with sex workers and sex worker-led organizations.

Evidence-based recommendations

1. Offer a package of interventions to enhance community empowerment among sex workers.
2. Promote correct and consistent condom use among sex workers and their clients.
3. Offer periodic screening for asymptomatic STIs to female sex workers.
4. Offer female sex workers, in settings with high prevalence and limited clinical services, periodic presumptive treatment for asymptomatic STIs.
5. Offer voluntary HIV testing and counselling to sex workers.
6. Use the current WHO recommendations on the use of antiretroviral therapy for HIV positive general populations for sex workers.
7. Use the current WHO recommendations on harm reduction for sex workers who inject drugs (in particular needle and syringe programme and opioid substitution therapy).
8. Include sex workers as targets of catch-up hepatitis B immunization strategies in settings where infant immunization has not reached full coverage.

Source: WHO, 2012

Prisons

Key WHO recommendations around testing services for people in prisons and closed settings have, to date, mostly focused on HIV prevention, testing and treatment. In 2013, UNODC and partners developed a comprehensive package of 15 key interventions for HIV prevention and treatment in prisons and other closed settings. Due to common transmission routes, these recommendations are equally applicable to viral hepatitis, and HBV vaccination and diagnosis and treatment of viral hepatitis is one of the specific recommendations (see box). This recommendation stipulates that

"prisons should have a comprehensive hepatitis programme, including the provision of free hepatitis B vaccination for all prisoners, free hepatitis A vaccination to those at risk, and other interventions to prevent, diagnose and treat hepatitis B and C equivalent to those available in the community (including condom, needle and syringe programmes and drug dependence treatment as needed)."

Existing recommendations

1. Information, education and communication
2. Condom programmes
3. Prevention of sexual violence
4. Drug dependence treatment, including opioid substitution therapy
5. Needle and syringe programmes
6. Prevention of transmission through medical or dental services
7. Prevention of transmission through tattooing, piercing and other forms of skin penetration
8. Post-exposure prophylaxis
9. HIV testing and counselling
10. HIV treatment, care and support
11. Prevention, diagnosis and treatment of tuberculosis
12. Prevention of mother-to-child transmission of HIV
13. Prevention and treatment of sexually transmitted infections
14. Vaccination, diagnosis and treatment of viral hepatitis
15. Protecting staff from occupational hazards

Source: WHO, 2013

Recommendations and principles surrounding HIV testing in prisons apply equally to HBV and HCV testing in prisons (see box).

Existing recommendations

- It is important to guard against negative consequences of testing in prisons – for example, segregation of prisoners – and to respect confidentiality.
- It is also important that people who test positive have access and are linked to HIV care and treatment services.
- HIV testing and counselling should be voluntary.
- The use of HIV rapid testing can increase the likelihood of prisoners receiving their results.
- Testing in conjunction with other risk-reduction services such as the provision of condoms with lubricants and STI screening can increase the benefits of testing and counselling.

Source: WHO, 2014.

Summary and quality of evidence

Summary of evidence base for different screening approaches

Cost–effectiveness evidence summary: overview of report

☐ High
☐ Moderate
☐ Low
☐ Very low

Cost–effectiveness analyses of screening for HCV

1. **Gleue systematic search identified 19 studies.** Majority of studies evaluated the cost–effectiveness of screening for HCV in Europe or the US; one study was carried out in Japan and another one in Italy. Ten studies evaluated screening in the general population; eleven studies screening IDUs; three studies looked at recipients of blood transfusions; one study evaluated screening in women during pregnancy and a further study looked at HCWs. Studies evaluated a one-off screening intervention, with the exception of one study that analysed screening every five years. Comparators were either: no screening or the status quo or different strategies were compared with each other. Recent studies concentrated on birth cohort screening in the US, evaluating the cost–effectiveness of one-off screening for a cohort born between 1946 and 1970 and a cohort born between 1945 and 1965. HCV prevalence in this population was comparatively high. The initiation of a one-off screening intervention was assessed and compared with current risk-based screening interventions in these US studies.

2. **Targeted review of the literature** to determine the state of evidence about the cost–effectiveness of testing for HCV in different types of epidemics and among different risk groups. We provide a qualitative assessment of conclusions.

- Testing in high-risk groups such as PWID, MSM, prisoners, HIV-infected persons, and commercial sex workers is likely cost–effective. Testing in settings with a high prevalence of high-risk patients is almost certainly cost–effective in all locations. It is important, however, to ensure adequate follow up after diagnosis.
- The best approach to testing outside of high-risk risk groups depends a great deal on a country's unique HCV epidemiology. Most countries have at least some component of "birth cohort" epidemic, and "birth cohort" testing is likely cost–effective in most settings.
- In most epidemics, routine screening in the entire population is likely not to be cost–effective. The specific threshold at which a country should alter its approach to routine testing, however, is a

function of multiple factors and cannot be identified more generally.

This report does not represent the results of a full systematic review. It is meant to serve as a summary of existing studies on cost–effectiveness of screening and treatment for HCV, with an analytic summary of key considerations. Due to the lack of relevant literature from low- and middle-income countries (LMICs), existing studies from high-income countries (HICs) are described and their potential uses and limitations, when drawing conclusions are discussed.

Summary of findings and conclusions

- Testing in high-risk groups such as PWID, MSM, prisoners, HIV-infected persons, and commercial sex workers is likely cost–effective. Testing in settings with a high prevalence of high-risk patients is almost certainly cost–effective in all locations. It is important, however, to ensure adequate follow up after diagnosis.
- Persons who inject drugs: Multiple analyses in many geographical regions concur that routine testing for HCV in venues with a high prevalence of PWID is cost–effective, even when the studies assume very poor follow-up rates and limited access to therapy. Further, dynamic HCV transmission models suggest that aggressive diagnosis and treatment among current drug users could reduce the incidence of HCV – "cure as prevention". With typical prevalence estimates of 40%, but ranging as high as 75% in some cohorts, routine screening for HCV is almost certainly cost–effective.

1. *Men who have sex with men:* Men who have sex with men (MSM) are also at an increased risk of HCV incidence, particularly if they are also HIV-positive. Cost–effectiveness modelling has found testing using liver function tests in combination with HCV Ab testing to be cost–effective in the HIV-positive MSM population. The results of these studies are dependent on appropriate linkage to effective therapy and retention in care.
2. *Prisoners:* Prisons are likely to have a high HCV prevalence as the result of a high prevalence of PWID in prisons. A UK-based study, however, found that HCV case detection, using dried blood spot testing, was cost–effective, even when the model assumed low rates of HCV treatment initiation. A second study concurs that screening in prisons can be cost–effective, but this study concluded that targeting to screening to those prisoners with a history of injection drug use improves cost–effectiveness.

3. HIV-infected persons: Although nearly every guideline for HCV care recommends HCV screening at enrolment in care, there are no cost–effectiveness analyses that address the specific question of the cost–effectiveness of HCV testing at enrollment in HCV care. Because the prevalence of HCV is known to be high in HIV-infected persons, the pace of fibrosis progression in HIV/HCV-coinfected patients is high, and new therapies to treat HCV are effective in HIV/HCV coinfection, testing for HCV at enrolment in HCV is almost certainly cost–effective.
4. *Sex workers:* Because many sex workers are also PWID or non-injection drug users, the prevalence of HCV in this group is likely high. No studies were identified that address cost–effectiveness of HCV testing in sex workers, and therefore uncertain whether cost–effective to routinely screen all sex workers, compared to an approach that targets testing to sex workers who report a history of injection drug use.

- **Birth-cohort:** i.e. testing among easily identified age or demographic groups known to have high HCV prevalence ("birth-cohort testing"). Most countries have at least some component of "birth cohort" epidemic, and "birth cohort" testing is likely cost–effective in most settings**.** Whenever there is an easily identified demographic group that has high HCV prevalence (for example, all individuals born in a certain time period) it is likely cost–effective to routinely test for HCV within that cohort. Several cost–effectiveness studies in the US and in Portugal show that birth cohort testing was cost–effective.
- **Routine testing of the entire population:** Routine general population screening: testing among the general population without attempt to identify high-risk behaviours or characteristics ("routine testing"). The data about population screening typically come from HICs such as the US and UK, and such studies find that routine testing in the general population is not cost–effective. When compared to "birth cohort testing", however, universal testing resulted in worse outcomes and higher costs than the birth cohort approach. This analysis raises the spectre that in countries whose HCV epidemic is largely concentrated to a specific birth cohort or demographics group, attempting to identify cases by routine testing of the entire population can dilute the testing effort and result in fewer cases of HCV being identified. An older study, conducted in the UK, also found that although screening high-risk groups in primary care settings was cost–effective, extending screening beyond high-risk individuals was not.

Importantly, all of the above studies reflect the epidemiology of HCV in HICs. One recent paper explicitly studied the cost–effectiveness in Egypt of one-time, routine screening for HCV followed by treatment with either pegylated interferon and ribavirin (PEG-RBV) or PEG-RBV plus an HCV protease inhibitor. Given the very high prevalence of disease, screening was always cost–effective, and often cost-saving. **Drivers of cost–effectiveness** **HCV prevalence**: Screening provides increasing value as prevalence rises. In one U.S.-based study, screening was cost–effective (compared to no screening) at a US willingness-to-pay threshold down to a prevalence of 0.53%. In addition to the underlying prevalence of HCV infection, studies identified the **rate of progression from chronic HCV to cirrhosis** as an important factor together with levels of linkage to care and treatment that substantially influenced cost–effectiveness results.	
Risks/benefits **Targeted risk-based testing** **Benefits** • Key populations are disproportionately affected by hepatitis in all regions. • Key populations are less likely to have received HBV vaccination, and offer of HBV testing will facilitate higher rates of completion of vaccination. • *In HIV-infected persons:* Benefits of testing and treatment greatest in HIV-coinfected persons as they have more rapid progression of liver disease (HCV-associated liver disease in coinfected patients is emerging as a major cause of morbidity and mortality in HIV-infected persons) and risk of onward transmission than those without HIV infection. • Leads to earlier diagnosis and access to treatment before development of cirrhosis. • Some studies of PWID have shown that HCV testing and knowledge of serostatus results in reduction in injecting risk behaviours, including frequency of injecting and number of people injected with, in addition to other important risk behaviours, such as heavy alcohol consumption, and increases treatment uptake. • Modelling studies suggest that scaling up treatment with DAAs, across different prevalence settings, would have a major impact on the prevalence of HCV.	☐ Benefits clearly outweigh harms ☐ Benefits and harms are balanced ☐ Potential harms clearly outweigh potential benefits *Are the desirable anticipated effects large?* ☐ No ☐ Probably ☐ Uncertain ☐ Yes ☐ Varies

- HCV treatment outcomes among people who actively inject drugs have been found to be acceptable.
- Community-based testing is a critical approach for reaching people from key populations and vulnerable populations who are unlikely to go to a facility, particularly those who are asymptomatic.

Risks

- Challenging to identify and engage high-risk groups
- People may be reluctant to admit risk behaviours, or may be unaware they are at risk, and so a screening approach that relies on history may miss a substantial proportion of cases.
- Much high-risk behaviour is stigmatized and underreported.
- Health-care workers are not always skilled at identifying high-risk behaviours.
- Because HCV risk behaviours are stigmatized and underreported, trying to identify high-risk individuals is difficult and prone to under testing high-risk patients.
- For key populations, especially those whose behaviour is criminalized, testing services are sometimes misused in punitive or coercive ways. As a result, people from key populations avoid the health services that they need.
- In addition, stigma, discrimination, lack of confidentiality, coercion and fear of repercussions, as well as lack of appropriate health services, resources and supplies, prevent people from testing and, if positive, linking to care.
- Provider-initiated testing and counselling (PITC) among key populations and vulnerable populations is recommended, so long as it is not compulsory or coercive and it is linked to treatment and care.
- In many settings, and in spite of guidelines, service providers are often reluctant to offer antiviral treatment to PWID.

PITC in health-care facilities

Benefits

- 89/117 LMICs recommend HIV PITC in all patient encounters.
- High HIV PITC acceptance in antenatal care (ANC) and TB settings
- Introduction of PITC increased paediatric HIV testing
- Many clinical settings in generalized epidemic settings not offering hepatitis testing – e.g. STI clinics, primary care, and so many missed opportunities for HBV diagnosis in health care facilities.

Partner testing

Benefits

- Participating in couples and partner HBV testing has a number of benefits. These include adoption of prevention strategies by the couple (for example, condom use, safe injecting practices) and promotion of linkage to and retention in appropriate health-care services.
- Also applies to opportunistically offering HBV testing and vaccination to family members and other close household contacts of people diagnosed with CHB re access to vaccination and care.
- Couples and partner testing helps more people know their HBV and/or HCV status, particularly men, who in generalized epidemic settings may be less likely to test than women.
- Partners: <5% of people currently HIV test with their partners and similar low rates for HBV. Note: HIV serodiscordancy is common (half to two thirds of HIV-positive adults with cohabiting relationship have HIV-negative partner.
- Offering partner testing for persons with HBV and HCV – highest possible yield. Although risk of infection may be low, a negative test in the partner provides reassurance and the opportunity to provide counselling on reducing future risk, including vaccination.

Birth cohort testing

Benefits

- Being a member of a birth cohort is easily determined.
- Screening by age group is less stigmatizing.
- Targeting testing to birth cohorts is feasible and often cost–effective.
- In countries with a strong birth cohort dynamic, birth cohort screening is likely preferred.

Risks

- Feasibility and successful implementation of birth cohort screening not well established

Routine generalized testing

Benefits

- Leads to earlier diagnosis and access to treatment before development of cirrhosis

• Way of accessing missing populations, such as men, key populations and young women who are not pregnant • Community-based testing is a critical approach for reaching people from key populations and vulnerable populations who are unlikely to go to a facility, particularly those who are asymptomatic. **Risks** • When the HCV epidemic is concentrated to a specific age or risk group, routine generalized testing and screening may dilute the screening effort in the cohort with the highest prevalence of HCV and result in fewer cases of HCV identified and higher cost than "birth cohort testing." • If an epidemic is highly concentrated with a specific risk or demographic group, screening outside of that group can be inefficient and increase costs. • In most epidemics, routine screening in the entire population may not be cost–effective. • May lead to lower-than-expected positivity rates with home-based testing, testing within campaigns, key population outreach and testing of index partners. • Countries with high HCV prevalence across the entire population should implement routine screening, but • The specific threshold at which a country should alter its approach to routine testing, however, is a function of multiple factors and cannot be identified more generally. • Suboptimal linkage to care is highly variable and may be problematic	
Acceptability, values and preferences A values and preferences survey of implementers and users of hepatitis B and C testing services was carried out by FIND in September 2015. A total of 104 respondents from 43 (20 high-income, 23 low- and middle-income) countries. Relating to this PICO, • Respondents from LMICs identified following target populations as priority for hepatitis B and C testing: blood donors (>85% for B and C), children born to HCV-infected mothers (55% vs 75% for HBV), persons living with HIV (50% vs 65%), and pregnant women (40% vs 78%), MSM (25% vs 45% for HBV), sex workers (<10% for HCV and 45% for HBV), and prisoners (25% for HCV and HBV) and those chronically ill (around 25% for HCV and HBV%).	☐ No major variability ☐ Major variability Is the option acceptable to key stakeholders? ☐ No ☐ Probably ☐ Uncertain ☐ Yes ☐ Varies

• Population – wide screening was suggested by less than 10% for HCV and around 15% for HBV (30% of respondents), and in blood donors (22%) was less supported. **PITC** • There is generally high acceptance of testing in ANC settings **Partner testing** • Offering partner testing for persons with HBV and HCV – highest possible yield • Need to overcome reluctance to provide partner testing/index partner testing. **Targeted testing in drug treatment programmes, STI clinics, HIV clinics** • Routine HIV, hepatitis B and C testing in these clinic settings less stigmatizing	
Equity, ethics and human right implications *Will recommendation raise questions around equity?* • As for all testing services, programmes for key populations need to emphasize WHO's "5 Cs" – particularly consent, confidentiality and connection to comprehensive prevention, care and treatment. • The use of community-based and hepatitis B and C rapid testing can increase the likelihood of some key populations, such as prisoners receiving their results. • Testing in certain populations, such as in prisons may increase chance for stigmatization. *Are there ethical implications to this recommendation?* • No major concerns.	☐ Less equitable ☐ More equitable
Resource use and financial implications **Resource use (*see* parameter matrix for sample HIV testing programme costs)** Estimating the costs associated with a given hepatitis testing approach can be challenging. Costs for similar hepatitis testing may differ significantly between countries and by programme type within a country. Differences in	*1.1.9* *Are the resources required small?* ☐ No ☐ Probably ☐ Uncertain ☐ Yes ☐ Varies

programme costs may be due to general cost differences between countries, in what specific services are provided (referral to clinic for those testing hepatitis-positive vs enhanced linkage support), cadre of staff employed (nurses vs community health workers), the ease of reaching different populations, the capacity of the health system, and the level of HIV testing coverage. **Standardized approach to costing of hepatitis testing:** A common approach to estimating costs involves identifying and estimating costs incurred by the health-care provider within the following broad categories: • personnel (for example, health-care providers at facilities, counsellors, other paid programme staff, volunteers); • recurrent costs (for example, HIV test kits and commodities, printed materials, office supplies); • capital expenses, often amortized over their useful life and discounted annually at 3% (for example, office space, transportation, equipment); **Materials:** • Cost of testing kits, buffer/reagents • Cost of sterile lancets, pipettes, gloves, sharps-bins or other method of disposal of used kits • Cost of automated reading machine, if applicable • Quality-control reagents, if applicable (some kits are supplied with positive and negative controls) **Training and supervision:** • Cost of training testing providers and appropriate assessment, validation and revalidation of their skills • From included studies, excellent robust specificity of all tests is reassuring in terms of ensuring cost effective initiation of algorithms for further investigation and treatment. • If being utilized at the point of care, it will be the responsibility of the testing provider to record and report the result appropriately.	

- **Feasibility and constraints to implementation**

Are any major barriers expected for the implementation of this recommendation?

- As for all testing services, programmes for key populations need to emphasize WHO's "5 Cs" – particularly consent, confidentiality and connection to comprehensive prevention, care and treatment.
- Need to overcome reluctance to provide partner testing/index partner testing
- Make use of lay providers/peer testing for outreach especially among key populations
- Viral hepatitis testing for key populations needs to be delivered alongside other key primary prevention interventions.
- Accessibility and coverage of testing would need to be high to have an impact on the prevalence of HBV among PWID and other key populations. Offering dried blood spot (DBS) testing for HCV to PWID attending drug treatment programmes increased uptake of testing services.

Is the option feasible to implement?

☐ No
☐ Probably
☐ Uncertain
☐ Yes
☐ Varies

Relevance to different settings/populations

Will this recommendation be most relevant for particular settings (e.g. endemicity)?

Key populations

1. In all settings, a number of social and structural barriers exist to PWID being able to access testing, health-care and harm reduction services, including inadequate coverage and delivery of interventions, stigma and discrimination, high incarceration rates, unstable living conditions, comorbid health problems, poor health literacy and social difficulties. This results in inadequate uptake of prevention, testing, treatment and HBV vaccination,
2. Viral hepatitis testing and treatment for PWID must always be delivered alongside other evidence-based essential primary prevention interventions also. Studies have shown that the uptake of opioid substitution therapy (OST) and adequate needle–syringe programme (NSP) coverage, in isolation, have been shown to reduce the odds of acquiring HCV, but the combination of both interventions together had a much larger impact. Additionally, the high prevalence of comorbidities in populations of PWID, including viral hepatitis/HIV coinfection, TB, mental health problems and poly-drug use alongside social and economic predictors of poor health means that it is particularly important that comprehensive prevention, treatment, care and social services are integrated and accessible to this population.
3. In many settings, responses to viral hepatitis, with particular regard for the need to reach key populations, can be integrated in order to be most effective. When this is not possible, strong links among health services working with priority populations should be established and maintained. Additionally, integration of viral hepatitis testing and treatment with existing services for HIV diagnosis and care is likely to be effective and less resource intensive.

4. Community-based testing is a critical approach for reaching people from key populations and vulnerable populations who are unlikely to go to a facility, particularly those who are asymptomatic. To improve access to and uptake of HBV, HCV and HIV testing, community-based testing services should be made available in settings acceptable and convenient to people from key populations and vulnerable populations
5. Accessibility and coverage of viral hepatitis testing will need to be high to have an impact on the prevalence of HBV and HCV among PWID. In order to achieve this, approaches must be acceptable to PWID populations. For example, studies from the UK suggest that offering DBS testing for HCV to PWID attending substance misuse services may increase uptake of testing services.

Prisoners

Many prisons around the world have implemented viral hepatitis and HIV prevention programmes, including HepTS and HTS as part of a comprehensive package of interventions; however, they are often small in scale and lack the necessary combination of essential interventions, greatly reducing their effectiveness.

Access to testing and counselling for viral hepatitis and HIV which is voluntary in nature and easily accessible alongside access to prevention, care and treatment interventions must be urgently scaled up in prisons. Particular attention must go to providing accurate information, obtaining informed consent and maintaining confidentiality. Additionally, there are often major challenges to continuity of care within closed settings and between prisons and the community that need to be addressed.

Pregnant women

Although the risk of mother-to-child transmission (MTCT) of HCV is much lower than that of HBV, perinatal transmission of HCV does occur in 4–6% of births, and the risk is two to three times higher if the mother is coinfected with HIV. MTCT is the most common cause of HCV infection in young children. In some settings, such as in west sub-Saharan Africa, there is a relatively high prevalence of HCV among young children, which may be a result of the high prevalence of HIV/HCV coinfection in that region.

HCV risk factor information should be elicited from pregnant women, and if present, testing of pregnant women for HCV should also be considered alongside testing initiatives for HIV and HBV. There is currently no effective public health intervention to decrease the risk of MTCT of HCV. However, as DAAs become more widely available, they may have a potential role to play in preventing MTCT of HCV if found to be safe and effective for use in pregnancy. Diagnosis of women with HCV before pregnancy should be prioritized so that appropriate treatment and potential HCV clearance can be achieved.

Children

Consideration should be given to testing children born to HCV-infected mothers, particularly if the

mother is coinfected with HIV or has other risk factors for HCV infection, such as injecting drug use. Similar to HBV, the progression of HCV liver disease is usually slow in infected children, but early diagnosis is important to enable monitoring for liver disease and for linkage to appropriate care and treatment when necessary. As DAAs become more widely available, HCV testing in infants and children will become important to be able to offer curative treatment at an early stage before progression of liver disease.

Most infants whose mothers have been diagnosed with HBV or HCV should be followed up and routinely offered EID, and those diagnosed with either with should be regularly monitored for signs of liver disease so that treatment can be offered when necessary. However, some infants are lost to follow up, so additional paediatric case-finding is important. This can be achieved through the routine offer of PITC in health facilities, particularly in high-prevalence settings, and also through testing the family members of index cases where appropriate.

Integration of HBV and HCV testing into child health programmes

In high-prevalence settings: HBV and HCV testing of mothers and infants should be routinely available through a variety of services – child health services, immunization clinics, under-5 clinics, malnutrition services, well-child services and services for hospitalized and all sick children, TB clinics, and services for orphans and vulnerable children. For example, in Malawi it was reported that integrated testing for HIV-exposed infants at six weeks of age into routine postnatal, under-5 and immunization clinics was acceptable and feasible.

Potential viral hepatitis testing approaches to improve case-finding among infants and children

In all settings

- Offer early infant diagnosis for HBV- and HCV-exposed infants.
- Offer testing to all children and adolescents presenting with indicator conditions/signs and symptoms that suggest acute HBV or HCV, including anorexia, nausea, jaundice, right upper quadrant discomfort and abnormal liver function tests.
- Consider offering viral hepatitis testing to all children and adolescents attending HIV services, STI clinics and TB clinics.

In high-prevalence settings and for high-risk individuals

- Offer viral hepatitis testing or retesting to mothers or infants in immunization clinics or under-5 clinics. If mothers are not available for testing or refuse testing, infant testing is an acceptable alternative.
- Offer viral hepatitis testing to all children with parents or siblings receiving any HIV service (for example, PMTCT, ART) through home-based or facility-based HTS.
- Consider viral hepatitis testing in all children and adolescents attending paediatric inpatient health services.

Rationale for recommendation

. Strength of recommendation
. Implementation considerations
16. Research gaps • Large-scale implementation studies in range of different LMICs should be performed to evaluate different testing approaches and the extent to which providers can accurately identify and test high risk patients when employing a targeted approach or birth cohort approach, as well as estimate of linkage to HCV care and the HCV cascade. Terms of cost, impact and cost–effectiveness and evaluation of key drivers in a range of different high-endemic, low-income settings. • A formal cost–effectiveness analysis that compares “targeted” vs “birth cohort” vs “routine” testing requires estimates of the prevalence of high-risk behaviours, stratified by age, the prevalence of HCV among those with high- and low-risk behaviours, stratified by age, and the age structure of the population. This will need to be informed by cost of both HCV therapy in a country, as well as the costs associated with untreated HCV and end-stage liver disease. • What proportion of HCV-positive cases will be missed by a testing policy based on screening for at-risk behaviours and exposures?

4.3. How to test HBV

Decision-making tables – PICO 1

HBsAg testing: Among persons identified for hepatitis B testing, what is the diagnostic accuracy of available assays for detecting HBsAg (RDT, EIA)?

Topic for analysis: How to test

Population: Persons identified for HBV testing

Intervention: Rapid diagnostic test for HBsAg detection

Comparison: Enzyme immunoassays for HBsAg detection

Outcomes: Diagnostic accuracy (Sensitivity, Specificity, Positive predictive value, Negative predictive value, TN, TP, FN, and FP).

Background:

- The most important marker for the diagnosis of hepatitis B infection that may require treatment remains the detection of hepatitis B surface antigen (HBsAg).
- Chronic hepatitis B infection is defined by the detection of HBsAg on two occasions six months apart.
- However, after initial testing, further characterization of the individual's HBV infection is based on a sequential testing strategy of for other markers of HBV infection (supplementary testing) triggered by the detection of HBsAg in the first instance.

Immunoassays (laboratory-based)

- The most widely used HBsAg assays are laboratory-based immunoassays.
- This can be in the form of an enzyme immunoassay (EIA), chemiluminescence immunoassay (CLIA) or electrochemiluminescence immunoassay (ECL).
- These are best suited to settings with high throughput of specimens and where infrastructure (electricity, cold storage, climate-controlled rooms) and skilled staff are consistently available.
- Other simple assays such as agglutination assays are also available for detection of HBsAg but these generally require serum/plasma specimens and cold storage. The results of simple assays may be read visually.

Rapid diagnostic tests (RDTs) – performed in-laboratory or at the point-of-care

- Many laboratories in resource-limited settings may not have access to specialized equipment and

process few specimens, per day. Hence, individual tests, including rapid diagnostic tests (RDTs), may be more appropriate.

- RDTs for detection of HBsAg come in immunofiltration (flow through) and immunochromatographic (lateral flow) formats.
- In general, RDTs do not require cold storage and may be tested using capillary (finger-stick) whole blood.
- The manufacturer's instructions for use should always be followed. The results of RDTs are read visually.
- RDTs may be deliverable at the point of care (POC).
- The expansion of their use depends on their performance and operational characteristics in the setting of intended use, ultimately with the aim being to reach resource-limited settings and offer cost-efficient testing services as an alternative to assays that require specific laboratory infrastructure and staff skills to perform.

The selection of EIA or RDTs should not be mutually exclusive. Choice of appropriate technology can be complex but can usually be distilled down to three main factors: performance, cost and accessibility. There are inevitably trade-offs, based not only on disease prevalence and the health-care infrastructure, but also on technical, socioeconomic, cultural, behavioural considerations.

DRAFT recommendation(s):

Summary pooled diagnostic accuracy of rapid HBsAg assays stratified by study, patient, index and reference test

□ High
□ Moderate
□ Low
□ Very low

	Sub-analysis	Pooled clinical accuracy			Likelihood ratios (REM)		Heterogeneity (Tau-squared)	
		n	*Sen (95% CI)*	*Spec (95% CI)*	*LR^+ (95%CI)*	*LR^- (95% CI)*	*PLR*	*NLR*
Study	*Pre 2005*	19	96.9 (96.0–97.7)	99.7 (99.6–99.8)	266 (106–665)	0.056 (0.033–0.095)	2.72	0.91
	Post 2005	44	86.4 (85.2–87.5)	99.4 (99.2–99.5)	84.6 (43.6–165)	0.126 (0.087–0.183)	4.10	1.27
	Case–control	21	96.7 (96.0–	99.3 (99.0–	105 (48.0–	0.028 (0.010–	2.23	4.86

			97.3)	99.5)	230)	0.076)		
Patient	*Blood donors*	19	91.6 (90.1–92.9)	99.5 (99.3–99.7)	89.2 (32.8–243)	0.106 (0.055–0.204)	3.82	1.86
	HIV+	6	72.3 (67.9 – 76.4)	99.8 (99.5–99.9)	193 (77.4–497)	0.29 (0.22–0.38)	0.384	0.0059
	HIV–	4	92.6 (89.8–94.8)	99.6 (99.0–99.9)	79.5 (11.6–545)	0.08 (0.05–0.13)	2.97	0.080
Index Test	*Whole blood*	11	91.7 (89.1–93.9)	99.9 (99.8–99.9)	347 (158–762)	0.089 (0.058–0.136)	0.81	0.24
	Determine	12	90.8 (88.9–92.4)	99.1 (98.9–99.4)	239 (17.1–33300)	0.077 (0.035–0.168)	20.2	1.56
	BinaxNOW	6	97.6 (96.2–98.6)	100 (99.7–100)	221 (36.1–1350)	0.045 (0.016–0.128)	3.53	1.20
	VIKIA	3	82.5 (77.5–86.7)	99.9 (99.8–100)	1070 (376–3060)	0.108 (0.026–0.458)	<0.005	1.472
	Serodia	3	82.5 (77.5–86.7)	99.9 (99.8–100)	285 (71.4–1140)	0.045 (0.029–0.069)	<0.005	<0.005
Reference Test	*CMIA*	9	80.4 (77.9–82.6)	99.0 (99.6–99.3)	58.5 (31.3–109)	0.141 (0.074–0.268)	0.44	0.73

EIA: enzyme immunoassay; RDT: rapid diagnostic test; CI: confidence interval; *with EIA reference

Quality of evidence

*Refer GRADE table in footnote

Conclusions:

- Rapid diagnostic tests, including those performed on whole blood specimens, have good clinical sensitivity and excellent clinical specificity compared to the reference standard (laboratory-based EIA for HBsAg detection). Improvement in both clinical and analytical sensitivity could potentially enhance their impact globally.
- Caution in HIV-positive individuals is important with significantly reduced clinical sensitivity compared to HIV-negative individuals

  Reassuring accuracy of whole blood specimens compared to plasma or serum specimens further facilitates use in the field.

Risks/benefits

□ Benefits clearly outweigh harms

Benefits **Advantages of testing by RDT compared to laboratory-exclusive EIAs** • Does not require capital investment in laboratory infrastructure, e.g. EIA plate washers, readers, incubators, analysers, cartridge or random-access analysers • Concurrent reduction in maintenance costs and reagents • May be deliverable at the point of care (POC). This may allow greater access to testing and eliminate need for mechanisms for transportation of specimens to the laboratory • If testing at POC, may reduce number of individuals "lost to follow up", i.e. never receive their test results • May be carried out by trained lay providers and health-care workers, in addition to trained laboratory scientists • Dedicated venepuncture may not be required. **Risks** Disadvantages of testing by RDT compared to laboratory-exclusive EIAs • Possible reduction in clinical sensitivity/specificity compared to laboratory-based methods. • RDTs appear to be less sensitive in HIV-positive individuals. • Higher cost per test after expense of laboratory infrastructure has been met. • User variability and subjectivity in reading of a visual assay, second reader suggested. • Performance characteristics may vary with environmental factors, e.g. heat, humidity, storage conditions. • Internal quality control measures may be inferior to standardised laboratory assays, e.g. lack of test kit controls, no specimen addition controls. • Although RDTs using capillary whole blood negate the need for venipuncture and maintenance of laboratory equipment, significant heterogeneity and sub-optimal clinical and analytical sensitivity must be considered. • Recording of results in a database which can be subsequently interrogated and audited as is the case with centralised laboratory testing may be compromised with testing at POC. This may impact on reporting and epidemiological surveillance of the burden of disease.	□ Benefits and harms are balanced □ Potential harms clearly outweigh potential benefits *Are the desirable anticipated effects large?* □ No □ Probably □ Uncertain □ Yes □ Varies
Acceptability, values and preferences A values and preferences survey of implementers and users of hepatitis B and	□ No major variability

C testing services was carried out by FIND in September 2015. A total of 104 respondents from 43 (20 high-income, 23 low- and middle-income) countries. Relating to this PICO, • 47% of respondents from low- and middle-income countries would prefer an RDT method of testing using capillary whole blood compared to dedicated venepuncture, even at the cost of reduced clinical sensitivity). • 50% of respondent would accept an assay with a minimal sensitivity of 95%, 43.5% would accept 98% and 4.3% would accept 90%. However, when the notion of cost was introduced, only 7% responded that 95% sensitivity would not be acceptable. • 77.3% of respondents preferred results of be available on the same day or sooner. Respondents commented that delay in individuals receiving results was likely to result in a loss to follow-up. **Community:** • Support for the most effective testing approach in order to impact on availability of testing, especially in resource-limited settings and remote areas and optimize access to at-risk groups. **Patients/caretakers:** • In the setting of HIV, use of RDTs has facilitated scaling up of testing services in terms of widening access to testing services. **Health-care workers:** • If RDTs are utilized at the POC, this will allow HCWs to carry out testing and organize follow up potentially in one consultation. There is a need for appropriate training of testing-providers and laboratory staff. • The intervention was considered likely to be acceptable to key stakeholders as the sensitivity and the specificity of RDT for screening of chronic HBV infections are comparable with EIAs.	□ Major variability Is the option acceptable to key stakeholders? □ No □ Probably □ Uncertain □ Yes □ Varies
Equity, ethics and human right implications *Will the recommendation raise questions around equity?* • No. The recommendation of the possibility of testing using RDT at POC offers new opportunities for enhancing screening, referral, and treatment for the individuals with chronic HBV infection especially in the resource-limited settings, thus will reduce transmission, morbidity and mortality associated with undetected and untreated HBV infection.	□ Less equitable □ More equitable

Are there ethical implications to this recommendation?

- No major concerns.

Resource use and financial implications

Materials:

- Cost of test kits
- Cost of sterile lancets, alcohol swabs, gloves, sharps bins or other method of disposal of used kits
- Cost of automated RDT readers, if applicable
- Quality control reagents, if applicable (some kits are supplied with positive and negative test kit controls).

Training and supervision:

- Cost of training testing providers and appropriate competency assessment, certification and re-certification of their skills
- From included studies, excellent clinical specificity of all assay formats is reassuring in terms of ensuring cost effective initiation of testing strategies for further investigation and treatment.
- If being utilized at the point-of-care, it will be the responsibility of the testing provider to record and report the result appropriately.

Other:

- Creation of a database into which results obtained by POC can be recorded
- Linkage to care, e.g. antenatal clinics

Possible test procurement cost:

Test	Cost (US$) per test	Source
RDT	0.3–0.95 (procurement cost)	WHO database
EIA	0.4–2.8 (procurement cost)	WHO database

Are the resources required small?

□ No
□ Probably
□ Uncertain
□ Yes
□ Varies

Feasibility and constraints to implementation *Are any major barriers expected for the implementation of this recommendation?* • High-throughput EIAs require certain laboratory infrastructure and equipment with precision and expertise required in its operation. • Delivery of RDTs requires appropriate training of test providers in performing and reading of the test result, storage of materials and recording and reporting of the status. Decentralisation of testing puts tremendous stress on already fragile health systems in terms of training needs, supply chain management, quality assurance, and monitoring and evaluation of effectiveness and impact. External quality assessment of quality of tests and testing possible but challenging when the need for proficiency panels is increased from a few laboratories to hundreds and possibly thousands of POC sites. **Feasibility survey report to be presented at meeting.**	1.1.10 *Is the option feasible to implement?* □ No □ Probably □ Uncertain □ Yes □ Varies

Relevance to different settings/populations

Will this recommendation be most relevant for particular settings (e.g. endemicity)?

- The introduction of HBsAg testing using RDTs will be most relevant in settings where there is poor access to existing laboratory testing-services, either access to centralised laboratory testing or lack of testing-infrastructure in existing laboratories.
- Delivery of RDTs at the point-of-care in remote or resource-limited settings, e.g. HBsAg testing in antenatal clinics may significantly affect the future burden of disease.
- Useful for testing of both symptomatic and asymptomatic individuals.
- It will be most relevant to key affected populations who may be at risk of infection but who may be reluctant to or have poor access to health-care services, such as individuals who attend drug-rehabilitation clinics or prisoners. These individuals require screening, may require treatment if infected or vaccination if not currently infected.
- It will be less relevant in individuals who have good access to health care and in settings where laboratory testing for hepatitis B is already well established.

Rationale for recommendation:

Strength of recommendation
Implementation considerations • Symptomatic vs asymptomatic individuals; in a symptomatic individual, you may not need such good analytical sensitivity than when screening an asymptomatic individual.
Research gaps • Impact of using RDTs for HBsAg at the point-of-care on delivery and implementation of testing services.

GRADE Summary of findings

Question: Should RDTs be used to diagnose HBsAg in HIV-negative individuals?

Sensitivity	0.88 to 0.95
Specificity	0.93 to 1.00

Prevalences	5%	20%

Outcome	№ of studies (№ of patients)	Study design	Factors that may decrease quality of evidence					Effect per 1000 patients/year		Test accuracy QoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 5%	pre-test probability of 20%	
True positives (patients with HBsAg)	4 studies 997 patients	cross-sectional (cohort type accuracy study)	serious [1]	not serious [2]	serious [3]	serious [4]	none	44 to 48	176 to 190	⊕◯◯◯ VERY LOW [1 2 3 4]
False negatives (patients incorrectly classified as not having HBsAg)								2 to 6	10 to 24	
True negatives (patients without HBsAg)	4 studies 997 patients	cross-sectional (cohort type accuracy study)	serious [1]	not serious [2]	serious [3]	not serious [5]	none	884 to 950	744 to 800	⊕⊕◯◯ LOW [1 2 3 5]
False positives (patients incorrectly classified as having HBsAg)								0 to 66	0 to 56	

1. Downgraded by one for risk of bias: all studies were prospective cohort studies *(1)*, although one was assessed as high risk of bias because patients were pre-selected based from known chronic hepatitis B patients.
2. Although study was not specifically designed in HIV-negative patients, clear testing and results were included
3. Downgraded by one for inconsistency: unexplained heterogeneity may arise from differences between studies in specimen condition (serum, whole blood), specimen processing (field vs laboratory), reference tests (CMIA; EIA on dried blood spots) and study population (e.g. known chronic hepatitis B patients, general community screen)
4. Downgraded by one for imprecision: confidence intervals extend below 90% accuracy, with tau-squared for PLR >1 (indicating substantial heterogeneity)

Question: Should RDTs be used to diagnose HBsAg in HIV-positive individuals?

Sensitivity	0.72 (95% CI: 0.68–0.76)
Specificity	1.00 (95% CI: 0.99–1.00)

Prevalence	5%	20%

Outcome	No. of studies (No of patients)	Study design	Factors that may decrease quality of evidence					Effect per 1000 patients/year		Test accuracy QoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 5%	pre-test probability of 20%	2
True positives (patients with HBsAg)	5 studies 2566 patients	cross-sectional (cohort type accuracy study)	serious[1]	not serious[2]	serious[3]	serious[4]	none	36 (34–38)	145 (136–153)	⊕◯◯◯ Very low[1–4]
False negatives (patients incorrectly classified as not having HBsAg)								14 (12–16)	55 (47–64)	
True negatives (patients without HBsAg)	5 studies 2566 patients	cross-sectional (cohort type accuracy study)	serious[1]	not serious[2]	not serious[5]	not serious[6]	none	948 (945–949)	798 (796–799)	⊕⊕⊕◯ Moderate[1–2, 5–6]
False positives (patients incorrectly classified as having HBsAg)								2 (1– 5)	2 (1–4)	

1. Downgraded for one for risk of bias: all studies were prospective cohort studies of consecutive patients. Studies used different specimens (serum, 2; capillary whole blood, 1; venous whole blood, 1), reference standards (CMIA, EIA confirmed by neutralization), and had patients with different ART status (four studies ART naive).
2. Not downgraded for indirectness: all studies performed in cohorts of consecutive patients in Tanzania *(2)*, Ghana *(3)*, Malawi *(4)*, South Africa *(5)* and Bissau *(6)*.

3. Downgraded by one for inconsistency with sensitivities ranging from 62% to 100%: unexplained heterogeneity may arise from differences between studies in specimen type, specimen processing and study population. Two studies had very high sensitivities (100%, 96%) while the remainder *(3,5,6)* had low sensitivities (range 62–70%). Tau-squared <1 for studies
4. Downgraded by one for imprecision: confidence intervals 67.9–76.4%. Two studies had very high sensitivities (100%, 96%) while the remainder *(3,5,6)* had low sensitivities (range 62–70%).
5. Not downgraded for inconsistency: specificities ranged from 99% to 100%, with tau-squared <1
6. Not downgraded for imprecision: narrow confidence interval

Question: Should Determine HBsAg be used to diagnose HBsAg in a global setting?

Sensitivity	0.91 (95% CI: 0.89–0.92)
Specificity	0.99 (95% CI: 0.99–0.99)

Prevalences	5%	20%

Outcome	No. of studies (No. of patients)	Study design	Factors that may decrease quality of evidence					Effect per 1000 patients/year		Test accuracy QoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 5%	pre-test probability of 20%	
True positives (patients with HBsAg)	12 studies 7552 patients	cohort and case–control type studies [1]	serious	not serious	very serious[2]	not serious	none	45 (44–46)	182 (178–185)	⊕◯◯◯ Very low[2]
False negatives (patients incorrectly classified as not having HBsAg)								5 (4–6)	18 (15–22)	
True negatives (patients without HBsAg)	12 studies 7552 patients	cohort and case–control type studies	serious	not serious	serious[3]	not serious	none	941 (940–944)	793 (791–795)	⊕⊕◯◯ Low[3]
False positives								9 (6–10)	7 (5–9)	

Outcome	No. of studies (No. of patients)	Study design	Factors that may decrease quality of evidence					Effect per 1000 patients/year		Test accuracy QoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 5%	pre-test probability of 20%	
(patients incorrectly classified as having HBsAg)										

1. Lin *(7)*, Lien *(8)* and Randrianna *(9)* used a case–control design.
2. Significant heterogeneity across studies for sensitivity; tau-squared 20.2.
3. Heterogeneity exists, but with lower clinical impact; tau-squared 1.56.

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4.4. How to test HCV

Decision-making tables – PICO 2

To ascertain exposure to HCV through anti-HCV testing: Among individuals identified for hepatitis C testing, what is the diagnostic accuracy of available assays for detecting anti-HCV (RDT, EIA)?

1. Topic for analysis: How to test

Population: Individuals identified for HCV testing to ascertain exposure to HCV

Intervention: Rapid diagnostic tests and enzyme immunoassays for detection of antibodies to HCV

Comparison:

1. Nucleic acid testing (NAT)
2. EIA and immunoblot
3. EIA only

Outcomes: Diagnostic accuracy (Sensitivity, Specificity, Positive predictive value, Negative predictive value, TN, TP, FN and FP).

2. Background:

- Screening for exposure to hepatitis C virus (HCV) is dependent on assays that detect antibodies to HCV (anti-HCV) in the first instance.
- Once antibody status is confirmed, the individual should undergo supplementary testing for active HCV infection using an assay designed to detect viral replication, such as HCV RNA or core antigen (HCV cAg).
- Assays designed solely to detect antibodies to viral antigens will inevitably have a "window period" of infectivity in early infection in an individual who has been recently infected whose infection will not be detected by a given serological assay. This diagnostic window period can be shortened by direct detection of viral antigen or nucleic acid.
- The improvements in assay performance over time, in particular the EIAs, have been termed as "generations" of the assays. **(see footnote)*
- It is important to note that the latest generation of assays designed to detect anti-HCV also are combined with cAg in order in increase seroconversion sensitivity of the assay, but these "4th generation" or "combination" assays should not be used to differentiate exposure from chronic infection.

- Note that sensitivity of antibody-only assays may be reduced if the patient is immunocompromised, e.g. HIV, immunosuppressive therapy, renal dialysis.

Immunoassays (laboratory-based)

- The most widely used anti-HCV assays are laboratory-based immunoassays.
- They detect antibodies to core and non-structural antigens. In the case of 4th generation assays, the assay combines detection of antibodies to HCV along with detection of hepatitis C core (P22 Ag) antigen directly.
- These can be in the form of an enzyme immunoassay (EIA), chemiluminescence immunoassay (CLIA), electrochemiluminescence immunoassay (ECL) or recombinant immunoblot assay.
- These are best suited to settings with high throughput of specimens and where infrastructure (electricity, cold storage, climate-controlled rooms) and skilled staff are consistently available.

Rapid diagnostic tests (RDTs)

- Many laboratories in resource-limited settings may not have access to this specialized equipment and process fewer specimens, per day. Hence, individual tests, including rapid diagnostic tests (RDTs), may be more appropriate.
- RDTs for detection of anti-HCV are simple to perform and do not require instrumentation, they come in immunofiltration (flow through) and immunochromatographic (lateral flow) formats and may be read visually.
- In general, RDTs do not require cold storage and may be tested using capillary (fingerstick) whole blood or oral fluid. However, the manufacturer's instructions for use should always be followed.
- RDTs may be deliverable at the point of care (POC).
- The expansion of their use depends on their performance and operational characteristics in the setting of intended use, ultimately with the aim being to reach resource-limited settings and offer cost-efficient testing services as an alternative to assays that require specific laboratory infrastructure and staff skills to perform.

*** Summary of assay "generations"**

1st generation assays:

- Detection of antibodies to NS4 antigen only
- Becomes detectable 12–26 weeks after exposure
- High false-positive rate, i.e. poor positive-predictive value in low-prevalence populations.

2nd generation assays:

- Detects antibodies to NS3, NS4 and core antigen
- Decreased window period of infectivity to 10–24 weeks.

3rd generation assays:

- Detects antibodies to NS3, NS4, NS5 and core antigen
- Further decreased window period of infectivity.

4th generation assays A.K.A. combination assays:

- Combination of detection of circulating antibodies to viral antigens as above, but also addition of monoclonal antibodies to detect hepatitis C antigens (P22 Ag) directly.
- Immunoassays solely for the detection of HCV core antigen were developed initially to close the diagnostic window in seronegative infection and subsequently for the detection of antigenaemia in the presence of antibody.

3. Draft recommendation(s):

4. Summary and quality of evidence

□ High
□ Moderate
□ Low
□ Very low

<u>Systematic review report</u>

A systematic review was commissioned in order to assess this PICO question (*see* SR PICO 2). The purpose of this review was to determine the sensitivity and specificity of assays used to detect hepatitis C antibody using multiple specimen types, including serum, whole blood and oral fluid.

Summary of the evidence

Method: A literature search was conducted focused on hepatitis C, diagnostic tests, and diagnostic accuracy. Studies were included if they evaluated an assay to determine the sensitivity and specificity of a single hepatitis C antibody (HCVAb) test among humans. Two reviewers performed a quality assessment of the studies and extracted data for estimating test accuracy.

Results:

A total of 52 studies were included, evaluating 30 RDT devices of varying generation of assay.

- **RDTs vs EIA only:**
  Pooled clinical sensitivity and specificity were 0.98 and 1.00, respectively.
- **RDTs vs EIA, immunoblot and NAT:**
  Pooled clinical sensitivity and specificity were 0.96 and 1.00, respectively.
- **RDTs vs NAT or immunoblot:**
  Pooled clinical sensitivity and specificity were 0.93 and 0.98, respectively.
- **RDTs vs Ag/Ab combination assay:**
  Pooled clinical sensitivity and specificity were 0.86 and 0.99, respectively.
- **RDTs on oral fluid compared to a serological reference standard using serum/plasma:**
  Pooled clinical sensitivity and clinical specificity were 0.94 and 1.00, respectively.
- Results were comparable across general populations, hospital patients and key populations.

Pooled diagnostic accuracy for HCV antibody tests

Pooled test accuracy for different tests (52 research studies).

Comparison (number of studies)	**Pooled SE**	**95%CI**		**Tau-square *P*-value for hetero-geneity**	**Pooled SP**	**95% CI**		**Tau-square *P*-value for hetero-geneity**
RDT versus EIA only (*n* = 5)	0.99	0.98	1.00	<0.001	1.00	1.00	1.00	<0.001
RDT versus NAT or Immunoblot (*n* = 13)	0.93	0.91	0.95	<0.001	0.98	0.97	0.99	<0.001
RDT versus EIA, NAT or Immunoblot (*n* = 14)	0.97	0.96	0.98	<0.001	1.00	1.00	1.00	<0.001
Oral RDT versus blood reference (*n* = 12)	0.94	0.93	0.96	<0.001	1.00	1.00	1.00	<0.001
Sample type								
Blood samples *(n* = 45)	0.98	0.97	0.98	<0.001	0.98	0.98	0.99	1.1.11
Oral samples (*n* = 12)	0.94	0.93	0.96	<0.001	1.00	1.00	1.00	<0.001
Source population								
General screening (*n* = 17)	0.95	0.94	0.96	<0.001	0.99	0.98	0.99	<0.001
Key population (*n* = 19)	0.97	0.96	0.98	<0.001	0.94	0.94	0.95	<0.001
Hospital patients (n = 16)	0.97	0.96	0.98	<0.001	1.00	1.00	1.00	<0.001
Antibody and Antigen Combo testing (*n*=6)	0.86	0.79	0.94	<0.001	0.99	0.98	1.00	<0.001
Oral kits brand								
OraQuick (n = 8)	0.98	0.97	0.99	<0.001	1.00	1.00	1.00	<0.001

Other brands (n = 6)	0.88	0.84	0.92	<0.001	0.99	0.99	1.00	<0.001

SE: sensitivity; SP: specificity; CI: confidential interval; RDT: rapid diagnostic test; EIA: enzyme immunoassay; NAT: nucleic acid testing

Note:*

[#] Studies conducted across these regions were not included here.

Conclusions:

- Rapid diagnostic tests, including RDTs for oral fluid, have excellent sensitivity and specificity compared to laboratory-based methods, across different populations for detection of antibodies to HCV. This suggests that RDTs can be used to test for HCV antibody.
- Sensitivity/specificity was less for RDTs for anti-HCV compared to newer combination antibody/antigen assays.

Issues raised from the review:

- The comparison of RDT versus immunoblot would include HCV-cleared person: HCV Ab^{+} but HCV RNA^{-}.
- Publication bias, as studies with poor test performance were less likely to be published, lead to exaggerated estimates of the accuracy.

Quality of evidence

*Refer GRADE table in footnote

5. Risks/benefits

Benefits

Advantages of testing by RDT compared to laboratory-exclusive EIAs

- Does not require capital investment in laboratory infrastructure, e.g. EIA washers, readers, incubators, analysers, cartridge or random-access analysers
- Concurrent reduction in maintenance costs and reagents
- May be deliverable at the point of care (POC). This may allow greater access to testing and eliminate need for mechanisms for transportation of specimens to the laboratory
- If testing at POC, may reduce number of individuals "lost to follow-up", i.e. never receive their test results
- May be carried out by trained lay-providers and health-care workers, in addition to trained laboratory scientists
- Dedicated venepuncture may not be required as some assays are validated

□ Benefits clearly outweigh harms
□ Benefits and harms are balanced
□ Potential harms clearly outweigh potential benefits

Are the desirable anticipated effects large?

for capillary whole blood or oral fluid. • Rapid diagnostic tests (RDTs) have potential for scaling up access to hepatitis B testing, particularly for key populations. **Risks** Disadvantages of testing by RDTs compared to laboratory-exclusive EIAs • Increased cost per test after expense of laboratory infrastructure has been met • User variability and subjectivity in reading the visual assay, suggest second reader • Performance characteristics may vary with environmental factors, e.g. heat, humidity, storage conditions. • Internal quality control measures may be inferior to standardized laboratory assays, e.g. lack of test kit controls, no specimen addition controls. • Performance characteristics may vary in certain individuals, e.g. HIV infection, immunosuppressed – lower sensitivity of anti-HCV compared to NAT testing. • Recording of results in a database which can be subsequently interrogated and audited as is the case with centralized laboratory testing may be compromised with testing at POC. This may impact on reporting and epidemiological surveillance of the burden of disease.	□ No □ Probably □ Uncertain □ Yes □ Varies
6. Acceptability, values and preferences A values and preferences survey of implementers and users of hepatitis B and C testing services was carried out by FIND in September 2015. A total of 104 respondents from 43 (20 high-income, 23 low- and middle-income) countries participated. Relating to this PICO, • 47% of respondents from low- and middle-income countries would prefer an RDT method of testing, even at the cost of reduced sensitivity). **Community:** • Support for the most effective testing approach in order to impact on availability of testing, especially in resource-limited and remote areas and optimize access to at-risk groups. **Patients/caretakers:** • In the setting of HIV, use of RDTs has facilitated scaling up of testing services in terms of widening access to testing services. **Health-care workers:** • If RDTs are utilized at POC, this will allow HCWs to carry out testing and	□ No major variability □ Major variability Is the option acceptable to key stakeholders? □ No □ Probably □ Uncertain □ Yes □ Varies

organize follow up potentially in one consultation. There is a need for appropriate training of testing providers and laboratory staff. • Appropriate pre- and post-test counselling was mentioned in the values and preferences survey, which suggested that in low- and middle-income countries, not all individuals were being offered this service. • 47% of respondents (*n* = 23) preferred POC testing using capillary whole blood even at the expense of clinical sensitivity. • The intervention was considered likely to be acceptable to key stakeholders as clinical sensitivity and clinical specificity of RDTs for ascertaining HCV exposure are comparable with EIAs.	
7. Equity, ethics and human right implications *Will the recommendation raise questions around equity?* • No. The recommendation of the possibility of testing using RDTs offers new opportunities for enhancing screening, referral, and treatment for the individuals with chronic HCV infection especially in the resource-limited settings, thus will reduce transmission, morbidity and mortality associated with undetected and untreated HCV infection. *Are there ethical implications to this recommendation?* • No major concerns.	□ Less equitable □ More equitable
Resource use and financial implications **Materials:** • Cost of test kits • Cost of sterile lancets, alcohol swabs, gloves, sharps-bins or other method of disposal of used-kits • Cost of automated RDT readers, if applicable • Quality control reagents, if applicable (some kits are supplied with positive and negative test kit controls) **Training and supervision:** • Cost of training testing providers and appropriate competency assessment, certification and re-certification of their skills • From included studies, excellent specificity of all assays is reassuring in	*Are the resources required small?* □ No □ Probably □ Uncertain □ Yes □ Varies

terms of ensuring cost–effective initiation of algorithms for further investigation and treatment.

Other:

- Creation of a database into which results obtained by POC can be recorded
- Linkage to care, e.g. antenatal clinics

Possible test-procurement costs:

RDTs	Cost (US$) per test	Source
	0.50–1.70 (>10 for oral fluid)	MSF, WHO database
Laboratory-based assays		
HCV EIA	2.60–4.30 (procurement costs)	WHO database
HCV RNA	~20	

8. Feasibility and constraints to implementation

Are any major barriers expected for the implementation of this recommendation?

- High-throughput EIAs require extensive laboratory infrastructure and equipment with staff expertise in its operation. Batching can lead to long delays before results are available.
- Delivery of RDTs requires appropriate training of test providers in performing and reading of the test result, storage of materials and recording and reporting of status.
- Decentralization of testing puts tremendous stress on already fragile health systems in terms of training needs, supply chain management, quality assurance, and monitoring and evaluation of effectiveness and impact. External quality assessment of quality of tests and testing possible but challenging when the need for proficiency panels is increased from a few laboratories to hundreds and possibly thousands of POC sites.

Feasibility survey report to be presented at meeting.

Is the option feasible to implement?

□ No
□ Probably
□ Uncertain
□ Yes
□ Varies

9. Relevance to different settings/populations

Will this recommendation be most relevant for particular settings (e.g. endemicity)?

- This recommendation of the introduction of RDTs will be most relevant in settings where there is poor provision of laboratory testing-services, either access to centralized laboratory testing or lack of testing infrastructure in existing laboratories.
- Delivery of RDTs at the POC will have relevance more in remote or resource-limited settings,

compared to settings where there is good access to health care and established screening programmes.

- It will be most relevant to groups of patients at risk of infection but who may be reluctant to or have poor access to health-care services, such as individuals who attend drug-rehabilitation clinics or closed settings such as prisoners. These individuals require screening and may require assessment and treatment if found to be infected.
- It will be less relevant in individuals who have good access to health care and in settings where laboratory testing for hepatitis C is already well established.

10. Rationale for recommendation:

11. Strength of recommendation

12. Implementation considerations

13. Research Gaps

- Impact of testing at POC using RDTs to rule out/rule in HCV exposure on HCV-associated morbidity and mortality and onward transmission testing and treatment programmes.

GRADE Summary of findings

Table: Strength of evidence for diagnostic accuracy

	Unit of analysis	Type of samples	Studies, *n*	Risk of bias	Consistency	Directness	Precision	# of samples	Strength of evidence	Sen (95% CI)	Sp (95% CI)	Pretest probability (%)	Positive LR (95% CI)	PPV	Negative LR (95% CI)	NPV	Strength evidence
RDT versus EIA, or immunoblot	General population, hospital patients, blood donors, injection drug users and other high-risk populations	Oral fluid, serum or plasma	14	Mod	Se: Inconsistent	Indirect	Precise	42,239	Se: Mod	0.97 (0.0.96 –0.98)	1.00 (1.00 –1.00)	0.05	372.92 (267.56 –574.12)	0.95	0.03 (0.02 –0.04)	1.00	
					Sp: Consistent				Sp: Mod								
Oral RDT versus blood reference	General population, hospital patients, blood donors, injection drug users and other high-risk populations	Oral fluid, serum or plasma	12	Mod	Se: Inconsistent	Indirect	Precise	14,547	Se: Mod	0.94 (0.93 –0.96)	1.00 (1.00 –1.00)	0.05	314.5 (202.02 –684.07)	0.94	0.06 (0.04 –0.07)	1.00	
					Sp: Consistent				Sp: Mod								

Mod: Moderate; Sen: sensitivity; Sp: specific

4.5. How to test - testing strategy HBV

Decision-making tables – PICO 3

Testing strategy to diagnose chronic HBV infection through detection of HBsAg: Among individuals identified for hepatitis B testing, what is the best testing strategy (diagnostic accuracy and other outcomes) for detection of HBsAg? (One-assay versus two-assay strategy)

1. Topic for analysis: How to test

Population: Persons identified for HBV testing

Intervention: One-assay testing strategy; one HBsAg assay

Comparison: Two-assay testing strategy; two different HBsAg assays

Outcomes: Diagnostic accuracy (Sensitivity, Specificity, Positive predictive value, Negative predictive value, TN, TP, FN, and FP).

Background:

HIGH CONCERN HIV	MODERATE HBV	LOW HCV
Lifelong ARVs initiated on basis of serologic test alone Major implications to false positive results	Initiation of lifelong TDF would usually require HBV DNA or presence of cirrhosis	Initiation of DAAs would always require confirmation of ACTIVE infection with HCV NAT or Ag

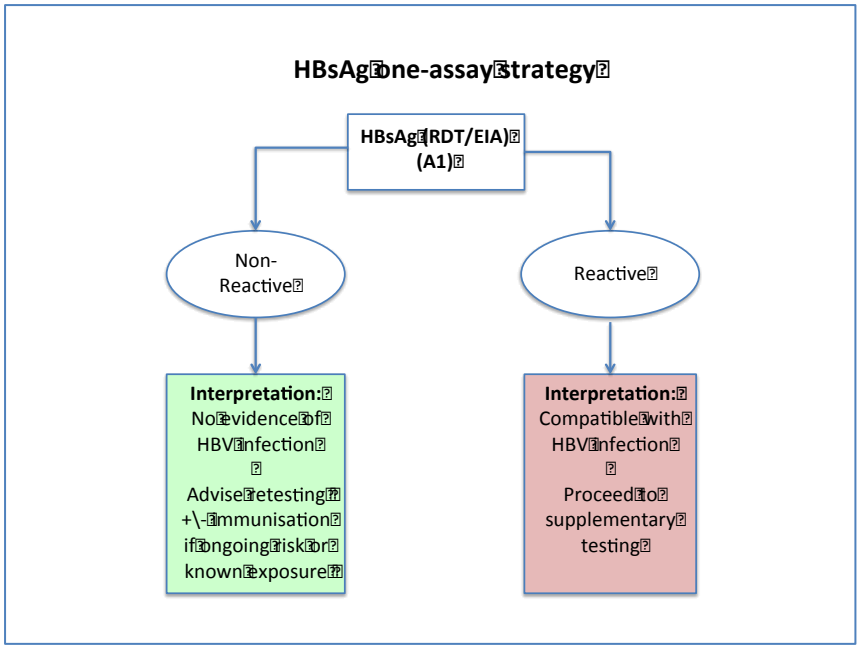
- The most important marker for the diagnosis of chronic hepatitis B infection that may require treatment remains the detection of hepatitis B surface antigen (HBsAg).
- Although the case definition of chronic hepatitis B is the detection of HBsAg twice six months apart, if facilities exist, after an initial positive result, supplementary testing can be undertaken in order to facilitate entry into a care pathway.
- HBsAg will be detectable in the blood if there is current hepatitis B infection. Confirmation of the specificity of a reactive HBsAg first-line test result is usually carried out by either;
  i) repeating the HBsAg testing in a different assay of similar sensitivity, or
  ii) performing a neutralization test using a specific anti-HBs-containing reagent in the same first-line assay after appropriate dilution of the specimen under test. Specificity is confirmed when this reagent abolishes reactivity in the assay.
- This PICO question addresses the issue of whether a positive result from a single HBsAg assay has sufficient specificity in order to proceed to supplementary testing and/or entry into a care pathway, or whether confirmatory testing on the same specimen with a different HBsAg assay

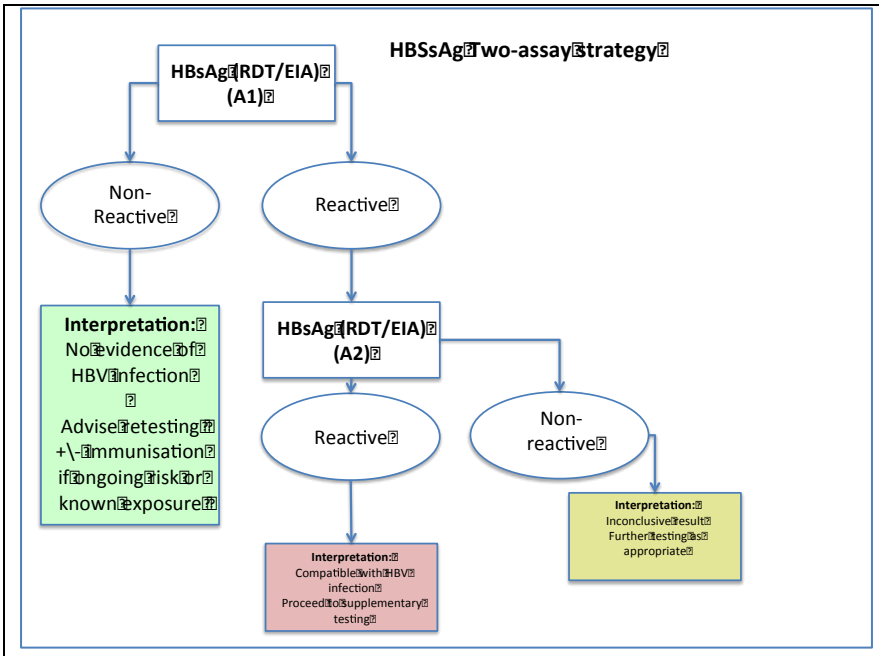
(or neutralization), performed sequentially after the first assay is required.

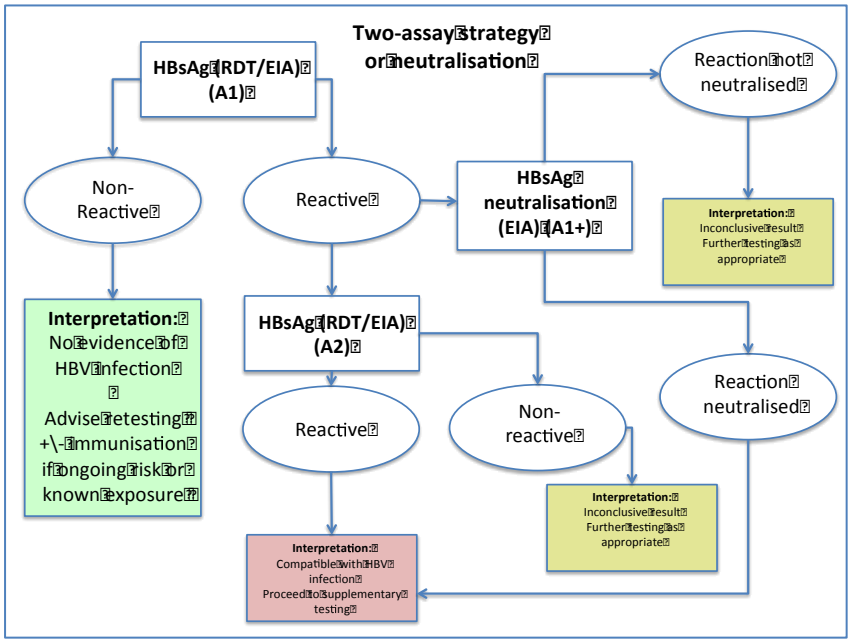
Testing strategies:

- WHO recommends standardized testing strategies to maximize the accuracy of hepatitis B and C testing while minimizing cost and increasing simplicity.
- A testing strategy describes a testing sequence for a specific testing objective, taking into consideration the presumed prevalence of the analyte to be tested in the population. In both high and low prevalence settings, more than one assay may be required.
  See footnote for further detailed explanation of the one- and two-assay strategies.

Figs 1–3: Possible testing strategies for detecting HBsAg







Since the decision for PICO 3 critically depends on whether currently available tests for detecting HBsAg show acceptable sensitivity and specificity, the following conclusions from PICO 1 should be considered:

- Overall compared to an EIA reference, RDTs had pooled clinical sensitivity 90% (95% CI: 89, 91) and clinical specificity 100% (95% CI: 99, 100).
- RDTs were more accurate among HIV-negative patients, with pooled clinical sensitivity of 93% (95% CI: 90, 95), and pooled clinical specificity of 100% (95% CI: 99, 100) compared to results in HIV-positive patients in whom RDTs are 72% sensitive (95% CI: 68, 76) and 100% specific (95% CI: 100, 100) compared to an EIA reference.
- Results for capillary whole blood specimens were comparable to serum and less heterogeneous.

The two-test strategy involves the use of a more sensitive test as the screening test and a second test that is more specific to reduce false-positive results. Since the review in PICO 1 showed that HBsAg serological assays have excellent specificity, then there is no need for the use of another test if a simple testing strategy is desired.

2. DRAFT recommendation(s):

Summary and quality of evidence

Systematic review

- A systematic review by the London School of Hygiene and Tropical Medicine (LSHTM) was commissioned to assess this PICO question.

Summary of results

- No studies directly compared diagnostic accuracy, cost, cost-effectiveness of one- vs two-assay HBsAg testing strategies.
- The reviewers found four documents that demonstrated a strategy for hepatitis B testing.
- The Public Health England UK standard for microbiology investigation (SMI) (under review) suggested that either neutralization or repeat testing of the same specimen with a different HBsAg assay would be acceptable, but did not directly quote any evidence for this.
- The Australian "National Testing Policy for hepatitis B" suggested that HBsAg be confirmed with neutralization but also anti-HBc and anti-HBs should be assessed in order to form a profile and suggested management, e.g. immunization.
- Fan et al. (2014) used a decision-tree model of sequential HBeAg and HBV-DNA in a cohort of pregnant women in order to calculate cost-effectiveness.

□ High
□ Moderate
□ Low
□ Very low

Conclusions • Diagnosis of HBV is very complex and there may not be 1–2 simple algorithms that will cover all settings. **Quality of evidence** • Study quality was not evaluated using the QUADAS-2 tool and the STARD checklist, as it was not applicable. • None of the studies met inclusion criteria. **GRADE Summary of findings** Not applicable as no studies met the inclusion criteria for the systematic review. • All PICOs related to HBV will need to be looked at together in order to try and answer PICO 3. **<u>Modelling studies of one-assay vs two-assay strategies</u>** *To be added at meeting.*	
3. Risks/benefits **Benefits** • The intervention of testing using a single HBsAg assay would simplify the process of testing. • Sensitivity, i.e. detecting those individuals with HBV infection should not be compromised with a one-assay strategy. This will identify those who require further assessment and possible treatment. • Cost of overall testing may be reduced. • May allow more rapid reporting of the result, so that the patient can be appropriately followed up for further assessment, vaccination or given health protection advice, e.g. measures to reduce onward transmission. **Risks** • Possible reduced positive predictive value of a single test result on one	□ Benefits clearly outweigh harms □ Benefits and harms are balanced □ Potential harms clearly outweigh potential benefits *Are the desirable anticipated effects large?* □ No □ Probably □ Uncertain □ Yes □ Varies

assay may lead to more false-positives and associated anxiety and cost associated with follow up, before the correct diagnosis is ultimately made. This is especially relevant in low-prevalence settings at which even a test of 99% specificity may yield more false-positive than true-positive results. • If analytical or clinical sensitivity of the first-line assay is lower than the combination of two different assays, this may result in more false-negatives, resulting in individuals not referred for the appropriate further assessment and potential ongoing transmission • The recommendation of confirmation with neutralization is reliant on adequate facilities being available. If performed incorrectly, may result in indeterminate results, leading to increased cost of further testing.	
4. Acceptability, values and preferences **Community/patients/caretakers** • Patients require a simple and rapid testing strategy, yet one that is acceptably accurate for the purpose of testing **Health-care workers** • HCWs will need to understand the strengths and limitations of any given testing strategy and appropriately counsel patients who are screened. • HCWs will need to appropriately act on results, either positive and negative. • If performing testing at POC, HCWs need to be adequately trained in the use of RDTs and record/report results appropriately. **Other** • Laboratory staff need to be appropriately trained in performance of the various assays according to the manufacturers' instructions for use, especially if HBsAg neutralization is being utilized	□ No major variability □ Major variability Is the option acceptable to key stakeholders? □ No □ Probably □ Uncertain □ Yes □ Varies
Equity, ethics and human right implications *Will recommendation raise questions around equity?* • Yes, availability of HBsAg neutralization assays or other supplementary testing, such as nucleic acid testing and/or further hepatitis B markers with be more available in settings with established hepatitis B testing	□ Less equitable □ More equitable

facilities.

- However, note that where an RDT for HBsAg is used and no neutralization reagents are available, confirmation of an acute or chronic infection for diagnostic purposes may be concluded by repeat testing for HBsAg after a period of time. Consecutive reports of HBsAg-positive status twice at least 6 months apart will confirm a diagnosis of chronic HBV infection.

Are there ethical implications to this recommendation?

- The ethical implication of recommending a strategy with possible suboptimal PPV.

5. Resource use and financial implications

Diagnostics/laboratory monitoring

- Cost of a one-test vs two-test strategy will be less due to fewer tests being used.

Assay format	Indicative cost (US$) per test	Source
RDT	0.30–0.95 (procurement cost)	WHO database
EIA	0.40–2.80 (procurement cost)	WHO database

Training and supervision

- Appropriate training for laboratory staff and health-care workers delivering testing services at the point of care.

Are the resources required small?

□ No
□ Probably
□ Uncertain
□ Yes
□ Varies

6. Feasibility and constraints to implementation

Are any major barriers expected for the implementation of this recommendation?

- Procurement of appropriate test kits meeting required sensitivity/specificity/analytical sensitivity

Is the option feasible to implement?

□ No
□ Probably

• Delivery of testing services to remote/resource-limited settings **<u>Feasibility survey report to be presented at meeting.</u>**	□ Uncertain □ Yes □ Varies

7. Relevance to different settings/populations

Will this recommendation be most relevant for particular settings (e.g. endemicity)?

- This recommendation will be most relevant in settings that do not have established hepatitis B testing programmes and of less relevance where laboratory testing for hepatitis B is already well established.

8. Rationale for recommendation:

9. Strength of recommendation

10. Implementation considerations

11. Research gaps

- Impact of a one- vs two-step testing strategy on hepatitis B testing programmes
- Cost-effectiveness studies of one- versus two-assay or neutralization testing strategies

Footnote: Explanation of one- and two-assay strategies and PPV and NPV calculation method based on prevalence

One-assay strategy

- A single test is performed. If the test result is reactive, an "HBsAg positive" status is reported, with need for complementary testing and follow-up HBsAg testing in 6 months to diagnose chronic infection recommended.
- If the initial test result is non-reactive, an "HBsAg negative" status is reported.
- This strategy efficiently identifies most uninfected individuals and rules out chronic HBV infection; it identifies those likely to be infected and in need of additional testing.

- It is a suitable testing strategy for resource-limited settings because only a single test or one assay is performed as long as an assay that meets high standards for analytical and clinical sensitivity and clinical specificity is used.
- Limitations of this approach are that a small percentage of test results may be false positive, so appropriate procedures to follow up individuals need to be in place.

Two-assay strategy

- The test results of two different assays are used sequentially (i.e. not in parallel), to increase the positive predictive value of the overall test strategy.
- If the test result on the first-line assay is non-reactive, an "HBsAg negative" result is reported. However, if the test result on the first-line assay is reactive, a second test with an assay from a different manufacturer is performed.
- If both test results are reactive, the status is reported as: "HBsAg positive" with need for supplementary testing and follow-up HBsAg testing in 6 months to diagnose chronic infection recommended." If the test result on the second-line assay is non-reactive, the result is reported as "HBsAg inconclusive; requires additional testing."
- This strategy efficiently identifies most uninfected individuals and more definitively rules out chronic HBV infection than a one-assay testing strategy. It improves the positive predictive value when the test results of two different assays are both reactive. It can be used by non-laboratory staff, provided that adequate quality assurance standards are in place.
- The two-assay strategy may produce a small number of false-positive results (particularly in low-prevalence settings); some persons with recent HBV infection may receive false-negative results, which will depend on the analytical sensitivity of the assays used.

Worked example to illustrate the effect of prevalence on predictive values for the two different testing strategies

Assuming the following assay performance characteristics:

- If Assay 1 has sensitivity of 99% and specificity of 98%.
- If Assay 2 has sensitivity of 99.4 and specificity of 99.5%.

	Prevalence of analyte		
	0.1%	1%	10%
Positive predictive values			
Assay 1	4.7%	33.3%	84.6%
Assay 1 + Assay 2 (serial)	90.7%	99%	99.9%
Negative predictive values			
Assay 1	99.9%	99.99%	99.99%

Using the following equation for PPV and NPV that incorporates prevalence more correctly,

$$\mathrm{PPV} = \frac{\text{sensitivity} \times \text{prevalence}}{\text{sensitivity} \times \text{prevalence} + (1 - \text{specificity}) \times (1 - \text{prevalence})}$$

$$\mathrm{NPV} = \frac{\text{specificity} \times (1 - \text{prevalence})}{(1 - \text{sensitivity}) \times \text{prevalence} + \text{specificity} \times (1 - \text{prevalence})}$$

Reference: Altman DG, Bland JM. Diagnostic tests 2: predictive values. BMJ. 1994 Jul 9; 309(6947): 102. Available at: https://www.ncbi.nlm.nih.gov/pmc/ articles/PMC2540558 /pdf/bmj00448-0038a.pdf

4.6. How to test - testing strategy HCV

Decision-making tables – PICO 4

Testing strategy to ascertain exposure to HCV: Among persons identified for hepatitis C testing, what is the best testing strategy (diagnostic accuracy and other outcomes) for detection of antibodies to HCV? (One-assay versus two-assay strategy)

1. Topic for analysis: How to treat

Population: Persons identified for hepatitis C virus (HCV) testing

Intervention: One-assay testing strategy; one HCV serological assay

Comparison: Two-assay testing; two different HCV serological assays

Outcomes: Diagnostic accuracy (Sensitivity, specificity, positive predictive value, Negative predictive value, TN, TP, FN, and FP).

2. Background:

HIGH CONCERN HIV	MODERATE HBV	LOW HCV
Lifelong ARVs initiated on basis of serologic test alone Major implications to false positive results	Initiation of lifelong TDF would usually require HBV DNA or presence of cirrhosis	Initiation of DAAs would always require confirmation of ACTIVE infection with HCV NAT or Ag

- WHO recommends standardized testing strategies to maximize accuracy while minimizing cost and increasing simplicity.
- A testing strategy describes a testing sequence for a specific testing objective, taking into consideration the presumed prevalence of the analyte to be tested in the population. In both high- and low-prevalence settings, more than one serological assay may be required to establish exposure to HCV.
- Screening for exposure to HCV is dependent on assays that detect antibodies to HCV (anti-HCV) in the first instance. Once antibody status is confirmed, the patient will undergo supplementary testing for active HCV infection using an assay designed to detect viral replication, such as HCV RNA or core antigen (HCV cAg).
- It is important to note that the latest generation of assays designed to detect anti-HCV also are combined with cAg in order in increase sensitivity of the assay, but these "combo" assays should not be used to differentiate HCV exposure from active HCV infection.
- The question this PICO aims to address is whether one or two serological assays (anti-HCV or HCV Ag/Ab combo assays) performed sequentially are required in terms of specificity and positive predictive value in order to proceed to supplementary testing.

See footnotes for explanation of one-test and two-test strategies.

Figs 1–4: Possible testing strategies for detection of anti-HCV and diagnosis of active HCV infection

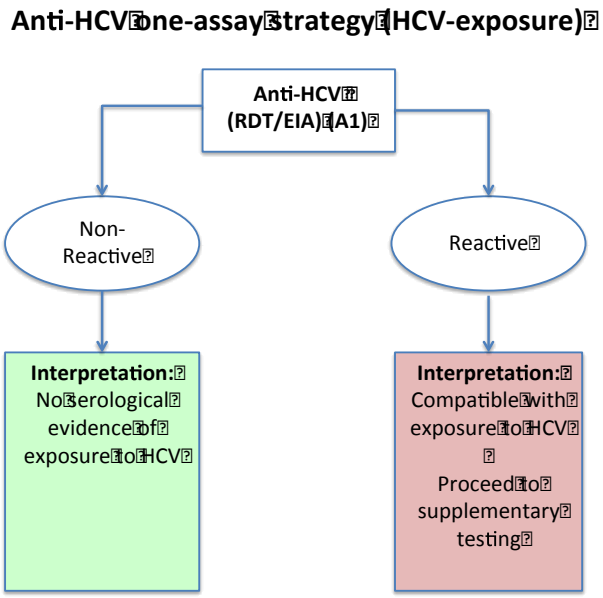


Fig. 1. One-assay testing strategy for exposure to HCV (detection of anti-HCV)

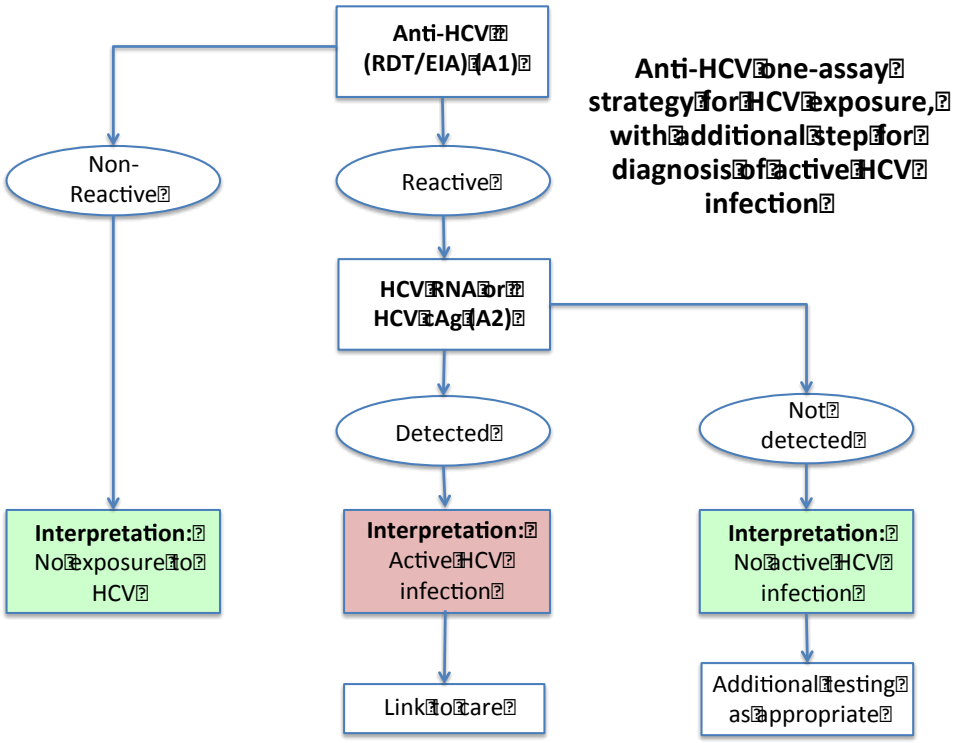


Fig. 2. One-assay testing strategy for diagnosis of HCV (detection of anti-HCV, followed by HCV RNA/core Ag)

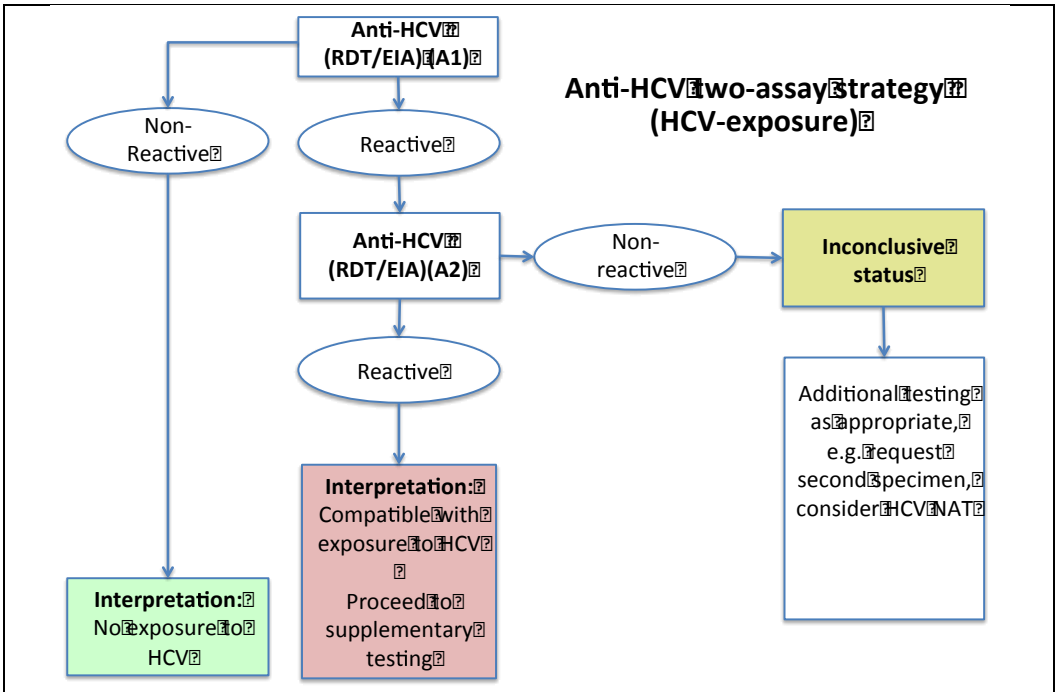


Fig. 3. Two-assay testing strategy for exposure to HCV (detection of anti-HCV)

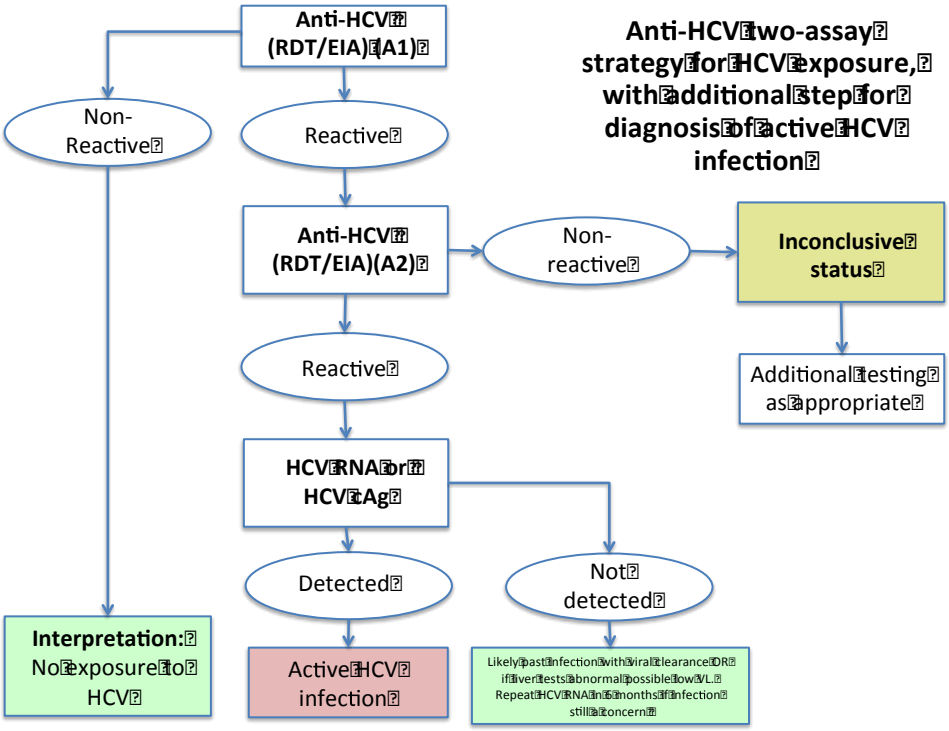


Fig. 4. Two-assay testing strategy for diagnosis of HCV (detection of anti-HCV, followed by HCV RNA/core Ag)

3. Draft recommendation(s):

4. Summary and quality of evidence

□ High
□ Moderate
□ Low
□ Very low

Systematic review:

A systematic review was commissioned in order to assess this PICO question. The purpose of this review was to determine from the published literature the evidence for a one-assay strategy for one-assay testing compared to two assays (sequential), before testing to diagnose active HCV infection is undertaken.

Summary of results

No study compared the diagnostic accuracy, cost, or cost-effectiveness of one- versus two-assay testing strategies for HCV exposure.

The result of the PICO 2 review showed that the overall pooled clinical sensitivity and clinical specificity for HCVAb RDT versus EIA were 0.98 (95% CI 0.98–1.00) and 1.00 (95% CI 1.00–1.00). The overall pooled clinical sensitivity and clinical specificity for RDTs that use oral fluid compared to a serological reference standard using serum/plasma were 0.94 (95% CI 0.93–0.96) and 1.00 (95% CI 1.00–1.00).

See footnote for tables illustrating the impact of prevalence and one- versus two-assay strategies on PPV.

Quality of evidence

- Study quality was not evaluated using the QUADAS-2 tool and the STARD checklist, as it was not applicable.
- None of the studies met inclusion criteria.

GRADE Summary of findings

Not applicable as no studies met the inclusion criteria for the systematic review.

Predictive modelling analysis: (Parry, Public Health England)

- There is a strong influence of prevalence and assay specificity on positive predictive value (PPV).
- At high prevalence, the use of a highly specific single screening test yields a PPV in excess of 0.99, and the ratio of true-positive (TP):false-positive (FP) results is high (164).
- At the lowest prevalence (e.g. 0.4%), PPV might be expected to fall below 0.5, yielding more false-positive diagnoses than true ones, particularly if the test specificity falls below 0.995.
- Even in middling prevalence, the PPV will struggle to exceed 0.9 should the specificity performance of the test employed fall below 0.99.
- The negative predictive value (NPV) is generally high except in high-prevalence populations (e.g. 45%) when a test with relatively poor sensitivity (<99%) is employed.

• In all but the highest population prevalence, the use of less sensitive test kits in the two-test strategy had only a modest impact on NPVs. In all but the highest prevalence, the calculations demonstrate the potential for substantial gains in diagnostic accuracy in return for a modest investment in a second, independent, test kit to be applied to initially reactive individuals. • For example, taking a model population of 100 000 with an anti-HCV prevalence of 2%, the PPV of an algorithm employing two independent test kits with modest sensitivities and specificities of 0.98 is improved from 0.500 to 0.980 in return for the supplementary use of <4000 of Test Kit B. **<u>Predictive modelling analysis: (Linas, Boston University)</u>** *To be added at meeting*	
5. Risks/benefits **Benefits** • The intervention of testing using a single test on one anti-HCV/core Ag assay will simplify the process of testing. • Cost of overall testing may be reduced. • May allow more rapid reporting of the result, so that the patient can be appropriately followed up for further assessment or given health protection advice. **Risks** • Possible reduced PPV of a single test on one assay may lead to more false-positives, especially in low-prevalence settings, with associated anxiety and cost associated with follow-up testing or treatment.	□ Benefits clearly outweigh harms □ Benefits and harms are balanced □ Potential harms clearly outweigh potential benefits *Are the desirable anticipated effects large?* □ No □ Probably □ Uncertain □ Yes □ Varies

6. Acceptability, values and preferences A values and preferences survey of implementers and users of hepatitis B and C testing services was carried out by FIND in September 2015. A total of 104 respondents from 43 (20 high-income, 23 low- and middle-income) countries participated. Relating to this PICO, • 50% of respondents to the values and preference survey from low- and middle-income settings (*n* = 23) preferred a one-assay screening strategy before confirmatory testing (compared to a one-assay strategy for diagnosis of HCV active infection). **Patients/caretakers** • Patients require a simple and rapid testing strategy, yet one that is acceptably accurate for the purpose of testing. **Health-care workers** • HCWs will need to understand the strengths and limitations of any given testing strategy and appropriately counsel patients who are screened. • HCWs will need to appropriately act on results, either positive or negative. **Other** • Laboratory staff needs to be appropriately trained in performance of the various assays according to the manufacturers' instructions for use.	□ No major variability □ Major variability Is the option acceptable to key stakeholders? □ No □ Probably □ Uncertain □ Yes □ Varies
Equity, ethics and human right implications *Will recommendation raise questions around equity?* • Yes, availability of HCV RNA NAT assays or other supplemental testing, such as core antigen testing, will be more easily available in settings with established hepatitis C testing facilities. *Are there ethical implications to this recommendation?*	□ Less equitable □ More equitable

7. Resource use and financial implications

Are the resources required small?

□ No
□ Probably
□ Uncertain
□ Yes
□ Varies

Diagnostics/Laboratory monitoring

- Cost of a one-test vs two-assay strategy will be less due to fewer tests being used but the positive predictive value is higher and therefore the status will be more accurately assigned.

Assay format	Indicative cost (US$) per test	Source
RDTs	0.50–2.00 (10 for oral fluid RDTs)	MSF, WHO
EIA	0.50–1.70	WHO
HCV Ag	25–50	MSF
Quantitative NAT for HCV RNA	10–45	MSF, UNITAID
Qualitative NAT for HCV RNA)	43–51	UNITAID

8. Feasibility and constraints to implementation

Are any major barriers expected for the implementation of this recommendation?

Is the option feasible to implement?

□ No
□ Probably
□ Uncertain
□ Yes
□ Varies

- None, provided there is internal and country commitment to HCV testing
- Regional and country variability in access to treatment and procurement of testing equipment and services
- With regard to any diagnostic assay, availability of a local laboratory, which is able to procure the testing platform and reagents required for testing.

Feasibility survey report to be presented at meeting

9. Relevance to different settings/populations

Will this recommendation be most relevant for particular settings (e.g. endemicity)?

10. Rationale for recommendation:
11. Strength of recommendation
12. Implementation considerations
13. Research gaps • Development/implementation projects evaluating use of HCV core antigen as a one-stop diagnostic test • Impact of one-test vs two-test screening on hepatitis C testing services

GRADE Summary of findings

Footnote: Explanation of one- and two-assay strategies

One-assay testing strategy

- A single test is performed. If the test result is reactive, an "anti-HCV positive" status is reported. A specimen should be collected for supplemental laboratory testing to pursue the testing algorithm. If the initial test is non-reactive, an "anti-HCV negative" status is reported.
- This testing strategy efficiently identifies most uninfected individuals, it identifies those likely to be infected and in need of additional testing.
- It is suitable for resource-limited settings because one single test on one assay is performed as long as the assay used has high clinical sensitivity and clinical specificity. Limitations of this approach are that a small percentage of test results may be false positive, so appropriate procedures to follow up individuals need to be in place.

Two-assay testing strategy

- The test results of two different assays are used sequentially (i.e. not in parallel), to improve the positive predictive value of overall testing strategy.
- If the test result for the first-line assay is non-reactive, an "anti-HCV negative" status is reported.
- If the test result for the first-line assay is reactive, a second test performed on an assay from a different manufacturer is performed.
- If both test results are reactive, the result is reported as: "anti-HCV positive for further diagnostic testing." If the test result of the second-line assay is non-reactive, the result is reported as "anti-HCV inconclusive; requires additional testing".
- This testing strategy efficiently identifies most uninfected individuals and more definitively rules out exposure to HCV than a one-assay testing strategy. It improves the positive predictive value when the test results for two different assays are both reactive. It can be used by non-laboratory staff, provided that adequate quality assurance measures are in place.

- The two-assay testing strategy may still produce a small number of false-positive results (particularly in low-prevalence settings); some persons with recent exposure may receive false-negative results which will depend on the analytical sensitivity of the assays used

Modelling tables:

Table 1: Outcomes for single-test strategy based on typical estimates of test accuracy

Anti-HCV Prevalence: 45%		Population		100,000								
Test A		Test B										
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
1.000	0.995	n/a	n/a	45,000	0	275	54,725	100,000	0.994	1.000	164	1.000	0.995
0.995	0.995	n/a	n/a	44,775	225	275	54,725	100,000	0.994	0.996	163	0.995	0.995
0.995	0.980	n/a	n/a	44,775	225	1,100	53,900	100,000	0.976	0.996	41	0.995	0.980
0.980	0.980	n/a	n/a	44,100	900	1,100	53,900	100,000	0.976	0.984	40	0.980	0.980

Anti-HCV Prevalence: 10%		Population		100,000								
Test A		Test B										
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
1.000	0.995	n/a	n/a	10,000	0	450	89,550	100,000	0.957	1.000	22	1.000	0.995
0.995	0.995	n/a	n/a	9,950	50	450	89,550	100,000	0.957	0.999	22	0.995	0.995
0.995	0.980	n/a	n/a	9,950	50	1,800	88,200	100,000	0.847	0.999	5.5	0.995	0.980
0.980	0.980	n/a	n/a	9,800	200	1,800	88,200	100,000	0.845	0.998	5.4	0.980	0.980

Anti-HCV Prevalence: 2%		Population		100,000								
Test A		Test B										
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
1.000	0.995	n/a	n/a	2,000	0	490	97,510	100,000	0.803	1.000	4.1	1.000	0.995
0.995	0.995	n/a	n/a	1,990	10	490	97,510	100,000	0.802	1.000	4.1	0.995	0.995
0.995	0.980	n/a	n/a	1,990	10	1,960	96,040	100,000	0.504	1.000	1.0	0.995	0.980
0.980	0.980	n/a	n/a	1,960	40	1,960	96,040	100,000	0.500	1.000	1.0	0.980	0.980

Anti-HCV Prevalence: 0.4%		Population		100,000								
Test A		Test B										
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
1.000	0.995	n/a	n/a	400	0	498	99,102	100,000	0.445	1.000	0.8	1.000	0.995
0.995	0.995	n/a	n/a	398	2	498	99,102	100,000	0.444	1.000	0.8	0.995	0.995
0.995	0.980	n/a	n/a	398	2	1,992	97,608	100,000	0.167	1.000	0.2	0.995	0.980
0.980	0.980	n/a	n/a	392	8	1,992	97,608	100,000	0.164	1.000	0.2	0.980	0.980

Table 2: Outcomes for two-test strategy based on typical estimates of test accuracy (see Fig. 4)

Anti-HCV Prevalence: 45%		Population		100,000								
Test Kit A		Test Kit B										
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
1.000	0.995	1.000	0.995	45,000	0	1	54,999	145,275	1.000	1.000	32,727	1.000	1.000
1.000	0.995	0.980	0.980	44,100	900	6	54,995	145,275	1.000	0.984	8,018	0.980	1.000
0.980	0.980	1.000	0.995	44,100	900	6	54,995	145,200	1.000	0.984	8,018	0.980	1.000
0.980	0.980	0.980	0.980	43,218	1,782	22	54,978	145,200	0.999	0.969	1,964	0.960	1.000

Anti-HCV Prevalence: 10%		Population		100,000								
Test Kit A		Test Kit B										
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
1.000	0.995	1.000	0.995	10,000	0	2	89,998	110,450	1.000	1.000	4,444	1.000	1.000
1.000	0.995	0.980	0.980	9,800	200	9	89,991	110,450	0.999	0.998	1,089	0.980	1.000
0.980	0.980	1.000	0.995	9,800	200	9	89,991	111,600	0.999	0.998	1,089	0.980	1.000
0.980	0.980	0.980	0.980	9,604	396	36	89,964	111,600	0.996	0.996	267	0.960	1.000

Anti-HCV Prevalence: 2%		Population		100,000								
Test Kit A		Test Kit B										
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
1.000	0.995	1.000	0.995	2,000	0	2	97,998	102,490	0.999	1.000	816	1.000	1.000
1.000	0.995	0.980	0.980	1,960	40	10	97,990	102,490	0.995	1.000	200	0.980	1.000
0.980	0.980	1.000	0.995	1,960	40	10	97,990	103,920	0.995	1.000	200	0.980	1.000
0.980	0.980	0.980	0.980	1,921	79	39	97,961	103,920	0.980	0.999	49	0.960	1.000

Anti-HCV Prevalence: 0.4%		Population		100,000								
Test Kit A		Test Kit B										
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
1.000	0.995	1.000	0.995	400	0	2	99,598	100,898	0.994	1.000	161	1.000	1.000
1.000	0.995	0.980	0.980	392	8	10	99,590	100,898	0.975	1.000	39	0.980	1.000
0.980	0.980	1.000	0.995	392	8	10	99,590	102,384	0.975	1.000	39	0.980	1.000
0.980	0.980	0.980	0.980	384	16	40	99,560	102,384	0.906	1.000	10	0.960	1.000

4.7. How to test – confirmation of viraemia HCV

Decision-making tables – PICO 5a

Testing strategy for diagnosis of HCV active infection: Among individuals with confirmed exposure to HCV (HCV Ab positive), what is best testing strategy (diagnostic accuracy and other outcomes); comparing HCV core antigen versus NAT for HCV RNA to diagnose active HCV infection?

Decision-making tables – PICO 6

Testing strategy for diagnosis of HCV active infection (quantitative or qualitative NAT): Among individuals with confirmed exposure to HCV (Ab positive), what is the diagnostic test accuracy of qualitative NAT methods versus quantitative NAT methods to diagnose active HCV infection?

1. Topic for analysis: How to test

PICO 5a

Population: Individuals with detectable HCV RNA with or without confirmed exposure to HCV (HCV seropositivity)

Intervention: HCV core antigen

Comparison: HCV RNA NAT

Outcomes: Diagnostic accuracy (Sensitivity, Specificity, Positive predictive value, Negative predictive value, TN, TP, FN, and FP).

PICO 6

Population: Persons with confirmed exposure to HCV (HCV seropositivity)

Intervention: Qualitative HCV RNA NAT

Comparison: Quantitative HCV RNA NAT

Outcomes: Diagnostic accuracy (Sensitivity, Specificity, Positive predictive value, Negative predictive value, TN, TP, FN, and FP).

2. Background:

- Determination of exposure to HCV through detection of antibodies to HCV (anti-HCV) is a commonly used first-line diagnostic tool to identify those who are infected with HCV who might benefit from antiviral treatment, yet diagnosis of active HCV infection requires evidence of active viral replication, traditionally ascertained by the detection of HCV ribonucleic acid (RNA) by nucleic acid testing (NAT).
- Detection of HCV RNA may also be used as the preferred first-line investigation in individuals who may be persistently seronegative due to underlying immunosuppression, e.g. poorly-controlled HIV infection, renal dialysis.
- Qualitative NAT for HCV allows for detection of the virus as well as evidence of the level of HCV RNA circulating in the peripheral blood falling below a clinically relevant threshold, i.e. a qualitative measurement.

- Assays to detect HCV-RNA have been developed for the near point-of-care (POC) setting.
- NAT assays can be very sensitive and specific but more costly than serological methods such as HCV core antigen testing, and require sophisticated laboratory equipment and therefore skilled staff.
- HCV core antigen (HCVcAg) testing was developed as an alternative to NAT for the diagnosis of active HCV infection. HCV nucleocapsid peptides 22 (p22) are released into plasma during viral assembly and can be detected throughout the course of HCV infection. There are also several assays that have been commercialized for stand-alone detection of HCV cAg as a replacement to NAT.
- Furthermore, HCV cAg is detectable earlier than antibodies and therefore detection of HCV cAg has also been applied as an additional analyte for serological assays for use to ascertain exposure to HCV as a combination HCV Ag/Ab (4th generation) assay. The addition of cAg in combination was intended to increase sensitivity of the assay in early infection. Although the output may be interrogated in order to ascertain whether antigen, antibody or both were detected, the purpose of the assays is not to differentiate seropositivity from active infection.
- Both HCV cAg and HCV RNA (either qualitative or quantitative detection) have been shown to have clinical utility for the detection of active HCV infection though there is scarce research comparing the HCVcAg, and qualitative and quantitative NAT for this purpose.

3. Draft recommendation(s):

- **Summary and quality of evidence**

□ High
□ Moderate
□ Low
□ Very low

1. Distribution of viraemia in persistent infection:

A threshold of >10 000 IU/mL will capture 95% of persistent infections (except, temporarily, for a minority of those with partial viral control) between 5 and 12 months post infection.

In 95% of cases of hepatitis C infections:

- Those with persistent infection and viral plateau will have a viral load (VL) >100 000 IU/mL at month 5 and remain at least >10 000 IU/mL between months 5 and 12.
- Those with persistent infection but partial viral control will also have >100 000 IU/mL at month 5 and remain having a VL at least >1000 IU/mL temporarily, going back to a viral load >100 000 IU/mL between months 10 and 12.
- Between 2 and 3–4 months after infection, acute infection (giving VLs up to >100

000 IU/mL) may be captured that would spontaneously resolve but would likely be antibody negative.

Publications are:

Hajarizadeh B, Grady B, Page K, Kim AY, McGovern BH, Cox AL et al. Patterns of hepatitis C RNA levels during acute infection: the C3 study. PLOS One. 2015;10 (4):e 0122232.

Hajarizadeh B, Grebely J, Applegate T, Matthews GV, Amin J, Petoumenos K et al. J Med Virol. 2014;86 (10):1722–9.

Glynn SA, Wright DJ, Kleinman SH, Hirschkorn D, Tu Y, Heldebrant C et al. Transfusion. 2005;45 (6):994–1002.

2. Systematic reviews of the evidence:

Two systematic reviews were commissioned to address the above PICO questions. These aimed to summarize (1) the diagnostic accuracy of HCV cAg testing (PICO 5a), (2) the diagnostic accuracy of qualitative versus quantitative RNA for diagnosis of active HCV infection (PICO 6).

Summary of results of HCV cAg review

Diagnostic accuracy of HCV cAg for diagnosis of active infection (Fig. 1)

- 7 assays utilizing HCV core antigen were assessed. Note that two of these were HCV antigen/antibody combination assays and not designed to differentiate active infection from seropositivity, but are included as the antigen/antibody components of the assays were reported separately.

			Effect Accuracy (95% CI)		Effect LR	
Index test	***n*** **(samples)**	**Unit of analysis**	**Sensitivity**	**Specificity**	**Positive LR**	**Negative LR**
Abbott ARCHITECT	20 (11,820)	Sample	93.4% (88.7, 96.2)	98.7% (96.9, 99.4)	71.8 (28.6, 160.3)	0.07 (0.04, 0.12)

HCV Ag Assay						
Ortho ELISA-Ag	5 (1,177)	Sample	93.2% (81.6, 97.7)	99.2% (87.9, 99.9)	116.5 (6.7, 977)	0.06 (0.02, 0.07)
Bio-RAD Monolisa HCV Ag-Ab ULTRA	5 (525)	Sample	28.6–95%*	94.9% (89.9, 99.8)**	NA	NA
EIKEN Lumispot HCV Ag	2 (235)	Sample	97.5–98.1%*	ND	NA	NA
Fujirebio Lumipulse Ortho HCV Ag	1 (80)	Sample	95% (90.2, 99.8)**	ND	NA	NA
Hunan Jynda HCV Core Ag ELISA	4 (524)	Sample	59.5% (46, 71.7)	82.9% (58.6, 94.3)	3.5 (1.1, 12.6)	0.28 (0.2, 0.3)
DiaSorin S.A. Murex Ag/Ab EIA	4 (730)	Sample	50–100%*	83.8–100%*	NA	NA

n: study number, CI: confidence interval; LR: likelihood ratio; ND: no data, NA: not applicable — if sensitivity and specificity results were not available from meta-analysis, likelihood ratios were not calculated.

* Meta-analysis not possible. Range of results seen across studies reported.

**Result from one study only.

<u>Limits of detection of HCV cAg assays</u>

- The limit of detection for the most evaluated and best performing assay (Abbott Architect) is 3 fmol/L HCV cAg or 0.06 pg/mL, which equals to a limit of detection of about ~1000–3000 IU/mL with a NAT.

Impact of findings in different prevalence settings (for assays where a meta-analysis was possible) (Fig. 2)

Outcome	Effect per 1000 patients with presumed HCV for varying prevalence settings comparing HCV core Ag against HCV RNA		
	Prevalence 2%*	**Prevalence 10%***	**Prevalence 30%***
Abbott ARCHITECT HCV Ag Assay			
TP	19 (18, 19)	93 (89, 96)	279 (267,288)
TN	967 (951, 974)	888 (873, 895)	691 (697, 696)
FP	13 (6, 29)	12 (5, 27)	9 (4, 21)
FN	1 (1, 2)	7 (4, 11)	21 (12, 33)
Ortho ELISA-Ag			
TP	19 (16, 20)	93 (82, 98)	279 (246, 294)
TN	970 (862,980)	891 (792, 900)	693 (616, 700)
FP	10 (0, 118)	9 (0, 108)	7 (0, 84)
FN	1 (0,4)	7 (2, 18)	21 (6, 54)
Hunan Jynda HCV Core Ag ELISA			
TP	12 (9, 14)	60 (46, 72)	179 (138, 216)
TN	813 (578, 921)	747 (531, 846)	581 (413, 658)
FP	167 (59, 402)	153 (54, 369)	119 (42, 287)
FN	8 (6, 11)	41 (28, 54)	122 (84, 162)

TR: true positives (individuals with active HCV); TN: true negatives (individuals without active HCV); FP: false positives (individuals incorrectly classified as having active HCV); FN: false negatives (individuals incorrectly classified as not having active HCV)

*Numbers in parentheses consider 95% confidence interval of accuracy estimate.

Limits of detection of hepatitis C NAT assays

This systematic review shows that for diagnosis of active HCV infection, the lower limit of detection of most commercial qualitative assays was in the 10–15 IU/mL range measured against a WHO standard, whereas the lower limit of detection for quantitative assays is at 600–1100 IU/mL.

Additional information:

Newer quantitative viral load assays report limits of detection similar to qualitative viral load but might not quantitate results at that level.

NAT are in the pipeline for detection on capillary whole blood. Furthermore, detection from dried blood spot is also promoted to increase access. For both those strategies, the limit of detection is likely to be substantially higher (e.g. in the range of 2000 IU/mL) than for existing assays (because of volume tested and technical feasibility).

Fig. 3: Limit of detection of qualitative vs quantitative NAT

	Limits of detection							
Study	**Qualitative NAT (IU/mL)**				**Quantitative NAT (IU/mL)**			
Lee 2000	COBAS AMPLICOR™ HCV Test v2.0 assay. *Roche*	15	25	>50	AMPLICOR HCV test, v 2.0. *Roche*	300	600	1100
		95%	95%	100%		57%	95%	100%
Sarrazin 2008	CAP/CTM *Roche*	100%	–	–				
	RealTime HCV assay *Abbott*	87%						

Lee SC, Antony A, Lee N, Leibow J, Yang JQ, Soveiro S et al. Improved version 2.0 qualitative and quantitative AMPLICOR reverse transcription-PCR tests for hepatitis C virus RNA: calibration to international units, enhanced genotype reactivity, and performance characteristics. J Clin Microbiol. 2000;38 (11):4171–9.

Sarrazin C, Dragan A, Gartner BC, Forman MS, Traver S, Zeuzem S et al. Evaluation of an automated, highly sensitive, real-time PCR-based assay (COBAS Ampliprep/COBAS TaqMan) for quantification of HCV RNA. J Clin Virol. 2008;43 (2):162–8.

Conclusions:

- HCV core Ag assays can have high sensitivity (up to 93.4% for certain commercialized assays), high specificity, and good correlation with HCV RNA to a detection limit of roughly 1000–3000 IU/mL.
- NAT on plasma and serum are able to achieve higher sensitivity than cAg. Qualitative assays from published data are more sensitive than quantitative assays. This might not be the case for newer assays.

Quality of evidence

*Refer GRADE table in footnote

Predictive modelling (Linas, Boston University)

To be added at meeting

4. Risks/benefits **<u>cAG vs NAT</u>** **Benefits** • HCV cAg testing by immunoassay format has the potential to be less costly given that the cost of goods is lower. • Also HCV cAg is more stable and thus does not require a cold chain. • Further assessment and treatment may be administered more promptly to individuals when diagnosis of active HCV infection is undertaken in a more decentralized manner. There are both HCV cAG and NAT tests in the pipeline that might be possible on capillary blood at the point of care. However, current cAg immunoassays still require sophisticated laboratory equipment, electricity and therefore skilled staff to operate, while there are already some NAT assays available that can be done near the patient (however, still require plasma). • HCV cAg assay can conceivably be used in a one-step testing strategy (i.e. without an antibody test) particularly in high-prevalence settings. With such a strategy, patients can be identified earlier in their infection (than with antibody testing), results may be available faster to the patient and provider, resulting in less loss to follow up and faster treatment initiation. **Risks** • The patient with low-level viraemia (<3000 IU/mL) could be missed with HCV cAg assays or HCV RNA NAT with lower sensitivity. The clinical implications for the individual person and on a population level are not well understood. However, if the test does reach more patients (e.g. because it can be done on capillary blood or is less costly), then this risk might be outweighed by the benefits. • Although data exist on the utility of HCV cAg in seronegative individuals, no studies examined the use of HCV cAg and HCV RNA NAT in diagnosing HCV infection in key affected populations, in a one-step testing strategy rather than using an antibody assay. Such a strategy would only be cost–effective in certain high-prevalence settings. **Qual versus Quant NAT** **Benefits** • Qualitative NATs generally are more or at least as sensitive than quantitative assays.	□ Benefits clearly outweigh harms □ Benefits and harms are balanced □ Potential harms clearly outweigh potential benefits *Are the desirable anticipated effects large?* □ No □ Probably □ Uncertain □ Yes □ Varies

• The cost of a qualitative NAT assay may be lower than that of a quantitative assay. **Risk** • Recent publications suggest that monitoring for HCV will not be necessary with direct-acting antivirals (DAA) (as viral load at EVR is not predictive of cure). Therefore, quantitation at baseline will not be necessary. This is currently not yet widely confirmed or reflected in guidelines.	
5. Acceptability, values and preferences A values and preferences survey of implementers and users of hepatitis B and C testing services was carried out by FIND in September 2015. A total of 104 respondents from 43 (20 high-income, 23 low- and middle-income) countries. Relating to this PICO, • As assay (platform) for detection of HCV cAg is currently available in India, Indonesia, Macedonia, Viet Nam, Turkey (although the representativeness of these data are limited). The platform is available in South Africa but not currently being used for HCV cAg detection. • 50% found a diagnostic sensitivity of >95% acceptable, particularly if the test cost is lower, therefore increasing access to testing (respondents felt that then the test cost should be less than US$ 10 (83%) with a sensitivity of 95%. For a test with a sensitivity of 98%, forty-one per cent of respondents would accept a cost of US$ 11–20). • Free text comments suggested that HCV cAg was easier to do. • A larger number of respondents felt that the cost of testing for HCV RNA by NAT was considered more of a barrier than that of HCV cAg. • 47% of respondents in low- and middle-income countries favoured a decentralized test and a test on capillary blood even at the cost of sensitivity. • Also, 50% of patients preferred a test result in <2 hours (which could only be achieved at the point of care) while 27% found a result on the same day acceptable. **Patient:** • Patients at risk of progressive liver disease will benefit from reduced disease progression and related mortality and morbidity, if treatment is provided as a result of wider access to testing programmes.	□ No major variability □ Major variability Is the option acceptable to key stakeholder? □ No □ Probably □ Uncertain □ Yes □ Varies

Community: • To identify the individuals who require assessment and treatment would be an effective use of resources. • As testing and treatment programmes are scaled up, the numbers developing progressive disease and serious outcomes (HCC and complications of advanced liver disease), premature morbidity and mortality within the community will be reduced, and so also the burden of disease to societies where the disease is most prevalent. **Health-care workers:** • Appropriate use of resources to channel treatment to patients with higher risk of complications in the medium- and short term • Will require training in the use of testing equipment if being used in the near-POC setting • Appropriate reporting and recording of results. **Laboratory:** • Will require training for HCV cAg test: careful sample processing is necessary for HCV cAg assay to lyse viral particles, expose antigen and dissociate antibody from antigen and optimize the detection for HCV cAg.	
6. Equity, ethics and human right implications *Will the recommendation raise questions around equity?* • Equity will improve as a result of decreased cost and increased decentralization of testing; however, still improvement of access to testing facilities is necessary. • Regional and country variability in access to treatment. *Are there ethical implications to this recommendation?* • Ethical consideration for the possibility that WHO could recommend a testing strategy.	□ Less equitable □ More equitable
7. Resource use and financial implications **Input from modelling team**	*Are the resources required*

Materials/equipment:

- Cost of testing platform and reagents
- Other laboratory consumables

Training and supervision:

- Appropriate training of laboratory staff
- Quality control programmes
- If using near-POC assays, appropriate training of testing providers.

Other:

- Cost of transportation of specimens to the laboratory

Possible procurement costs (Fig. 4):

EXW / FCA PRICING FROM MSF PRODUCT GUIDE "PUTTING HIV AND HCV TO THE TEST"
***or UNITAID Hepatitis C Diagnostics Technology Landscape 1st Edition**

Lab-based tests	Cost for HCV (USD)	Polyvalency
Abbott Architect core Ag	$25-30	Extensive test menu including anti-HCV, anti-HBsAg
Abbott m2000 VL & GT	$13-35 each	Bundled pricing: HIV VL & EID, MTB, HCV, HBV, HPV, CT/NG Also: HCV GT, CMV, MTB RIF/INH
Biocentric VL	$23	HIV-2, MTB + resistance, HBV
Hologic Aptima VL	Awaiting launch	CT/NG, T. vaginalis, HPV Others in development incl HBV
Qiagen artus VL	$16-45	HBV, CMV, EBV, BKV, VZV, HSV, CT, MTB, C. Diff, VanR, GBS
Qiagen careHCV VL	$22 (China)*	-
Roche CAP/CTM qual & VL	$36-38*	CMV, B*5701, CT, MTB, HBV Global Access Program HIV VL & EID: $9.40
Roche GT (LPA)	$43-51*	-
Sacace VL & GT	>$20	Extensive test menu incl HIV & HBV
Siemens VL & GT (LPA)	$72-100 & $132-350	-

Cepheid: <20 NAT. With volume-based pricing down to~15

Note that new POC or near-POC assay platforms for HCV RNA are in development. See figures at the end of the table.

small?

□ No
□ Probably
□ Uncertain
□ Yes
□ Varies

Feasibility and constraints to implementation *Are any major barriers expected for the implementation of this recommendation?* • None, provided there is internal and country commitment to HCV testing. • Regional and country variability in access to treatment and procurement of testing equipment and services • With regard to any diagnostic assay, availability of a local laboratory, which is able to procure the testing platform and reagents required for testing. **Feasibility survey report to be presented at meeting.**	*Is the option feasible to implement?* □ No □ Probably □ Uncertain □ Yes □ Varies

8. Relevance to different settings/populations

Will this recommendation be most relevant for particular settings (e.g. endemicity)?

- Currently available HCV cAg testing will be more relevant to populations that presently rely on centralized laboratory testing for HCV RNA for confirmation of status.
- One-step strategies for testing with HCV cAg or NAT may be cost-effective only in high-prevalence settings.
- The recommendations are less likely to be relevant in high-income settings where there is already access to established hepatitis C testing and treatment programmes.

9. Rationale for recommendation:

10. Strength of recommendation

11. Implementation considerations

- Optimize test for asymptomatic patients in primary-care settings or in the community where the HCV endemic is high.

12. Research gaps

- Development/implementation projects evaluating use of HCV core antigen or HCV RNA as a one-step diagnostic strategy.
- Surveillance data: how many patients are missed by assays that have limits of detection of 2000 IU.
- Outcomes of patients with low viral loads
- More information on patients with high viral loads and negative HCV cAg to inform the

optimization of antigen detection.

- The kinetics of HCVcAg with treatment needs to be evaluated further, particularly in the context of new DAA regimens.
- More rigorous assessment of covariates in accuracy studies is required, such as HIV or HBV coinfection or genotype (particularly genotypes 5 and 6 where there are limited data).
- Development of muliplex instrument with other disease diagnosis with HIV, HBV, and TB at health centre.

GRADE Summary of findings

Table: Strength of evidence for diagnostic accuracy

SR outcome: diagnostic accuracy				**Quality**				**Strength of evidence**
Index Test	**Outcome Measure**	**# Studies (# samples)**	**Design**	**Risk of Bias**	**Inconsistency**	**Indirectness**	**Imprecision**	
Abbott ARCHITECT HCV Ag Assay	Sensitivity	30 (12 788)	Cohort and cross-sectional	Low[1]	Low[2]	Moderate[3] (–1)	Low[4]	Moderate ⊕⊕⊕◯
	Specificity	20 (11 820)	Cohort and cross-sectional	Low[1]	Low[2]	Moderate[3] (–1)	Low[4]	Moderate ⊕⊕⊕◯
Ortho ELISA-Ag	Sensitivity	6 (1 423)	Cohort and cross-sectional	High[1] (–2)	Moderate[2] (–1)	Moderate[3] (–1)	Low[4]	Very low ⊕◯◯◯
	Specificity	5 (1 177)	Cohort and cross-sectional	High[1] (–2)	Moderate[2] (–1)	Moderate[3] (–1)	Low[4]	Very low ⊕◯◯◯
Bio-RAD Monolisa HCV Ag-Ab ULTRA:	Sensitivity	5 (525)	Cohort and cross-sectional	Low[1]	High[2] (–2)	Moderate[3] (–1)	Low[4]	Very low ⊕◯◯◯

Sensitivity	Specificity	1 (337)	Cross-sectional	Moderate[1] (–1)	NA[2] (–1)	Moderate[3] (–1)	NA[4]	Very low ⊕◯◯◯
EIKEN Lumispot HCV Ag: Sensitivity	Sensitivity	2 (235)	Cross-sectional	Moderate[1] (–1)	Low[2]	Moderate[3] (–1)	Low[4]	Low ⊕⊕◯◯
	Specificity	0	NA	NA	NA	NA	NA	NA
Fujirebio Lumipulse Ortho HCV Ag: Sensitivity	Sensitivity	1 (80)	Cross-sectional	Moderate[1] (–1)	NA[2] (–1)	Moderate[3] (–1)	NA[4]	Very low ⊕◯◯◯
	Specificity	0	NA	NA	NA	NA	NA	NA
Hunan Jynda HCV Core Ag ELISA	Sensitivity	4 (524)	Cohort and cross-sectional	Moderate[1] (–1)	High[2] (–2)	Moderate[3] (–1)	Low[4]	Very low ⊕◯◯◯
	Specificity	4 (524)	Cohort and cross-sectional	Moderate[1] (–1)	High[2] (–2)	Moderate[3] (–1)	Low[4]	Very low ⊕◯◯◯
DiaSorin S.A. Murex Ag/Ab EIA	Sensitivity	4 (770)	Cohort and cross-sectional	Low[1]	High[2] (–2)	Moderate[3] (–1)	None[4]	Very low ⊕◯◯◯
	Specificity	3	Cohort	Low[1]	Moderate[2]	Moderate[3]	Low[4]	Low

		(658)			(–1)	(–1)		⊕⊕◯◯

NA: not applicable

Footnotes:

For each index test, quality of evidence started high when there were several high-quality observational studies (prospective cohort studies, cross-sectional studies with direct comparison of index test results with a reference standard). We then downgraded one point when there was moderate concern identified and two points when a there was a high concern identified in any of the four factors that may decrease the quality of evidence: risk of bias, inconsistency, indirectness, and imprecision.

[1] We used QUADAS-2 to assess risk of bias.

- For ARCHITECT, in half of the studies it was unclear how participants were selected and one study used only healthy blood donors, however, the data from all studies are consistent and unclear selection does not appear to cause bias thus we did not downgrade.
- For the Ortho ELISA, two studies of five used convenience enrolment for participant selection, and one enrolled only healthy blood donors thus we downgraded 2 points.
- For the Monolisa, four of five studies had unclear patient selection. For one it was unclear if the index and reference tests were performed within 30 days. Given that there were no high-risk concerns for bias we did not downgrade. For specificity, there was only one study with data that had unclear participant selection, thus we downgraded one point as there were no data from studies with random or consecutive selection to compare to and identify possible selection bias (as was possible with the ARCHITECT).
- For the Lumispot, both studies had unclear patient selection. As there were no data from studies with random or consecutive selection to compare, we downgraded one point.
- The Lumipulse only included one study with unclear participant selection and was downgraded one point.
- The Hunan Jynda had one of four studies with unclear participant selection, one in only healthy blood donors, and one for which it was unclear whether the index and reference were performed within 30 days. As the use of only healthy blood donors was considered a high-risk category, in combination with the other unclear factors, we downgraded one point.
- For the Murex test, three of four studies had unclear participant selection but no other high-risk concerns for bias and thus we did not downgrade.

[2] Unexplained heterogeneity in the remaining studies may be related to covariates that could not be adjusted for in meta-regression due to limited data (HIV and HBV coinfections, HCV genotype). Additionally, not all studies identified HCV antibody status or stratified by acute and chronic infection thus variability of HCV replication could contribute to higher false-negative HCV cAg.

- There was little heterogeneity noted in the ARCHITECT studies thus we did not downgrade.
- For the Ortho ELISA, there was moderate heterogeneity with largely one outlier study, thus we downgraded 1 point.
- For the Monolisa sensitivity outcome, heterogeneity between studies precluded meta-analysis and thus we downgraded 2 points. For specificity, there is only 1 study and we cannot assess heterogeneity and downgraded 1 point.
- For the Murex sensitivity outcome there was too much heterogeneity to pool the data, and thus we downgraded 2 points. For specificity, there were not enough studies to perform meta-analysis and heterogeneity could not be formally assessed; however, there is a broad range among results and thus we downgraded one point.
- The EIKEN Lumispot was only used in 2 studies. Sensitivity was similar in both studies suggesting little heterogeneity, thus we did not downgrade.
- For the Fujirebio Lumipulse, there is only 1 study and we cannot assess heterogeneity and downgrade 1 point.

[3] All studies were performed in reference laboratories, and the majorities were in high- and middle-income countries. Thus the patient population, the viral population tested (e.g. genotype distribution), and the test users are not representative of the limited-resource settings for which these guidelines are envisioned. All were downgraded 1 point.

[4] We considered imprecision as present when the pooled confidence intervals were >10% and when there were fewer than 250 samples in the analysis. As such, we downgraded the Ortho ELISA, and Hunan Jynda one point for wide confidence intervals, and downgraded the Lumispot one point for small sample size. Additionally, imprecision could not be graded for the Monolisa specificity outcome, and the Lumipulse test as these only included one study.

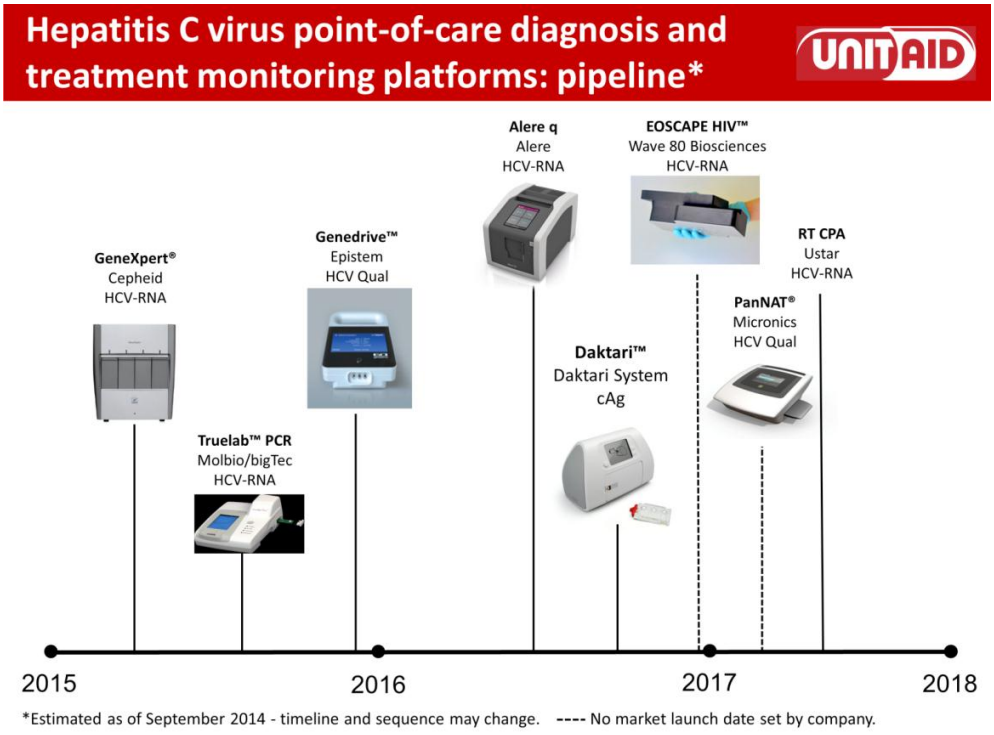
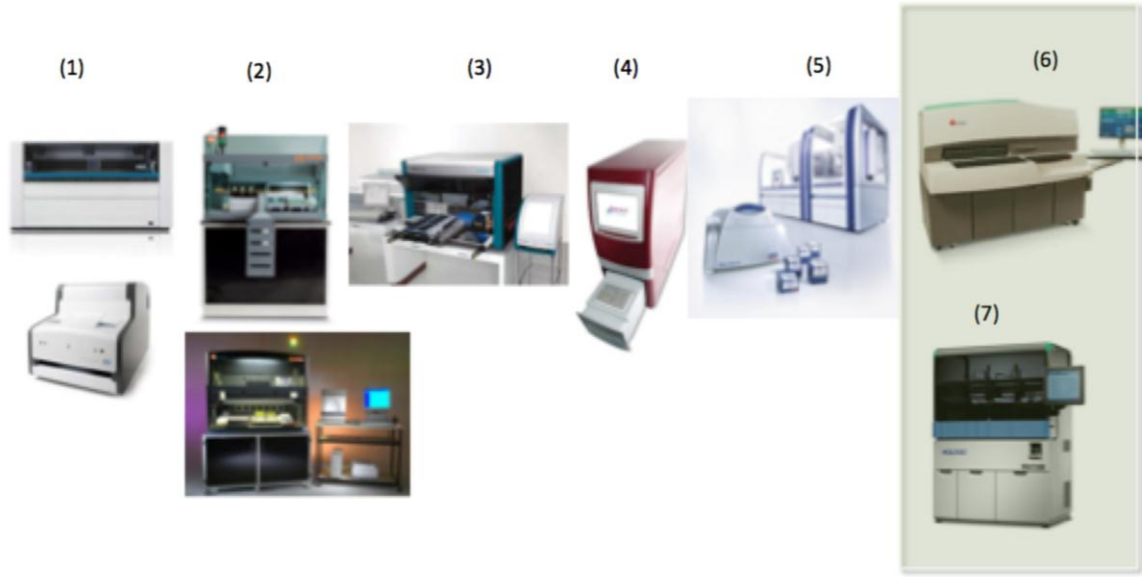


Fig. 1: POC HCV platforms available within the next 2 years

Fig. 2 and Table 2:

	Roche Molecular Systems (1)	Abbott Diagnostics (2)	Siemens Healthcare Diagnostics Inc. (3)	Sacace Biotechnologies (4)	QIAGEN (5)	Beckman Coulter (6)	Hologic Inc (7)
Qualitative	Roche COBAS AmpliPrep/COBAS TaqMan HCV Qualitative Test v.2						
Quantitative	Roche COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test v.2	Abbott RealTime HCV Assay	VERSANT HCV RNA 1.0 Assay (kPCR)	HCV Real-TM Quant Dx Assay	Artus HCV QS-RCQ Kit	VERIS MDx	RT-TMA Technology for the Panther® System
Lower limit of Detection	15 IU/mL	12 IU/mL –0.5 mL sample; 30 IU/mL –0.2 mL sample	15 IU/mL	13 IU/mL with 1 mL sample	35 IU/mL with automated extraction		
Sample type	Plasma or serum	Plasma or serum	Plasma or serum	Plasma	Plasma	Plasma, serum and culture	**Plasma**
Cost/test (US$)	36–38/43–51	13–35	72–100	>20	16–45		10–15
Price of instrument	80 000–100 000	248 000 (45 000 + 162 000 + 80 000)	Pricing for the assay and instrument is available from Siemens.	113 000 (95 000+18 645)	Pricing for the assay and instrument is available from Qiagen.	Pricing for the assay and instrument is available from Qiagen.	Pricing for the assay and instrument is available from Qiagen.
# Specimen/run	24 specimens in two hours, but it can process up to 72 samples at one time	96 samples at a time in about three hours of cycling time	89 samples per run with a total time to result of less than six hours	5–6 hours for a run of 24 samples	Continuous loading in batches of up to 24 samples plus internal controls	48 samples can be lined up on 12 racks. DNA tests is approximately 70 minutes and for RNA tests is 110 minutes	First reportable results available within three hours after loading samples and five results after every five minutes thereafter. Samples can be continuously loaded, with up to 120 samples on the Panther® System

Qualitative/Quantitative HCV RNA platforms currently available (1–5) and soon to be available (6–7)



4.8. Dried blot spots

Decision-making tables – PICO 7

Dried blood spots as sample collection method for serology/NAT for HBV/HCV: Among persons identified for (1) hepatitis B, or (2) hepatitis C testing, what is the diagnostic accuracy and impact of detecting HBsAg/HCV Ab or NAT from DBS samples versus venous samples?

1. Topic for analysis: How to test

Population:

- Samples for serology for HBV (HBsAg)
- Samples for serology for HCV (HCV Ab)
- Samples for HBV DNA
- Samples for HCV RNA

Intervention: Using dried blood spot (DBS) samples

Comparison: Using venous samples

Outcomes: Diagnostic accuracy (Sensitivity, specificity, positive predictive value, negative predictive value, TN, TP, FN, and FP)

2. Background:

In high-prevalence of hepatitis B virus (HBV) among low- and middle-income countries (LMICs) there is a need for improved HBV screening, especially in decentralized settings. And the entry of new all-oral direct acting antiviral therapy for hepatitis C provides an opportunity to scale up HCV care in LMICs and dramatically simplify diagnosis and monitoring.

In HIV, use of DBS has facilitated the diagnosis of HIV in children under 18 months and is a promising tool for HIV management in resource-limited settings (viral load monitoring is strongly recommended by WHO as the preferred ART monitoring test). While commercial rapid diagnostic tests (RDTs) exist for HBsAg and HCV antibody for HBV diagnosis and HCV screening, respectively, the use of DBS sent to centralized lab facilities for diagnosis or screening purposes may be useful in certain contexts where RDTs are not available or not feasible due to human resource, procurement, quality, regulatory or other constraints, particularly as they can be prepared from capillary blood, thus obviating the need for phlebotomy services.

Molecular tests for HBV DNA and HCV RNA must currently be performed in centralized facilities, where barriers to sample collection and transport, such as phlebotomy services, plasma separation

and cold chain, may make testing less feasible. One decentralized molecular platform for HCV RNA does exist but it too relies on plasma as a sample type. Thus DBS may be used in a similar way to HIV to facilitate diagnosis and monitoring in certain contexts.

Two main advantages of using DBS for HCV compared to HIV are that (1) there is no proviral DNA to overestimate the quantity of virus in the blood compared to plasma (although cell-associated RNA may still contribute) and (2) qualitative testing should suffice, both for diagnosis and measurement of SVR, as (i) viral load monitoring of DAA-based treatment is not useful and, (ii) since DAA therapy is non-toxic, a log drop calculation for treatment continuation at week 12 (EVR), as was the case for IFN-based therapy, is not needed.

In prioritizing the validation of serological testing on DBS versus NAT testing on DBS, depending on the context, they may be equally useful. If affordable, good-quality RDTs are available that can be performed off capillary blood then the impetus may be to prioritize that validation of NAT testing for HBV DNA and HCV RNA. However, if RDTs are not available then DBS testing may be equally important to increase access to serological testing, for example, for hard-to-reach populations and those with poor venous access. Equally, DBS may be useful where polyvalent screening for multiple diseases, such as HIV/HBV/HCV, is useful and where multiplex RDTs for this purpose are not available or more costly. Thus the choice and combination of test may be context specific whereby different programmes may opt for difference combinations of: (1) DBS serology + DBS NAT (remote settings), (2) RDT serology + DBS NAT (clinics, e.g. antental), or (3) EIA serology + plasma-based NAT (urban settings or more central hospitals).

See Feasibility section for current use of DBS.

3. Draft recommendation(s):

4. Summary and quality of evidence

Summary of results

□ High
□ Moderate
□ Low
□ Very low

7a: Meta-analysis for HBsAg

No. of included studies	10 (SR) 9 (meta-anal)
Total sample size	2481
Overall sensitivity	92.9% [86.2–96.5]
Overall specificity	99.0% [96.2–99.7]

Positive LR	92.9
Negative LR	0.072
Impact of cut-off	DBS LOD cut-off may be higher due to smaller sample volume (50 µL) but OD cut-off lower to maximize sensitivity (ROC curve needed to set cut-off) Std: SN 88% [74–95] SP 98.6% [89–100] High: SN 95.6% [91–98] SP 99.1% [97–100]
Impact of storage	Cold chain: SN 78.7% [70–85] SP 98.6% [68–100] ≥RT: SN 96.1% [92–98] SP 99.7% [98–100]
Impact of duration of storage	Accuracy not affected if RT –33°C for ≤15 d (1 study) or 63 d (another study)
Impact of assay	DBS provides good rule in test for diagnosing HBV but may not be able to rule out HBV in a minority of cases.

LR: likelihood ratio

7b: Meta-analysis for HCV Ab

No. of included studies	18 (SR) 14 (meta-anal)
Total sample size	6120
Overall sensitivity	98% [94–99]
Overall specificity	99% [97–100]
Positive LR	171
Negative LR	0.02
Impact of cut-off	None identified (varied widely, some used ROCs to set own cut-offs)
Impact of storage	–20°C = less variation compared to RT

Impact of duration of storage	Accuracy not affected if RT for ≤3 d (1 study) or ≤6 d (another study) or ≤60 d (another study)
Impact of assay	DBS provides good rule in and rule out test for screening for HCV.

7c: Meta-analysis for HBV DNA

No. of included studies	9 (SR) 7 (meta-anal)
Total sample size	905
Overall sensitivity	96% [91–98]
Overall specificity	100 [54–100]
Positive LR	287
Negative LR	0.04
Impact of threshold	LOD: 914 IU/mL (one study), 3000–4000 IU/mL (other studies) Clinical Tx threshold: 2000 IU/mL
Impact of storage	Not possible to calculate because all accuracy studies at –20°C.
Impact of duration of storage	No affect of accuracy if 4–37°C for ≤7 d (2 studies).
Impact of assay	DBS good to rule in HBV but may not rule out HBV in a minority of cases, particularly if viral loads are <3000 IU/mL.

7d: Meta-analysis for HCV RNA

No. of included studies	9 (SR & meta-anal)
Total sample size	1250
Overall sensitivity	96.0% [93.4–97.6]
Overall specificity	97.7% [94.7–99.0]
Positive LR	41.74
Negative LR	0.041
Impact of threshold	LOD: ≥150–250 IU/mL
Impact of storage	Better result at –20°C compared to RT; conflicting results re deterioration of sample at RT
Impact of duration of storage	Conflicting results re deterioration over time
Impact of assay	DBS good to rule in active HCV infection but may not rule out infection in a minority of cases with lower viral loads.

Quality of evidence

- Overall HBsAg: moderate (no significant indirectness, imprecision or inconsistency but significant risk of bias).
- Overall HCV Ag: moderate (no significant indirectness, imprecision or inconsistency but moderate risk of bias).
- Overall HBV DNA: low (no significant indirectness or imprecision but significant inconsistency and high risk of bias).
- Overall HCV RNA: moderate (no significant indirectness, imprecision or inconsistency but significant risk of bias).

*Refer GRADE table in footnote

5. Risks/Benefits

Benefits of DBS

□ Benefits clearly outweigh harms
□ Benefits and harms are balanced

- DBS is likely stable over time and maintains good accuracy in conditions with higher temperatures and with higher humidity.
- Lower biohazard, easier transport, no venepuncture needed
- Greater access to testing, especially for remote settings or specific programmes servicing key populations (people who inject drugs, prisoners, etc).

Risks of DBS

- Accuracy may be negatively affected when storing for prolonged durations (greater than 14 days) at higher temperatures (room temperature and above) and humidity.
- Use of DBS may require higher cut-offs to determine test positivity as DBS uses a small volume of blood, in order to maintain sensitivity, a higher cut-off may be required as compared to when using plasma samples. This may decrease the ability to rule out HBV/HCV in a minority of cases.
  DBS not regulatory approved as a sample type for HBsAg, HCV Ab, HBV DNA or HCV RNA testing.
- No guidance from manufacturers on use of commercial assays with DBS.
- The best type of filter paper to use is not known (but has been established for HIV DNA and RNA testing).
- Differences between capillary versus venous blood are not known (but has been established for HIV DNA and RNA testing with insignificant difference where training and proficiency testing are provided for capillary blood sampling).
- Most appropriate volume of capillary blood to be used (e.g. one versus more than one spot of 50 µL) is not known (but has been established for HIV DNA and RNA testing).
- Best types of commercial test kits to use with DBS are not known.

□ Potential harms clearly outweigh potential benefits

Are the desirable anticipated effects large?

□ No
□ Probably
□ Uncertain
□ Yes
□ Varies

6. Acceptability, values and preferences

1. Acceptable accuracy of DBS for testing HBsAg and HCV Ab as compared to use of plasma samples (as compared to WHO performance acceptance criteria):

HBsAg

	WHO EIA	WHO RDT	SR	Acceptable
Sensitivity	100	100	92	No
Specificity	≥98	≥98	99	Yes

□ No major variability
□ Major variability

Is the option acceptable

to key stakeholders?

□ No
□ Probably
□ Uncertain

EIA: enzyme immunoassays; SR: systematic review

□ Yes
□ Varies

HCV Ab

	WHO EIA	WHO RDT	SR	Acceptable
Sensitivity	100	≥98	98	Yes
Specificity	≥98	≥97	99	Yes

Accuracy is acceptable except for sensitivity of HBsAg testing, which may lead to a minority of cases being missed.

2. Acceptable accuracy of DBS for testing HBV DNA and HCV RNA as compared to use of plasma samples is not known because there are no WHO performance acceptance criteria. However accuracy should be measured in the context of clinical relevance, i.e.:

For HBV DNA:

1. Treatment thresholds of 2000 and 20 000 IU/mL
2. Ability to measure suppression in treatment monitoring (threshold not known but most people fail at viral loads >20 000 IU/mL).

For HCV RNA:

1. Confirming all those with chronic and active HCV infection (threshold not known but viral loads are generally high, with 95% of people having viral loads >1000 IU/mL if chronically infected and those with lower early viral loads more likely to clear infection).

Following new DAA therapy, confirming those with sustained virological response (SVR), whether at SVR 12/24 (threshold is not known but preliminary evidence suggests those failing therapy have higher viral loads).

7. Equity, ethics and human right implications

□ Less equitable
□ More equitable

Will the recommendation raise questions around equity?

DBS may allow more equitable access to testing.

Are there ethical implications to this recommendation? No.	
8. Resource use and financial implications • May reduce costs associated with sample collection, storage and transport. • May facilitate task shifting to lay workers to decrease human resource limitations.	*Are the resources required small?* □ No □ Probably □ Uncertain □ Yes □ Varies
9. Feasibility and constraints to implementation **10. Relevance to different settings/populations** *Are any major barriers expected for the implementation of this recommendation?* *Will this recommendation be most relevant for particular settings (e.g. endemicity)?* There is limited programmatic experience with the use of DBS for viral hepatitis testing, although this is well established for HIV DNA and RNA testing. 1. Studies are limited. 2. There are no current standards for test OD cut-offs for HBsAg or HCV Ab using DBS. 3. There is a dearth of studies that systematically examine the effects of storage and transport conditions on the accuracy of DBS. Hepatitis B and C testing using DBS have been used in several screening programmes in non-clinical settings and research studies. There is encouraging evidence from pilot schemes and where dried blood spot collection is used for hepatitis testing (detection of HBsAg and HCV Ab). DBS collection is view as an interesting alternative testing technology for peoples at increased risk of infection. For example, hepatitis testing using DBS is used by associations such as "Hepatitis C Trust" in the UK[1] or le "Réseau Hépatites LR" in France,[2] and by community pharmacists in the UK.[3] The "CheckPoint-Paris" from the "Kiosque", a	*Is the option feasible to implement?* □ No □ Probably □ Uncertain □ Yes □ Varies

voluntary counselling and testing service in France, offers rapid tests for screening and DBS for confirmation since 2010.[4] The National Institute for Health and Care Excellence (NICE) points out that DBS tests for hepatitis B and C can be useful in certain settings for people with poor venous access and where there may be no facilities or expertise to take venous blood samples.[3] The NICE recommends access to hepatitis B and C testing using DBS for prisoners and drug users.

In France, guidelines from the "Haute Autorité Sanitaire"[5] and AFEF-ANRS[6] underlined that DBS tests have good performance and are an alternative to venous blood tests. However, the absence of standardization limits the usefulness of DBS and thus there is no clear recommendation to expanded DBS testing.

Critically, as part of Scotland's Action Plan on HCV, "the introduction of DBS testing in the specialist drug services setting has had the greatest impact" in terms of increasing access to diagnosis.

References

1. Health Protection Agency. Hepatitis C in the UK: 2011 report. London: HPA; 2011.
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4. Le kiosque [webpage] (http://www.lekiosque.org/checkpoint, accessed 06 June 2016).
5. Haute Autorité Sanitaire, 2014. Place des tests rapides d'orientation diagnostique (TROD) dans la stratégie de dépistage de l'hépatite C (http://www.has-sante.fr/portail/upload/docs/application/pdf/2014-05/place_des_trod_dans_la_strategie_de_depistage_de_vhc-_rapport.pdf)
6. Prise en charge des personnes infectées par le virus de l'hépatite B ou de l'hépatite C (http://social-sante.gouv.fr/IMG/pdf/Rapport_Prise_en_charge_Hepatites_2014.pdf, accessed 06 June 2016).
7. Scotland's Action Plan on HCV. (http://www.inhsu.com/international-symposia.html.

11. Rationale for recommendation:

12. Strength of recommendation

13. Implementation considerations

- Regulatory approval for DBS as a sample type
- Where HR and phlebotomy services are lacking, training of lay workers to perform fingerprick DBS
- QA/QC; e-health/m-health to improve turnaround time of results
- Training clinicians to act on the result.

14. Research gaps

- Operational research to evaluate the accuracy of DBS using field specimens prepared and stored in real-life conditions will be needed.
- Validation of the use of DBS with commercially available HBsAg and HCV Ab tests and determining cut-off values with the use of ROC curves will be needed.
- The use of DBS for HCV RNA viral load measurement and of the rate of degradation of HCV RNA when stored in DBS at ambient temperatures and high humidity for different time periods will be needed as well as further study in HIV-coinfected patients and for use in treatment monitoring, specifically for measuring SVR 12/24 post DAA therapy.
- A systematic review on the LOD for HCV RNA testing on DBS that will serve to capture (1) everyone with chronic and active HCV infection, and (2) everyone failing DAA therapy at SVR12/24 (which will then inform the best time-point for SVR).
- A systematic review on the LOD for HBV DNA testing on DBS that will serve to capture (1) everyone who needs HBV treatment, and (2) everyone failing therapy.

GRADE Summary of findings for PICO7a

Number of studies	Type of study	Directness	Precision	Consistency	Risk of bias	Overall quality
Sensitivity 92.9% (95% CI 86.2–96.5)						
10 studies (370 HBsAg positive among 1516 samples)	Cross-sectional or cohort	No significant indirectness	No significant imprecision	Significant inconsistency (One paper reported lower sensitivity)	Significant risk of bias (patient enrolment not consecutive or random in some studies; pre-specified cut-off not used in	Moderate

					some studies)	
Specificity 99.9% (95% CI 97.6–100%)						
10 studies (370 HBsAg positive among 1516 samples)	Cross-sectional or cohort	No significant indirectness	No significant imprecision	No significant inconsistency	Significant risk of bias (patient enrolment not consecutive or random in some studies; pre-specified cut-off not used in some studies)	Moderate

DBS: dried blood spot

GRADE Summary of findings for PICO7b

Number of studies	Type of study	Directness	Precision	Consistency	Risk of bias	Overall quality
Sensitivity 98% (95% CI 93.0%–99.0%)						
14 studies (1549 HCV positive among 4304 samples)	Diagnostic accuracy	No significant indirectness	No significant imprecision	Significant inconsistency	Moderate risk of bias (patient enrolment only partly consecutive or random; several case–control studies)	Moderate
Specificity 99% (95% CI 97–100%)						
13 studies (2756 HCV positive among 4304 samples)	Diagnostic accuracy	No significant indirectness	No significant imprecision	Significant inconsistency	Moderate risk of bias (patient enrolment only partly consecutive or random; several case–control studies)	Moderate

GRADE Summary of Findings for PICO7c

Number of studies	Type of study	Directness	Precision	Consistency	Risk of bias	Overall quality
Sensitivity 98% (95% CI 92.0%–98.0%)						
7 studies (154 HBsAg positive among	Diagnostic accuracy	No significant indirectness	No significant imprecision	Significant inconsistency	High risk of bias (patient enrolment not consecutive or random in all studies;	Low

552 samples)					several case–control studies)	
Specificity 100% (95% CI 39–100%)						
4 studies (125 HBV DNA pos positive among 1648 samples)	Diagnostic accuracy	No significant indirectness	Significant imprecision with small sample size	Significant inconsistency	High risk of bias (patient enrolment not consecutive or random in all studies; several case control studies)	Low

GRADE Summary of findings for PICO7d

Number of studies	**Type of study**	**Directness**	**Precision**	**Consistency**	**Risk of bias**	**Overall quality**
Sensitivity of DBS for HCV VL: 96.0% (upper-lower bounds 93.4–97.6)						
9 studies 1335 samples	Cross-sectional, case–control or cohort	No significant indirectness	No significant imprecision	No significant inconsistency	Significant risk of bias (non-randomized or consecutive patient recruitment or case–control design)	Moderate
Specificity of DBS for HCV VL: 97.7% (upper-lower bounds 94.7–99.0)						
9 studies 1335 samples	Cross-sectional, case–control or cohort	No significant indirectness	No significant imprecision	No significant inconsistency	Significant risk of bias (non-randomized or consecutive patient recruitment or case–control design)	Moderate

4.9. Monitoring treatment response HCV

Decision-making tables – PICO 9

Monitoring for treatment response using HCV Ag testing in individuals with confirmed active HCV infection: Among individuals receiving antiviral treatment for HCV, what is the diagnostic accuracy of HCV core antigen versus NAT for HCV RNA qualitative detection (and/or) quantification to confirm successful treatment response with viral clearance?

Population: Patients receiving treatment for HCV

Intervention: HCV core antigen assay

Comparison: NAT for HCV RNA detection (and/or) quantification

Outcomes: Diagnostic accuracy (Sensitivity and specificity, TN, TP, FN, and FP)

1. **Background:**

- HCV core antigen (HCV cAg) testing was developed as an alternative to NAT for diagnosis of active HCV infection. HCV nucleocapsid peptides 22 (p22) are released into plasma during viral assembly and can be detected throughout the course of HCV infection.
- Detection of HCV viraemia is also important during treatment of chronic HCV infection.
- Current guidelines recommend verification of virological activity pre-treatment with the measurement of a baseline HCV RNA quantitative measurement (viral load) by NAT. For interferon-based treatments, HCV RNA viral load is assessed at week 4 of therapy for the "rapid viral response" (RVR) to help predict efficacy of therapy, and repeated at week 6 if elevated at week 4 to see further viral response and guide whether treatment should be continued.
- NAT for HCV RNA is performed again at week 12 (early viral response, EVR), at the end of treatment, and 12 and 24 weeks after therapy is completed to test for cure, "sustained viral response" (SVR).
- New, direct-acting antivirals (DAA) have made treatment for HCV much easier with oral rather than parenteral administration and shorter, more effective regimens that are likely to be easier to adhere to making access to affordable diagnostic and monitoring assays even more important. However, it is important to note that, ultimately, treatment monitoring may not be required with the routine use of DAAs.
- This PICO addresses the question of whether HCV cAg can be used as a tool for assessing response to treatment for HCV infection.

2. Draft recommendation(s):

3. Summary and quality of evidence

A systematic review (see SR_PICO 9) was commissioned to address the above PICO question. This aimed to examine the utility of HCV cAg monitoring for those on HCV treatment (PICO 9).

□ High
□ Moderate
□ Low
□ Very low

Summary of results

Sensitivity and specificity of Abbott ARCHITECT HCV cAg assay compared to HCV RNA assessed at baseline, at week 4 of interferon-based therapy (early viral response), and at week 24 after completion of treatment (sustained viral response)

		Baseline		**Early viral response (EVR)**		**Sustained viral response (SVR)**	
Author, Year	*N*	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
Feng, 2014	32	100%	N/A	100%	88.9% (68.4%, 100%)	100%	100%
Loggi, 2013	35	100%	N/A	73.5% (58.7%, 88.4%)	100%	100%	94.1% (82.9%, 100%)
Moscato, 2010	23	N/A	N/A	100%	70% (41.6%, 98.4%)	N/A	N/A

N: number of subjects; HCV: hepatitis C virus, Ag: antigen, Se: sensitivity, Sp: specificity, CI: confidence interval, N/A: not applicable as cannot be calculated from study data

Sensitivity and specificity of HCV core antigen in prediction of sustained viral response (SVR) after initiation of interferon-based treatment

Author, Year	*N* (N to achieve SVR)	Index test	Timing of test after treatment start	Change in HCVcAg	Sensitivity	Specificity
Feng, 2014	32 (21)	Abbott ARCHITECT	6 days	Log 10	95.2%	70%
Loggi, 2013	90 (57)	Fujirebio Lumipulse	7 days	Absolute	79.4%	88.5%
Moscato, 2010	44 (10)	Fujirebio Lumipulse	7 days	Absolute	57.1%	93.3%

HCV: hepatitis C virus; *N*: number

Conclusions:

- HCV core Ag assays can have high sensitivity (up to 93.4% for certain commercialized assays), high specificity, and good correlation with HCV RNA to a detection limit of roughly 3000 IU/mL.
- The data on HCV core Ag for treatment monitoring and as a test of cure is too limited to reach reliable conclusions.

Quality of evidence

*Refer GRADE table in footnote

4. Risks/benefits

Benefits

- HCV cAg testing by immunoassay format has the potential to be less costly and less complicated to perform than HCV RNA by NAT. However, these immunoassays still require sophisticated laboratory equipment and therefore skilled staff to operate. Access to cold storage and constant electricity is required for the current types of assays available for HCV cAg testing.
- Results for patients on antiviral treatment being monitored for sustained viral response may be available more rapidly as a result of decentralized testing.

□ Benefits clearly outweigh harms
□ Benefits and harms are balanced
□ Potential harms clearly outweigh potential benefits

Are the desirable anticipated

Risks • Due to reduced analytical sensitivity and limited understanding of kinetics of HCV cAg compared to HCV RNA by NAT, individuals on antiviral treatment may be misclassified as responding to treatment, but may have persisting viraemia below the limits of detection of the assay.	*effects large?* □ No □ Probably □ Uncertain □ Yes □ Varies
5. Acceptability, values and preferences A values and preferences survey of implementers and users of hepatitis B and C testing services was carried out by FIND in September 2015. A total of 104 respondents from 43 (20 high-income, 23 low- and middle-income) countries participated. Relating to this PICO, • As assay (platform) for detection of HCV cAg is available in India, Indonesia, Former Yugoslav Republic of Macedonia, Viet Nam, Turkey. The platform is available in South Africa but not currently being used for HCV cAg detection. • Currently only 11% of respondents are using HCV cAg as a test of cure. • 44% of respondents preferred a 12-week follow up for testing after completion of therapy, while 19% and 15% preferred a 4- or 8-week follow up. • 47% of patients preferred to have the same test for monitoring and detection, ideally in decentralized settings. • Free text comments from respondents included concerns regarding the sensitivity and specificity of cAg, but that it was potentially easier to do. One comment stated that it could be acceptable if it increased access to treatment. • A larger number of respondents felt that the cost of testing for HCV RNA by NAT was considered more of a barrier than that of HCV cAg. • As stated previously, 47% of respondents in low- and middle-income countries would prefer testing at POC, even at the cost of sensitivity. **Patients:** • Patients at risk of progressive liver disease will benefit from reduced disease progression and related mortality and morbidity, if treatment is provided as a result of wider access to testing programmes. **Community:** • To identify the individuals who require assessment and treatment would be an effective use of resources. • As testing and treatment programmes are scaled up, the numbers developing	□ No major variability □ Major variability Is the option acceptable to key stakeholders? □ No □ Probably □ Uncertain □ Yes □ Varies

progressive disease and serious outcomes (HCC and complications of advanced liver disease), premature morbidity and mortality within the community will be reduced, and so also the burden of disease to societies where the disease is most prevalent. **Health-care workers:** • Appropriate use of resources to channel treatment to patients with higher risk of complication in the medium- and short term • Will require training in the use of testing equipment if being used in the near-POC setting • Appropriate reporting and recording of results. **Laboratory:** • Will require purchasing of the appropriate platform and reagents for the HBsAg detection assay Will require training for HCV cAg test: careful sample processing is necessary for HCV cAg assay to lyse viral particles, expose antigen and dissociate antibody from antigen and optimize the detection for HCVcAg.	
6. Equity, ethics and human right implications *Will the recommendation raise questions around equity?* • Equity will improve as a result of decentralization of testing, however, still improvement of access to the testing facilities is necessary. • Regional and country variability in access to treatment. *Are there ethical implications to this recommendation?* • Ethical consideration for the possibility that WHO could recommend a suboptimal testing strategy.	□ Less equitable □ More equitable
7. Resource use and financial implications **Materials/equipment:** • Cost of testing platform and reagents • Other laboratory consumables	*Are the resources required small?* □ No □ Probably □ Uncertain

Training and supervision:

- Appropriate training of laboratory staff
- Quality control programmes
- If using near-POC assays, appropriate training of testing providers.

Other:

- Cost of transportation of specimens to the laboratory

Possible procurement costs:

Assay format	Indicative cost (US$) per test	Source
RDTs	0.50–2.00 (10 for oral fluid RDTs)	MSF, WHO
EIA	0.50–1.70	WHO
HCV Ag	25–50	MSF
Quantitative NAT for HCV RNA	10–45	MSF, UNITAID
Qualitative NAT for HCV RNA)	43–51	UNITAID

□ Yes
□ Varies

- **Feasibility and constraints to implementation**

Are any major barriers expected for the implementation of this recommendation?

- Regional and country variability in access to treatment and procurement of testing equipment and services
- With regard to HCV cAg, availability of a local laboratory, which is able to procure the testing platform and reagents required for testing.

Is the option feasible to implement?

□ No
□ Probably
□ Uncertain
□ Yes
□ Varies

8. Relevance to different settings/populations

Will this recommendation be most relevant for particular settings (e.g. endemicity)?

- HCV cAg testing will be more relevant to populations that presently rely on centralized laboratory testing for HCV RNA for confirmation of status.
- The recommendations are less likely to be relevant in high-income settings where there is already access to established hepatitis C testing and treatment programmes.

9. Rationale for recommendation:

10. Strength of recommendation

11. Implementation considerations

Optimize test for asymptomatic patients in primary-care settings or in the community where the HCV endemic is high.

12. Research gaps

- The kinetics of HCV cAg with treatment needs to be evaluated further, particularly in the context of new DAA regimens.
- More rigorous assessment of covariates is required in studies assessing HCV cAg or NATs for treatment monitoring or as a test of cure, such as HIV or HBV coinfection or genotype.
- Is treatment monitoring and/or confirmation of cure necessary with DAA regimens? If so, what would be the optimal timing of testing?
- When is the best time-point to test for cure with HCV core Ag?
- Development of muliplex instrument with other disease diagnosis such as HIV, HBV, and TB at health centre.
- HCVcAg assay to detect the variants of HCV.

GRADE Summary of findings

SR outcome 1: Diagnostic accuracy at SVR	1.1.16	1.1.17	1.1.18	Quality				Effect*	Strength of evidence
Index test	**Outcome measure**	**# Studies (# samples)**	**Design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**1.1.19**	**1.1.20**
Abbott ARCHITECT HCVAg Assay	Sensitivity	2 (67)	RCT, cohort	Low[1]	Low[2] 1.1.21	Moderate[3] (–1)	Low[4] 1.1.22	100%*	Moderate ⊕⊕⊕◯
	Specificity	2 (67)	RCT, cohort	Low[1]	Moderate[2] (–1)	Moderate[3] (–1)	Low[4] 1.1.23	94–100%*	Low ⊕⊕◯◯

SR outcome 2: Predictive accuracy of SVR				Quality				Effect*	Strength of evidence
Index test	**Outcome measure**	**# Studies (# individuals)**	**Design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**		
Abbott ARCHITECT HCV Ag Assay	Sensitivity	1 (23)	Cohort	Low[1]	NA[2] (–1)	Moderate[3] (–1)	NA[4]	95.2%**	Low ⊕⊕◯◯
	Specificity	1 (23)	Cohort	Low[1]	NA[2] (–1)	Moderate[3] (–1)	NA[4]	70%**	Low ⊕⊕◯◯
Fujirebio Lumipulse Ortho HCV Ag	Sensitivity	2 (134)	Cohort	Moderate[1] (–1)	Moderate[2] (–1)	Moderate[3] (–1)	Moderate[4] (–1)	57.1–79.4%*	Very low ⊕◯◯◯
	Specificity	2 (134)	Cohort	Moderate[1] (–1)	Moderate[2] (–1)	Moderate[3] (–1)	Moderate[4] (–1)	88.5–99.3%*	Very low ⊕◯◯◯

* Results reported are a range across studies or **individual result, NA: not applicable

References

1. Feng B, Yang RF, Xie Q, Shang J, Kong FY, Zhang HY et al. Hepatitis C virus core antigen, an earlier and stronger predictor on sustained virological response in patients with genotype 1 HCV infection. BMC Gastroenterol. 2014;14:47.
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4.10. Intervention to promote linkage to care

Decision-making tables – PICO 10

Interventions to optimize uptake of hepatitis testing and linkage to care across the viral hepatitis treatment cascade

17. Topic for analysis

Population: Individuals living with chronic hepatitis B or C (diagnosed or undiagnosed) or providers caring for these patients

Intervention: Psychosocial or structural interventions delivered in conjunction with screening, care, or treatment of hepatitis

Comparison: Standard of care or no intervention

Outcomes: Retention and progression along the continuum of care

18. Background:

Globally, 250 million people are chronically infected with hepatitis B virus (HBV), and 80–140 million are infected with chronic hepatitis C virus (HCV). Viral hepatitis is now responsible for 1.45 million deaths every year and is the seventh leading cause of mortality worldwide. Chronic HBV and HCV are responsible for over 90% of these deaths. But therapeutic advances are rapidly changing clinical management of both HBV and HCV infection, especially HCV infection is increasingly curable.

Reaping the clinical benefits of novel HBV and HCV therapies will require a continuum of care that start with screening and ultimately reaches and sustains viral suppression (Fig. 1). Similar to the HIV continuum of care, each step of the HBV/HCV continuum of care necessarily requires all prior steps and must be maintained over time. Screening is the critical entry way into the continuum and allows positive individuals to proceed and negative individuals to receive vaccination in the case of HBV. However, several barriers to screening have been reported, such as lack of knowledge, awareness and/or denial, lack of access to the infrastructure where screening is available, cultural beliefs, financial conditions including health insurance status, fear of blood taking and stigmatization as a patient aspects, and lack and gaps of awareness of risk factors, knowledge, experience of patient management, lack of infrastructure for testing and lack of access to treatment centres as a health-care aspect. Interventions can enhance chronic viral hepatitis management as part of each step along the continuum of care, including screening, linkage to care, treatment uptake, adherence, and viral suppression.

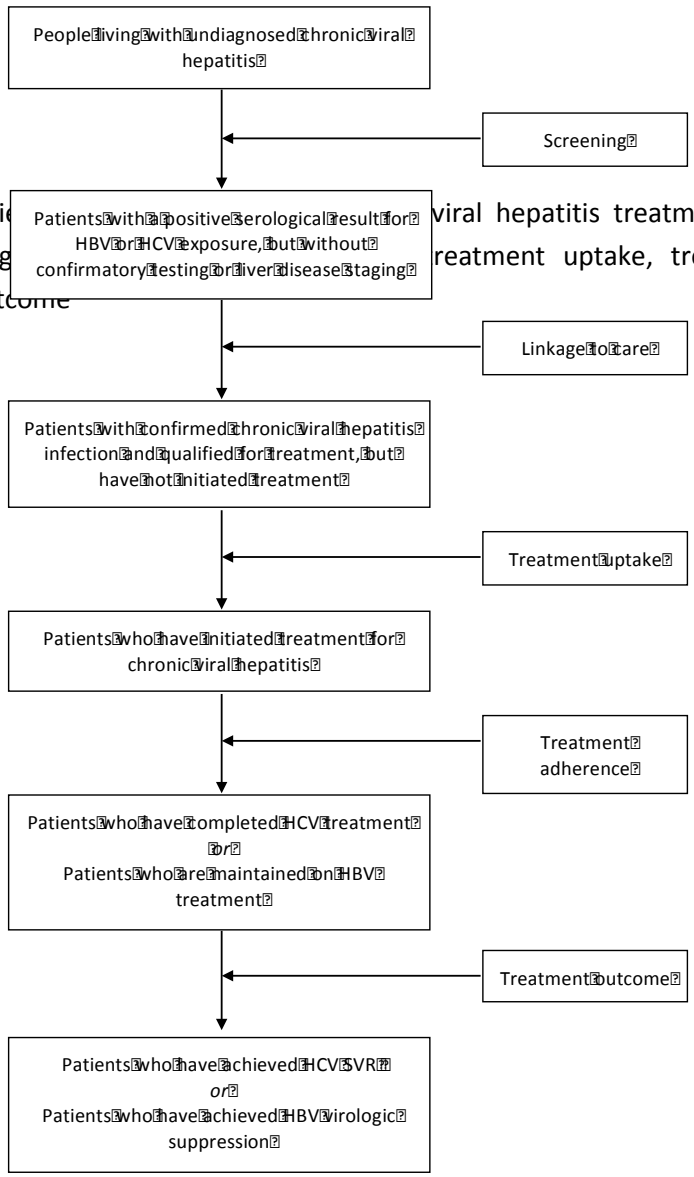


Fig. 1. Overvie viral hepatitis treatment continuum, including testing, linkag reatment uptake, treatment adherence, and treatment outcome

19. DRAFT recommendation(s):

20. Summary and quality of evidence

Summary of results

Evidence from the systematic review for linkage to care for HIV ARV GL 2015: among people living with HIV, what interventions facilitate linkage to care compared to standard of care? (PICO F.2.1)

Category	Summary	Quality
1. Counselling and support (*n* = 14)	Most interventions improved linkage to and engagement in care but not ART initiation	3 moderate, 6 low, 5 very low
2. Incentives (*n* = 4)	Mixed: one intervention worked, two did not	1 moderate, 2 low, 1 very low
3. Quality improvement (*n* = 7)	Most interventions increased outcomes targeted;	2 moderate, 5 very low

□ High
□ Moderate
□ Low
□ Very low

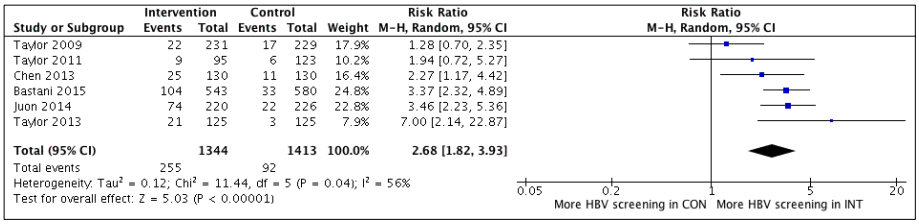
	interventions in category were multifaceted and incorporated interventions from other categories	

Evidence from the systematic review for linkage to care for hepatitis B and C testing GL:

We conducted a systematic review to identify interventions that improve the continuum of care in adults with chronic hepatitis B and C infection, quantify the effect size of these interventions, and recognize gaps in knowledge in interventional studies that target the chronic viral hepatitis continuum of care.

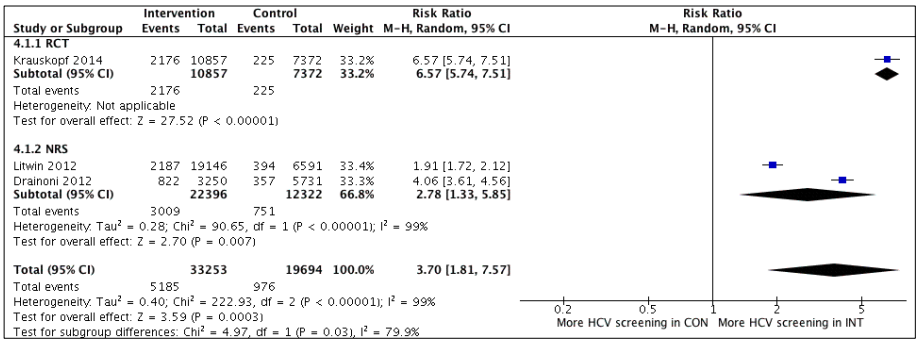
I. Meta-analyses for interventions to improve HBV screening

Single culturally tailored lay health worker educational session to improve HBV knowledge and promote testing vs no or unrelated educational session for self-reported HBV screening.



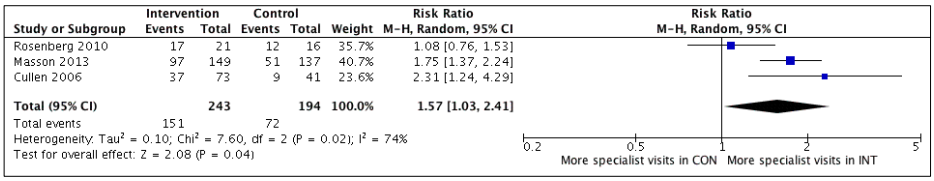
II. Meta-analyses for interventions to improve HCV screening

Clinician reminder to use HCV screening algorithm during clinical visit with or without supplementary provider education vs no clinician reminder for HCV screening.

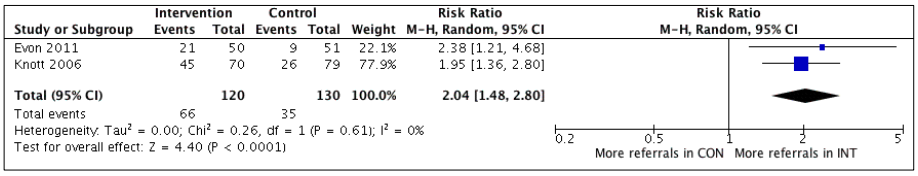


III. Meta-analyses for interventions to improve HCV linkage to care

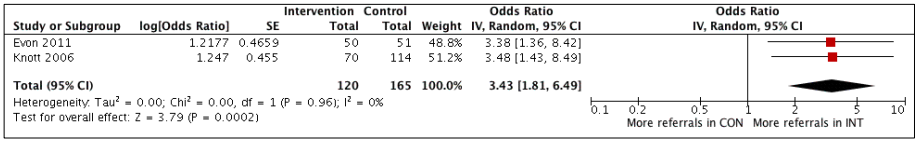
Facilitated referral and scheduling to specialist visit by staff at site of established care with or without supplementary HCV education and post-test counselling vs no facilitated referral for attendance at HCV specialist visit.



Individually tailored mental health counselling and motivational therapy for HCV^{+} patients with mental health and/or substance use comorbidities vs usual care for physician referral to initiate treatment.



Unadjusted results



Adjusted results

Results of educational interventions data from a systematic review:

For HBV

- Targeted population was HBV non-infected but at-risk individuals.
- Educational interventions increased: knowledge about the disease, HBsAg testing and HBV vaccination rates.

For HCV

- 50% of studies targeted HCV non-infected and the rest were HCV-infected individuals.
- Educational interventions increased: knowledge about the disease, the number of anti-HCVAb testing, willingness to undergo therapy, and treatment adherence.

Ref: Shah HA1, Abu-Amara M. Education provides significant benefits to patients with hepatitis B virus or hepatitis C virus infection: a systematic review. Clin Gastroenterol Hepatol. 2013;11(8):922–33. **Quality of evidence** *Refer GRADE table in footnote	
21. Risks/Benefits **Benefits of peer support, clinician reminder, and quality improvement initiatives/integration** • Increasing the rate for hepatitis B and C screening will increase the opportunity to link infected individuals to further hepatitis care and treatment. • The infected individuals will be offered health-protection advice to prevent disease progression and transmission. • Increase HCV treatment initiation, improve treatment completion, and increase SVR. **Risks** • Stigmatization by identifying HBV and/or HCV infection • Might be a challenge to differentially incentivize for receiving hepatitis care where poverty is prevalent and the rest of the population have limited access to health services in general. There are few studies on quality improvement initiatives/integration dealing with HBV.	□ Benefits clearly outweigh harms □ Benefits and harms are balanced □ Potential harms clearly outweigh potential benefits *Are the desirable anticipated effects large?* □ No □ Probably □ Uncertain □ Yes □ Varies
22. Acceptability, values and preferences **Counselling and peer support** • Counselling is recognized as standard of HIV-testing services and is also an essential intervention to support adherence. Although counselling for hepatitis testing services has not been established, simple educational interventions for lay health-care workers (HCWs) require little training, generate minimal costs, are highly feasible, and may substantially scale up HBV screening.	□ No major variability □ Major variability Is the option acceptable to key stakeholders?

Clinician reminder • HCWs will need to understand the strengths and limitations of appropriately counsel patients who are screened. • HCWs will need to aware the importance of reminder to enhance hepatitis screening during medical consultations. **Quality improvement initiatives/integration** • Coordinated mental health/substance abuse services within HCV service delivery will require new training and systems; however, linkage to initiation for HCV treatment, improved HCV treatment completion, and increased SVR will increase the rate of cure and prevent further HCV transmission.	□ No □ Probably □ Uncertain □ Yes □ Varies
23. Equity, ethics and human right implications **Counselling and peer support** *Will recommendation raise questions around equity?* • No. Increased rate for hepatitis B and C screening will increase equity to access for further hepatitis care and treatment, especially in resource-limited settings. *Are there ethical implications to this recommendation?* • No major concerns. **Clinician reminder** *Will recommendation raise questions around equity?* • No; increase in rate for hepatitis B and C screening will increase equity to access for further hepatitis care and treatment, especially in resource-limited settings. • Might be a challenge to differentially send clinician reminder to patients for receiving hepatitis care where poverty is prevalent and the rest of the population has limited access to health services in general. • Linkage to care of at-risk populations, e.g. antenatal clinics, tohose who attend drug-rehabilitation clinics, prisoners might be a challenge.	□ Less equitable □ More equitable

Are there ethical implications to this recommendation? • No major concerns. **Quality improvement initiatives/integration** *Will recommendation raise questions around equity?* • Some quality improvement initiatives may have a broader benefit for the health system. *Are there ethical implications to this recommendation?* • No major concerns.	
24. Resource use and financial implications **Counselling and peer support** • As testing and treatment programmes are scaled up, the numbers developing progressive disease and serious outcomes (HCC and complications of advanced liver disease), premature morbidity and mortality within the community will be reduced, and so also the burden of disease to societies where the disease is most prevalent. **Clinician reminder** • Creation of a new proper patient database in which the clinician reminder to prompt HCV testing require attention could be recorded might be costly; however, no extra cost is required to expand facility-based HCV screening in settings that have electronic records or analogous reminder systems. **Quality improvement initiatives/integration** • Variable and context specific, detailed costing of each intervention is not done	*Are the resources required small?* □ No □ Probably □ Uncertain □ Yes □ Varies
25. Feasibility and constraints to implementation *Are any major barriers expected for the implementation of this recommendation?* <u>Counselling and peer support and clinician reminder</u>	1.1.24 *Is the option feasible to implement?*

Feasible. • Simple educational intervention and clinician reminder, either electronic records or analogous reminder are highly feasible. <u>Quality improvement initiatives/integration</u> Feasible. • Coordinated mental health/substance abuse services within HCV service delivery will require involvement of stakeholders.	□ No □ Probably □ Uncertain □ Yes □ Varies
26. Relevance to different settings/populations *Will this recommendation be most relevant for particular settings (e.g. endemicity)?* • These recommendations will increase the opportunity of hepatitis screening and will be relevant to any circumstances.	
27. Rationale for recommendation:	
28. Strength of recommendation	
29. Implementation considerations • Establish linkages and referral pathways for the individuals infected with hepatitis viruses and suffering from mental health problems. • Ensure support from management and from service providers.	
30. Research gaps • Scientific implementation research, especially in low- and middle-income countries, is urgently needed to inform optimize chronic viral hepatitis service delivery systems. • Costing studies need to be performed.	

GRADE Summary of Findings

Evidence from the systematic review for linkage to care for HIV ARV GL 2015: interventions providing counselling and support (GRADE)

# (type) studies	Risk of:				N intervention (control)	Risk intervention (control)	Effect	Quality
	Bias	Inconsistency	Indirectness	Imprecision				
Linkage to care: trials								
1 (individual)	Not serious	Not serious	Not serious	Serious	200 (200)	0.67 (0.38)	RR 1.8 (1.4–2.1)	Moderate[1]
Linkage to care: observational								
7 (6 cohort, 1 other)	Serious	Not serious	Serious	Not serious	5271 (7195)*	0.81 (0.64)*	RR 1.25 (1.22–1.28)*	Very low[2]
Engagement in care: trials								
1 (individual)	Not serious	Not serious	Not serious	Not serious	188 (191)	0.92 (0.83)	pRR 1.1 (1.03–1.20)	Low[3]
Engagement in care: observational								
1 (Pre/post)	Serious	Serious	Serious	Not serious	1147 (1210)	0. 57 (0.45)	RR 1.28 (p<0.0001)	Low
ART initiation: trials								
2 (individual)	Not serious	Not serious	Not serious	Serious	806 (719)*	0.41 (0.43)*	RR 0.95 (0.84–1.07)*	Moderate[1]
PMTCT: trials								
1 (individual)	Not serious	Not serious	Not serious	Serious	197 (181)	0.64 (0.53)	aHR 1.39 (1.01–1.91)	Moderate[1]
PMTCT: observational								
1 (cohort)	Not serious	Not serious	Not serious	Serious	63 (332)	0.54 (0.25)	aOR 3.18 (1.76–5.73)	Low

Interventions offering incentives (GRADE)

# (type) studies	Risk of:				N intervention (control)	Risk intervention (control)	Effect	Quality
	Bias	Inconsistency	Indirectness	Imprecision				
Linkage to care: trials								
2 (1 individual, 1 cluster)	Not serious	Not serious	Not serious	Serious	60 (33)*	0.82 (0.48)*	RR 1.68 (1.16–2.44)*	Moderate[1]
Engagement in care: observational								
1 (cohort)	Serious	Not serious	Serious	Serious	100 (80)	0.94 (0.98)	2.00 (0.41–9.64)	Very low[1]

ART initiation: trials								
1 (individual)	Serious	Not serious	Not serious	Serious	60 (60)	0.45 (0.26)	aHR 2.93 (1.39–6.20)	Low[2]

Interventions introducing quality improvement (GRADE)

# (type) studies	Risk of:				N intervention (control)	Risk intervention (control)	Effect	Quality
	Bias	Inconsistency	Indirectness	Imprecision				
ART initiation: trials								
1 (1 cluster)	Not serious	Not serious	Not serious	Not serious	5390 (3862)	0.68 (0.62)	RR 1.24 (0.88–1.73)	Moderate[1]
Engagement in care: trials								
1 (1 cluster)	Not serious	Not serious	Not serious	Not serious	5390 (3862)	0.62 (0.58)	RR 1.1 (1.04–1.16)	High
PMTCT (ART initiation): observational								
3 (3 pre-post)	Serious	Not serious	Serious	Serious	619 (1296)*	0.36 (0.1)*	RR 3.48 (2.87– 4.22)*	Very low[3]
PMTCT (EID access): observational								
1 (1 pre-post)	Serious	Not serious	Serious	Serious	63 (332)	0.54 (0.25)	aOR 3.18 (1.76– 5.73)	Very low[2]
PMTCT (Receipt of AZT): trials								
1 (1 pre-post)	Serious	Not serious	Serious	Not serious	1258 (776)	0.87 (0.71)	RR 1.22 (1.16–1.28)	Very low[2]

Evidence from the systematic review for linkage to care for hepatitis B and C testing GL:

I. Meta-analyses for interventions to improve HBV screening

Quality assessment							No of patients		Effect		Quality
No. of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Single LHW educational session**	**No or unrelated educational session**	**Relative (95% CI)**	**Absolute (95% CI)**	
HBV screening											
6	Randomized trials	Serious[1]	Not serious	Not serious[2]	Not serious[3]	None	255/1344 (19.0%)	92/1413 (6.5%)	RR 2.68 (1.82–3.93)	109 more per 1000 (from 53 more to 191 more)	⨁⨁⨁◯ Moderate
								6.6%		110 more per 1000 (from 54 more to 192 more)	

1. 6/6 studies are at high risk of detection bias because the outcome was self-reported HBV screening 6 months post intervention. 5/6 studies are at high risk of attrition bias because the ratio of participants with missing data to participants with HBV screening outcome was high (>1.0).
2. Although all included studies involved Asian immigrants in North America, this was not judged to be a significant enough difference in populations to downgrade because the intervention strategies are not exclusive to Asian immigrant populations.
3. The confidence interval is not wide. The OIS was calculated to be 222, and the pooled sample size exceeded the OIS. 3/6 included studies were cluster RCTs, none of which performed analyses that accounted for clustering. Consequently, this meta-analysis commits a unit-of-analysis error and produces over-precise results. Additionally, no ICC were reported in the included studies, so statistical methods could not be used to reduce the effective sample size of the cluster RCTs. Despite this limitation, it is unlikely proper adjustment for cluster design would significantly impact the precision of the pooled results.

II. Meta-analyses for interventions to improve HCV screening

Quality assessment							No. of patients		Effect		Quality
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Clinical testing reminder**	**No reminder**	**Relative (95% CI)**	**Absolute (95% CI)**	
HCV screening											
3	Other design[1]	Serious[2]	Serious[3]	Not serious	Serious[4]	None[5]	5185/33253 (15.6%)	976/19694 (5.0%)	**RR 3.70** (1.81–7.57)	134 more per 1000 (from 40 more to 326 more)	⊕◯◯◯ Very low
								6.0%		161 more per 1000 (from 48 more to 393 more)	

1. This meta-analysis includes 1 cluster RCT and 2 NRS.
2. Drainoni (2012) is at high risk of performance bias and did not employ methods to adjust for confounding potentially introduced by its non-randomized study design. Krauskopf (2014) did not report comparability of randomized clusters and therefore was at high risk of bias.
3. All included studies report a risk ratio >1.0. However, I^2 = 99%. The high degree of heterogeneity may be due to differences between HCV screening algorithms used in each intervention.
4. Although the pooled sample size exceeds the calculated OIS, the confidence interval is wide. Additionally, Krauskopf (2014) was a cluster RCT that did not account for clustering in its analysis. Consequently, this meta-analysis commits a unit-of-analysis error and produces over-precise results. No ICC was reported, so statistical methods could not be used to reduce the effective sample size of the cluster RCT.
5. All included studies report a risk ratio >2.0. However, the pooled results have not been upgraded for large effect because the non-randomized design of 2/3 studies introduces a significant possibility of confounding.

III. Meta-analyses for interventions to improve HCV linkage to care

Quality assessment							No. of patients		Effect		Quality
No. of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Facilitated referral to specialist visit at site of established care**	**No facilitated referral**	**Relative (95% CI)**	**Absolute (95% CI)**	
Attended HCV specialist visit											
3	Randomized trials	Not serious[1]	Serious[2]	Not serious	Not serious[3]	None	151/243 (62.1%)	72/194 (37.1%)	**RR 1.57** (1.03–2.41)	212 more per 1000 (from 11 more to 523 more)	⨁⨁⨁◯ Moderate
								37.2%		212 more per 1000 (from 11 more to 525 more)	

1. Rosenberg (2010) relied on self-reported HCV status and self-reported attendance to an HCV specialist visit, putting the study at high risk of detection bias. However, because this study had a relatively small sample size it was not judged to put the entire meta-analysis at high risk of bias.
2. I^2 = 85%. This high degree of heterogeneity may be due to differences between the intensity of interventions in the included studies.
3. The confidence interval is not wide. The OIS was calculated to be 124, and the pooled sample size exceeded the OIS.

References

1. Drainoni ML, Litwin AH, Smith BD, Koppelman EA, McKee MD, Christiansen CL et al. Effectiveness of a risk screener in identifying hepatitis C virus in a primary care setting.Am J Public Health. 2012;102(11):e115–21.
2. Krauskopf K, Kil N, Sofianou A, Toribio W, Lyons J, Singer M et al. Evaluation of an electronic health record prompt for hepatitis c antibody screening of baby boomers in primary care – a cluster randomized control trial. J Gen Intern Med2014;29:S88–S89.
3. Rosenberg SD, Goldberg RW, Dixon LB, Wolford GL, Slade EP, HImelhoch S et al. Assessing the STIRR model of best practices for blood-borne infections of clients with severe mental illness. Psychiatr Serv. 2010;61(9):885–91.

Individually tailored mental health counselling and motivational therapy for HCV+ patients with mental health and/or substance use comorbidities vs usual care for physician referral to initiate treatment

Quality assessment							No. of patients		Effect		Quality
No. of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Individually tailored mental health counselling and motivational therapy**	**Usual care**	**Relative (95% CI)**	**Absolute (95% CI)**	
Physician referral to initiate treatment											
2	Other design[1]	Serious[2]	Not serious	Not serious[3]	Not serious[4]	None	66/120 (55.0%)	35/130 (26.9%)	**RR 2.04** (1.48–2.80)	280 more per 1000 (from 129 more to 485 more)	⨁◯◯◯ Very low
								(25.3%)		263 more per 1000 (from 121 more to 455 more)	
Adjusted physician referral to initiate treatment											
2	Other design [1]	Serious [5]	Not serious	Not serious [3]	Serious[6]	None	–/120	–/165	**OR 3.43** (1.81–6.49)	0 fewer per 1000 (from 0 fewer to 0 fewer)	⨁◯◯◯ Very low

1. Evon (2011) is a RCT, while Knott (2006) is an NRS.
2. Knott (2006) is at high risk of detection bias because the outcome was subjective and determined by the physician overseeing treatment who was not blinded. Unadjusted results from Knott (2006) were used in this meta-analysis that did not employ methods to adjust for confounding potentially introduced by its non-randomized study design.
3. The decision to not downgrade for indirectness assumes guidelines are applied to other contexts where mental health or substance use comorbidities are also contraindications to recommending HCV+ patients for treatment.
4. The confidence interval is not wide. The OIS was calculated to be 94, and the pooled sample size exceeded the OIS. Knott (2006) is at high risk of detection bias because the outcome was subjective and determined by the physician overseeing treatment who was not blinded.
5. The confidence interval for the pooled adjusted outcomes is wide.

References

4. Evon DM, Simpson K, Kixmiller S, Galanko J, Dougherty K, Golin C et al. A randomized controlled trial of an integrated care intervention to increase eligibility for chronic hepatitis C treatment. Am J Gastroenterol. 2011;106(10):1777–86.
5. Knott A, Dieperink E, Willenbring ML, Heit S, Durfee JM, WIngert M et al. Integrated psychiatric/medical care in a chronic hepatitis C clinic: effect on antiviral treatment evaluation and outcomes. Am J Gastroenterol. 2006;101(10):2254–62.

ANNEX 5: Systematic reviews and evidence summaries

5.1 SR who to test HBV: Literature review on cost-effectiveness of HBV screening, treatment strategies and applicability to LMICs
5.2 SR who to test HCV: Literature review on cost-effectiveness of HCV screening, treatment strategies and applicability to LMICs
5.3 SR1 How to test HBV: Diagnostic accuracy of tests to detect hepatitis B surface antigen: a meta-analysis and review of the literature
5.4 SR2 How to test HCV: Diagnostic accuracy of tests to detect hepatitis C antibody: a meta-analysis and review of the literature
5.5 SR3 How to test HBV: Diagnostic strategies for hepatitis B surface antigen detection: a meta-analysis and review of the literature
5.6 SR4 How to test HCV: Diagnostic strategies for hepatitis C antibody detection: a meta-analysis and review of the literature
5.7 SR6 How to test: Diagnostic accuracy of HCV RNA tests to detect active HCV infection: a meta-analysis and review of the literature
5.8 SR5a and 9 treatment monitoring HCV: HCV core antigen testing for presence of active HCV infection and monitoring for treatment response and cure: a systematic review
5.9.1 SR7a: Dried blood spots as a sample collection method for hepatitis B surface antigen serological testing
5.9.2 SR7b: Dried blood spots as a sample collection method for HBV DNA
5.9.3 SR7c: Dried blood spots as a sample collection method for hepatitis C virological testing: a systematic review and meta-analysis
5.9.4 SR7d: Dried blood spots as a sample collection method for HCV antibody: a systematic review and meta-analysis
5.10 SR 8: Diagnostic accuracy of HBsAg/HBeAg test versus NAT to confirm successful treatment response: a meta-analysis and review of the literature
5.11 SR Interventions to linkage to care - to optimize the chronic viral hepatitis care continuum: a systematic review and meta-analysis of interventions to improve hepatitis B and C screening, linkage to care, treatment uptake, treatment adherence, and viral suppression

Annex 5.1

PICO 1 - Who to test (HBV)

Literature review on cost-effectiveness of HBV screening, treatment strategies and applicability to LMICs

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1. Background

Hepatitis B virus (HBV) infection is highly prevalent worldwide, with a disproportionately high burden in low- and middle-income countries (LMICs).[1] There is mounting evidence regarding the efficacy of antiviral therapy in the reduction of disease progression to cirrhosis and hepatocellular carcinoma (HCC). However, this impact is not fully translated into practice as many people still remain unaware of their infection status, even in high-income countries (HICs),[2–4] and this value is likely to be even lower in LMICs. For example, in the Gambia, only 0.4% of screening participants in PROLIFICA had been tested in the past. Wilson and Jungner criteria have been used to assess whether a disease should screened.[5] However, despite fulfilling most of these criteria, screening for HBV is not performed systematically. The reasons surrounding this are likely multifactorial, including lack of awareness at all levels, lack of clear guidelines, competing health-care priorities, limited health-care budgets and political will. This leads to many people remaining undiagnosed until later stages of the disease, when prognosis is poor. Furthermore, even if diagnosed, access to appropriate antiviral therapy and ongoing clinical management is lacking.

Clarifications of terminology used in this report

1.1.1. High-risk group

For the purposes of HBV screening in LMIC, the categorisation of populations into “high-risk” groups is not helpful or informative in guiding policy. In most LMICs, the adult population prevalence (unvaccinated) falls into the intermediate- to high-endemicity categories.[1] Furthermore, within countries HBsAg prevalence is more homogenous within the population, than for example, with hepatitis C virus (HCV) infection. In this report, we therefore only refer to “high-risk” groups when referring to literature from HIC settings.

1.1.2. General population screening

This is used to refer to the fact that all members of the population have access to the screening programme under consideration. This can include community outreach screening, health-care facility-based screening, etc.

1.1.3. Targeted screening

This refers to screening of specific groups, e.g. pregnant women. Targeted groups are not necessary at higher risk of being infected than the general population.

2. Overview of the report

The purpose of this report is not to represent the results of a full systematic review. It is meant to serve as a summary of existing studies on cost-effectiveness of screening and treatment for HBV, with an analytic summary of key considerations. It was envisaged that there was a lack of relevant literature in LMICs, so existing studies from HICs are described and their potential uses and limitations when drawing conclusions are discussed.

3. Search strategy

We searched the bibliography of two previous systematic reviews on the cost-effectiveness of HBV screening by Hahné et al.[6] and Gueue et al. (unpublished, shared by WHO team) and included these in the discussion, where appropriate. Hahne and Gueue searches were performed upto 2011 and 2012, respectively. We therefore performed an updated search using PubMed to retrieve any further relevant articles to be included in this report. We searched PubMed for articles published between January 2000 and September 2015, with terms incorporating “hepatitis B”, “HBV”, or “CHB” and “cost” or “economic” and “screen”, “test” or “Diagn”. We excluded studies prior to 2000, as older studies were mainly studying cost-effectiveness of pre-vaccination screening, rather than screening for consideration of antiviral therapy. Furthermore, Geue et al. reports the low methodological standards of cost-effectiveness analyses in older studies. We selected articles published in English only. We did not search any databases other than PubMed, nor did we search the grey literature. However, we attempted to include any known ongoing HBV screening programmes in LMIC by consulting colleagues at WHO, in order to include any unpublished studies in this report.

We excluded studies that considered screening in the following groups, unless the study reported further linkage into care and treatment – blood banks and health-care workers. We excluded evaluations that included screening prior to vaccination, unless the analysis also considered antiviral therapy for the person found to be HBsAg-positive. We also excluded studies around screening for HBV prior to chemotherapy, as this was only likely to be relevant to higher income settings and would only concern a small subset of the populations in LMIC. We also excluded studies looking at coinfection with HIV and comparing diagnostic methods.

The PubMed search retrieved 32 studies, many of which overlapped with the bibliographies of the existing reviews. All studies were performed in HICs. We were unable to find any previous studies describing cost or cost-effectiveness of screening for HBV in LMICs. Due to the lack of published literature in LMICs, to better inform the report, we also included

data from the PROLIFICA study (forthcoming).[7] Finally, eight published studies and one unpublished study met inclusion criteria and are discussed in further detail below.

4. Summary of main literature

The existing published studies on the cost-effectiveness of screening and treatment for HBV have been performed in HICs where the prevalence in the general population is low.[1] We have also included discussion of unpublished PROLIFICA data, which is the only study in a LIC setting.

Two studies evaluated HBV screening in the general population[8,9] and seven studies in "high-risk" groups (all but one concerned screening in migrant or refugee populations).[10–16] We excluded studies of ANC screening as they did not consider antiviral therapy to the mother and only looked at the benefit of screening in order to guide vaccination strategies to reduce mother-to-child transmission. However, a brief summary is given below. The studies used different methods of screening the "high-risk" groups including, in the clinical setting,[10,14] community outreach methods[14] and overseas screening.[16] Various outcome measures were used including cost per quality-adjusted life-year (QALY) gained, cost per life-year (LY) saved and cost per case screened. Many of the models were simulated using hypothetical cohorts.

4.1. General population level screening

There was one previously published study in the USA and one forthcoming study in The Gambia, looking at the cost-effectiveness of offering screening and treatment to the general population.

Eckman et al.[8] looked at the cost-effectiveness of HBsAg testing of asymptomatic outpatients in primary care settings in the USA, using a hypothetical cohort (35-year-old male) with a general population prevalence of 2%. Screening was then followed by treatment with one of four regimens and compared to a no screening strategy. Screening and treatment were found to be cost-effective with an incremental cost-effectiveness ratio (ICER) of US$ 29 230/QALY. The ICER remained below their willingness to pay (WTP) threshold of US$ 50 000/QALY gained, even down to a population prevalence of 0.3%.

The feasibility of large-scale screening and treatment in sub-Saharan Africa (SSA) has been demonstrated by the ongoing "Prevention of liver fibrosis and liver cancer in Africa" (PROLIFICA) study in West Africa (Lemoine et al., forthcoming). This implementation study has screened nearly 10 000 adults for HBsAg at the community level in The Gambia and Senegal using an active outreach method. This is followed by full clinical assessment of those found to HBsAg positive and antiviral treatment if meeting eligibility criteria. A cost-effectiveness analysis of this community-based screen and treat strategy in The Gambia (Nayagam et al., forthcoming), compared to status quo, revealed an ICER of US$ 705/LY gained (other outcome measures also calculated: US$ 476/QALY gained or US$ 575/DALY averted). The authors

acknowledge that WTP thresholds levels, and their use, are highly debated in LMICs. However, it can be regarded as cost-effective if using the WHO WTP threshold of three times the country's GDP per capita to define a cost-effective intervention (3 times GDP per capita = US$ 1460 in The Gambia).[17] This is the only cost-effectiveness study of screening and treatment we have found in LMIC settings. Furthermore, it is furnished with real-life cost and effectiveness data from a large-scale screening and treatment intervention programme.

4.2. Screening of "high-risk" groups in HIC

There were six studies looking at the cost-effectiveness of screening and treatment in migrant or refugee populations in HICs,[10-14,16] and one looking at screening all groups classified as "high–risk" in Italy.[15]

The study by Wong and colleagues in 2011 looked at the cost-effectiveness of screening and treatment of immigrants for chronic hepatitis B (CHB) in Canada.[10] They considered a screen and treat strategy and a screen, treat or vaccinate strategy, with status quo (no screening). Screening was offered by the primary-care physician at a visit scheduled for another reason, described by the authors as a "case-finding" strategy. They used a hypothetical cohort (35-year-old male) with a baseline HBsAg prevalence among the immigrant population of 4.81%. The screen and treat strategy had an ICER of US$ 69 000/QALY gained. The authors acknowledge the uncertainty around WTP thresholds, but quotes range from US$ 50 000 to US$ 120 000 for Canada, implying a cost-effective intervention. This model is more clinically representative than many of the other models; however, it uses high and probably unrealistic uptake and adherence rates.

Another Canadian study by Rossi et al. (2013)[11] looked at combinations of scenarios involving screening, treatment and vaccination among newly arrived immigrants and refugees. The screen and treat scenario was found to be the most cost-effective with an ICER of US$ 40 880/QALY gained. This strategy exceeds the Canadian WTP threshold adopted in this study of US$ 50 000/QALY, when HBsAg prevalence is less than 3%. A societal perspective for the analysis was used. A hypothetical cohort of 250 000 immigrants was used, with baseline assumptions of 70% acceptance of screening, 60% linkage to care, 75% of those eligible will have treatment and annual cost of antiviral drugs at US$ 8089.

An earlier study by Hutton et al.[12] looked at the cost-effectiveness of screening and vaccination of Asian Pacific Islander adults for HBV by using a hypothetical cohort of 20–60 years old with a HBsAg prevalence of 10%. They compared four strategies of combinations of screening, treatment and vaccination, similar to the study described above. The screen and treat strategy was the most cost-effective with an ICER of US$ 36 000/QALY gained (compared to no screening), even down to an HBsAg prevalence of 1%. This study used a societal perspective.

Another, more recent, US study by Jezwa and colleagues[16] compared the cost-benefits of two overseas programmes for reducing HBV infection among refugees. They compared two strategies: (i) vaccination only, and (ii) screening and vaccination; and suggested onward

treatment on arrival in USA if HBsAg was positive. The strength of this study was the use of original data sets of refugee populations in two US states. Their baseline assumptions included a prevalence of 6.8%, 100% adherence with screening, 60% of those tested positive for HBsAg will link to specialist care and that 90% will adhere to treatment.

The study by Veldhuijzen et al.[13] was the only European study which looked at the cost-effectiveness of HBV screening and early treatment of migrants. An active screening method was used, where the target population is identified using the municipal population registry and receives a postal invitation to attend screening. Compared to status quo, screening and treatment had an ICER of €8966/QALY saved and was therefore reported as cost-effective compared to the authors' reported WTP threshold of €20 000/QALY. Their baseline HBsAg prevalence was 3.35%, 58% linkage to specialist care and 75% adherence.

A study by Rein et al.[14] looked at different methods of screening for HBV among the Asian migrant population in the USA. This was a descriptive rather than a formal cost-effectiveness analysis, with outcome measures given as cost per person screened. The screening methods analysed included testing at a community clinic and other more active community outreach models, where screening was performed at various events in the Asian community. The costs per person screened ranged from US$ 40 to US$ 280 depending on the method used. Integrating screening into clinical services was found to be the least costly method, but reached the least people, whereas extending screening outside the clinical setting was more costly as it included costs of organizing events and volunteer time, but reached more people. This study provides useful insights into the relative costs of various screening methods and, unlike some of the other studies, it includes full costs including those associated with recruiting patients. However, it does not provide long-term outcomes following on from a positive screening test and is therefore limited in its generalizability.

Ruggeri et al.[15] looked at screening of all groups defined as "high–risk" (according to local Italian guidelines), and compared the cost-effectiveness of screening followed by treatment for CHB using one of five alternative antiviral drugs. This was compared to the status quo strategy of no screening, but treatment for cirrhosis and HCC stages only. A hypothetic cohort of 100 000 individuals was considered and screening and treatment had an ICER of €17000/QALY.

4.3. Pregnant women

The screening of pregnant women for HBsAg (with or without HBeAg testing) in antenatal care (ANC) settings has also been considered in previous cost-effectiveness analyses. However, all these studies consider only the reduction in mother-to-child transmission and benefits to the child (using various outcome measures—cost per case detected, cost per infant carrier prevented or cost per LY gained). None of these ANC studies include onward linkage into care or treatment for the mother, to reduce her risk of progression of liver disease. A full discussion of these studies is therefore not included in this report. Furthermore, many of the studies are older studies published before 2000 (see Hahne review for summary of these

studies[18]) and performed in HICs (or one in upper-middle income category). They are also heterogeneous in terms of their research question and the baseline strategy under consideration, e.g. Barbosa study is comparing a comprehensive programme to a status quo which already includes screening and birth dose (BD), hepatitis B immune globulin (HBIG) and infant vaccination.[19] Fan compares whether to screen for HBeAg or HBV VL in order to guide the use of PPT antiviral therapy in USA.[20] Vimloket compared universal neonatal vaccination to screening for HBsAg and HBeAg to stratify whether HBIG is needed in Thailand, using cost per infection averted.[21] A full discussion of these studies is therefore not included in this report, as they were unlikely to be useful in helping guide these current recommendations for HBV screening and treatment in ANC settings in LMICs, but would be relevant to consider for reduction of HBV mother-to-child transmission strategies.

5. Drivers of cost-effectiveness

From the studies reviewed, some of the main drivers of whether a HBV screening and treatment strategy will be cost-effective are discussed below. This is not meant to provide an exhaustive list of drivers of cost-effectiveness but a descriptive analysis of key considerations, which will hopefully be useful in informing discussions. The main factors influencing the cost-effectiveness result are usually presented as the results of one-way sensitivity analyses, meant to be performed over plausible parameter ranges. However, it should be noted that the contribution of each parameter depends on the underlying type of model used and its baseline parameters.

5.1. HBsAg prevalence

Although the studies varied in the baseline HBsAg prevalence used in the model, they reported how the cost-effectiveness of the intervention would change over wide HBsAg prevalence ranges. HBsAg prevalence was found to have a relatively small influence on cost-effectiveness over the wide ranges tested in most of the studies. General population screening was found to remain cost-effective, i.e. ICER below the respective WTP threshold down to a HBsAg prevalence of 0.3% in the USA[8] and 2% in The Gambia (PROLIFICA). Screening of migrants in North America remained cost-effective down to a prevalence of 1–3%.[12,22] Other studies did not explicitly state a prevalence cut-off when the intervention is no longer cost-effective.[10,15]

It is important to note that “cost-effectiveness” is assessed using differing scales of cost and WTP thresholds between these studies. Therefore, extrapolation of the HIC results to LMICs is difficult, and absolute threshold cut-off for HBsAg prevalence should not be decided on the basis of this literature from HICs. However, the fact that all analyses revealed that a

screen and treat strategy remained cost-effective down to low HBsAg prevalence in the groups analysed increases the confidence of this finding.

This has important implications for strategy choice when considering screening in other countries with different prevalence profiles to the study in question. Also, importantly, as prevalence begins to fall as vaccination coverage increases, will it still remain cost-effective to continue screening once prevalence is low, and down to what HBsAg prevalence level does it still remain cost-effective to continue?

5.2. Costs

Cost components that need to be considered in economic evaluations of screening and treatment for HBV include costs of screening, diagnostics, monitoring and drugs. This should involve both the cost of consumables as well as other costs including human resource costs (which are included to various extents in different studies).

A key driver of cost-effectiveness of a screen and treat strategy reported in some studies is the cost of antiviral drugs.[9,11,12] The Rossi study used a drug cost of US$ 8089/year to represent the average cost of tenofovir and entecavir and varied this between US$ 7000 and US$ 9100, changing ICER by US$ 10 000, while still remaining cost-effective. Other costs were less important drivers of cost-effectiveness in their study. In the PROLIFICA study, the generic price of tenofovir (US$ 48) available for use in HIV programmes in SSA[23] was used as the base case. It should be noted that this price is not currently available for most countries to treat HBV mono-infection. Using the current pharmaceutical drug price of US$ 207[24] was reported to increase the ICER to US$ 1042/LY saved, whilst still remaining below the WTP threshold.

Screening costs varied between the studies, and were only found to be drivers of cost-effectiveness in the Wong[10] and PROLIFICA studies. In the PROLIFICA study, despite an active community-based screening campaign, screening costs were low (US$ 7.43 per person offered screening) and the intervention remained cost-effective even if there was a 3-fold increase in screening costs. The Rein[14] study in USA reported costs per person screened between US$ 40 and US$ 280, with the higher costs representing the more active outreach strategies.

It should also be noted that in HICs there are different cost components incurred (and included in these studies) for the management of end-stage liver disease, e.g. liver transplant. The cost-effectiveness of screen and treat strategies in HIC settings is partly due to the fact that early management reduces the risk of long-term sequelae, which can incur significant costs, e.g. estimated costs of managing cirrhosis is US$ 9000 per patient per year (pppy) and HCC is US$ 15 000 pppy in the Canadian study by Rossi et al.[11] However, in LICs, where there are currently limited options for management of end-stage liver disease (no transplant, limited endoscopy facilities, limited palliative care) and where patients often die at home, with the family as the primary care-giver, the costs of the intervention might not offset the cost avoided of end-stage liver disease. Furthermore, the annual costs of managing liver disease are variable and largely unknown.[25] The addition of a societal perspective analysis might be more appropriate in these settings.

5.3. Patient behaviour

Adherence to treatment and linkage to care were reported as key drivers of cost-effectiveness in some of the studies.[11,13] Veldhuijzen et al. reported that variation in rates of linkage to care and treatment adherence had the largest influence on ICER (ICER varied by about €3000 over the ranges tested—39–75%, 50–100%, for linkage and adherence, respectively). In the PROLIFICA study, variation in treatment adherence was also a key driver of cost-effectiveness. However, rates of linkage into care were reported to be less influential on ICER in this study. The baseline value of linkage into care was high at 81%, likely aided by re-imbursement of transportation fees, clinics held in rural sites to facilitate access to treatment, active reminders about appointments, as well as good sensitization and counselling of screened participants during the study.

Linkage into care and adherence rates being drivers of cost-effectiveness should be unsurprising if one considers that in order to gain the health benefits of a screening programme, the infected person needs to start antiviral therapy to reduce their chance of progression to end-stage liver disease. Furthermore, when people drop out at later stages of the care cascade, the impact is reduced, but the initial costs have already been incurred. This highlights the importance of educating patients on the need for continued treatment that has potential implications for successful programmatic implementation. Many barriers exist to successful linkage to care including both health service and patient factors – poor health infrastructure, distance from screening site to health facility, lack of education and patient fear.

Uptake of screening is not reported to be a key driver of ICER in the studies; however, this does not imply that high participation levels in screening is not important, as when considering health impact alone, increasing uptake is the key. The implication of this result is that it is likely to be worthwhile performing screening and treatment even if participation screening is assumed to be low. This could be because screening costs are low relative to the costs and health benefits of treatment for those who are infected.

5.4. Age of cohort

Age of the cohort screened was reported as a significant driver of cost-effectiveness in the Hutton and Wong studies. The former varied aged of screened cohort from 20 to 60 years, showing variation of ICER of US$ 23 000–US$ 58 000; the latter showed ICER between US$ 60 000 and US$ 136 000 over similar ranges, and Rossi found that the screen and treat intervention is no longer cost-effective if the cohort is over 55 years, with a non-linear relationship between ICER and age. However, despite the finding in HICs that it is more cost-effective to screen and treat younger, rather than older people, there are ethical

considerations around using age cut-offs and whether this should be used to guide these type of decisions.

5.5. Disease progression rates

Although the HBV models used slightly differing natural history structures and parameter assumptions, most of them showed that the cost-effectiveness was relatively sensitive to variations in disease progression rates used.

The Dutch study[13] showed that varying parameters between a range representing fast to slower disease progression showed significant variation in ICER between €5000 and €60 000/QALY gained, respectively, a trend which was also seen in other studies.[10,12] The Eckman study showed that the ICER was most sensitive to the rate of spontaneous HBeAg seroconversion assumed to be 5% at baseline, but exceeded the WTP threshold if increased to 10%. PROLIFICA study also showed that many of the transition rates were influential on ICER.

However, given the complex and heterogeneous natural history of HBV both within and between populations, and lack of natural history progression rate data specific to all populations, this is likely to remain an inherent limitation of all CE models for HBV. However, the ICER did remain below the WTP threshold used in the respective studies for most of the ranges used.

5.6. Effectiveness of antiviral therapy

Effectiveness of antiviral therapy was found to be influential on ICER in some studies.[10,12] However, different antivirals and different efficacy assumptions (which have often been superceded with more current data) were used by different authors (the older studies often included low-barrier to resistance drugs like lamivudine or interferon, whilst the newer studies mainly used tenofovir or entecavir). Therefore, conclusions as to the influence of these parameters on the result, as well as comparisons between studies have to be interpreted with caution.

With the recommendation of the use of newer drugs like tenofovir and entecavir, with similar high efficacy rates and better data on efficacy, model inconsistencies regarding efficacy assumptions should be less of a problem with economic analyses in the future. It needs to be noted that this will be dependent on the assumption that efficacy of antiviral therapy will be the same in HBV infected populations in LMICs as in HICs where most of the efficacy literature originates from.

5.7. Distribution of patients between different disease states

The proportion of HBsAg-positive patients with "stable infection", i.e. CHB not requiring treatment was seen as one of the drivers of cost-effectiveness in some studies. Rossi estimated that 50% of migrants diagnosed with CHB would be eligible for treatment, i.e. they had active chronic infection. They found that the ICER was sensitive to the proportion with stable infection, which when decreased from 70% to 30% increased ICER from US$ 37 000 to US$ 48 000/QALY saved. Veldhuijzen et al. assumed that 26% of HBeAg positive patients and 19% of HBeAg negative patients would be eligible for treatment according to Dutch HBV treatment guidelines, but did not comment on its influence on ICER. In contrast, within the PROLIFICA study, less than 10% of patients were considered eligible for treatment (in states of chronic active hepatitis, compensated cirrhosis or decompensated cirrhosis), and when a lower proportion of HBsAg positive people had stable CHB infection, the ICER decreased, i.e. the intervention became more cost-effective.

The explanation for the differences in eligibility criteria is beyond the scope of this current report, but might be partially explained by population characteristics (especially between HBV in Asian and African populations)[26,27] and the use of different local guidelines to classify treatment eligibility. The natural history structures are different between models, therefore direct conclusions cannot be drawn from these studies. The proportion of people who would benefit from treatment in a population, is likely to guide cost-effectiveness, but by how much is difficult to quantify based on current evidence and needs further research.

5.8. Others

Other drivers of cost-effectiveness included factors that are inherent to some of the techniques used in economic analysis, e.g. health utility values used for QALY assumptions[8–10] and discount rate used.[10,16] However, these are not discussed further in this report.

6. Limitations of comparing models/generalizability of results

WHO recommendations are primarily aimed for use in LMICs. Therefore, most of the studies summarized in this report have to be interpreted with extreme caution as they have mostly been conducted in HICs. The application of results from one setting cannot be translated into another setting. Conclusions drawn by making generalizations of results from cost-effectiveness analyses between countries or regions with such differing health-care structures, costs, patient behaviour, disease prevalence profiles and WTP thresholds can be misleading.

Comparison of model results are also hindered by differences in model structures, base-line scenarios used, populations under consideration, costs components included and varying assumptions around models parameters. The most useful health outcome measures to be used for cost-effectiveness analyses are also debated, and vary between studies, as do WTP thresholds.

In order to fully answer the question of what the most cost-effective approach is, ideally, a cost-effectiveness analysis is needed which is as specific as possible to the setting being considered as well as the strategies under consideration. However, this is obviously time and labour intensive.

7. Other considerations regarding place of screening

7.1. Community-level

Community-level screening could be considered the most active type of case-finding strategy with outreach components and therefore likely the most labour and resource intensive. However, within PROLIFICA, it has been found to be cost-effective, with low screening costs of US$ 7.43 per person offered screening. Various examples of community outreach programmes exist in the field of HIV,[28] and comparable strategies could be considered for HBV, with the caveat that "high-risk" groups will not be as applicable to HBV infection.

7.2. Health-care facilities

Screening at health-care facilities could include primary-care settings, inpatient and outpatient settings. It could include testing everyone, regardless of the reason for presentation or focus on only those with abnormal liver function tests, abnormal ultrasound scan, family history of liver disease or other clinical suspicion of liver function test. Testing could also be offered in special dedicated clinics, e.g. HIV, STD clinics.

A clinically guided testing approach is likely to reveal a higher proportion of people with HBV in highly endemic settings and therefore a lower cost per positive person found. Preliminary data from Mboup et al. (Senegal – verbal communication) where HBsAg screening is performed in the hospital guided by clinical reasons in the health facility (inpatient and outpatient settings), shows that out of 1000 people screened, 567 have been found to be HBsAg-positive (56.7% of those tested).

However, when considering performing a cost-effectiveness analysis of health facility-based screening, the difficulty arises in adjusting for background mortality among those seeking health care. It will depend on many factors, including underlying comorbidities and

age distribution and is likely to be highly heterogeneous between settings. Research into this is ongoing (Hess et al.).

7.2.1. ANC clinics

Cost-effectiveness of ANC screening, linkage into care and antiviral treatment for the mother (for the health benefit of the mother, rather than just the child), could be affected by the fact that women have been shown to have slower rates of progression to HCC[29] and have lower prevalence of HBsAg than men.[30] However, women attending ANC screening are likely to be of a younger age group than those reached by community-based screening, with a longer life expectancy, and therefore can potentially have more impact. The prevalence of HBsAg in women of childbearing age will also depend on the historical vaccination coverage in the country and the percentage of HBeAg-positive mothers will partly depend on the average childbearing age of the country and the rate of HBeAg loss in the region under consideration.[31] However, most importantly, since screening of mothers for HBV has benefits to both the mother and child, this is likely to be cost-effective.

Since there is variable percentage of attendance to antenatal care depending on the world region (ref), with this being the lowest in sub-Saharan Africa (SSA) (77% of women have at least one ANC visit, only 48% have four ANC visits),[32] this approach should also take into factors which will help strengthen ANC coverage in general and awareness campaigns.

7.2.2. Blood banks

Blood donor screening for HBV already forms part of WHO recommendations in order to prevent transmission of blood-borne viruses to the recipient.[33] However, this is rarely accompanied by the HBsAg positive donor being informed of this positive result, counselled and linked into care for clinical evaluation and treatment.[34]

As part of the PROLIFICA study, linkage into specialist care for blood donors who had tested HBsAg-positive at the blood bank was performed (Lemoine et al., forthcoming). The main difference found between the cohort of blood donors and those screened in the community were a higher proportion who were tested HBsAg-positive, a majority of whom were males, of younger ages, with a higher proportion requiring treatment and a lower proportion who linked to care. A formal cost-effectiveness analysis has not yet been performed, but these factors are likely to make it even more cost-effective for this cohort, compared to the cohorts who were screened in the community. However, as blood donors form only a small fraction of the population, this strategy is likely to be limited in its reach and population level effectiveness and probably should be seen as a complementary, rather than as an alternative to a wider screening strategy.

7.3. Workplace

Other ongoing research in West Africa as part of the PROLIFICA programme includes HBsAg screening in workplaces in Senegal (Mboup et al., unpublished data). Epidemiological and cost-effectiveness studies are underway. Provisional data shows that compared to community screening, there is a higher HBsAg prevalence, higher proportion of males uptaking screening and a higher proportion requiring treatment.

7.4. Others

Although other methods of screening are used, to varying levels, worldwide, including screening of health workers, couples pre-marriage, military recruits or pre-employment screening, etc., implementation and guidance of these methods are highly heterogeneous between countries;[35] and apart from the study in Iran (below), no data was found regarding their cost-effectiveness. Therefore, they will not be considered here in further detail. The study in Iran[36] looked at premarital HBsAg testing, but this was in order to determine whether to offer the partner of someone who is tested HBsAg positive vaccination. This does not include linkage for treatment. Mandatory premarital testing is not policy in many countries and would therefore have limited reach and applicability.

8. Further research needed to fill this information gap

More implementational research in LMICs needs to be done to assess feasibility, impact and cost-effectiveness of different screening methods. Further research into the simplification of care, as well as health systems research into integration of hepatitis programmes with other health services (e.g. HIV services), could also help guide how impact can be maximised and cost-effectiveness improved.

Ongoing HBV cost-effectiveness screening analyses that are being conducted are as follows:

- Screening in OPD settings – Sarah Hess, WHO
- Screening in ANC – benefits to the mother, Sarah Hess, WHO
- Screening in ANC – benefits to the child, Jess Howell, Imperial College
- Screening in work places, Senegal – Shevanthi Nayagam, Imperial College.

9. Conclusions

The data on the cost-effectiveness of screening for HBV is lacking, especially in LMICs. Therefore, it is hard to draw conclusions regarding the best screening strategy in terms of who to screen and where to screen, based on cost-effectiveness alone. However, the data that is

available shows that offering screening to the general population with subsequent antiviral treatment strategy is cost-effective in HICs[8] as well as LICs,[9] even down to a population prevalence as low as 0.3% and 2%, respectively in these studies. Furthermore, screening also has benefits that extend beyond the person screened but also others, e.g. prevention of mother-to-child transmission.

Relatively low screening costs, highly effective and relatively low-cost antiviral therapy at generic price and a fraction of HBsAg-positive persons requiring antiviral therapy should help drive the cost-effectiveness of a test and treat strategy. However, this has to be balanced against long-term treatment and the fact that a high proportion with CHB will survive without treatment. Finite treatment courses in certain patient groups are showing promising results and this could help increase cost-effectiveness further.[37] Improving country access to generic priced tenfovir for HBV mono-infection in all LMICs is vital to allowing adoption of wide scale HBV treatment programmes. Other strategies for reducing costs further include integration of HBV services into existing health-care structures, particularly in SSA where enormous progress has been made in the scale-up of HIV services, which may be expanded to also deliver HBV interventions using existing infrastructure, trained health-care professionals and field teams.

Although general guidance cannot be given based on the evidence, a pragmatic approach is to encourage screening anywhere that it is feasible within the country context, e.g. it can include ANCs, health-care facilities and blood banks. PROLIFICA has shown that population-level screening is feasible and cost-effective in The Gambia, but further research and large-scale implementation studies should be performed to evaluate this further in other high-endemic, low-income settings. Furthermore, HBV screening costs could be shared across other disease programmes, as there are overlapping benefits and synergies with maternal and child health goals and HIV infrastructure and experience.

This report aims to summarize key components of the existing literature which has highlighted that apart from the PROLIFICA study in West Africa, there is no data about the cost-effectiveness of screening and treatment in LMICs. Currently, there is not enough literature to make strong recommendations for screening based on cost-effectiveness arguments alone, and further research needs to be done to fill this gap, using similar real life screening data in LMICs like the PROLIFICA project. However, cost-effectiveness analyses form only a small part of guiding public health recommendations, and the overall health impact and key drivers should be considered.

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Annex 5.2

PICO 2 - Who to test (HCV)

Literature review on cost-effectiveness of HCV screening, treatment strategies and applicability to LMICs

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1. Executive summary

We conducted a targeted review of the literature to determine the state of evidence about the cost–effectiveness of testing for HCV in different types of epidemics and among different risk groups. We provide a qualitative assessment of conclusions.

5. Testing in high-risk groups such as persons who inject drugs (PWID), men who have sex with men (MSM), prisoners, HIV-infected persons, and commercial sex workers is likely to be cost–effective. Testing in settings with a high prevalence of high-risk patients is almost certainly cost–effective in all locations. It is important, however, to ensure adequate follow up after diagnosis.
6. The best approach to testing outside of high-risk risk groups depends a great deal on a country's unique HCV epidemiology. Most countries have at least some component of "birth cohort" epidemic, and "birth cohort" testing is likely cost–effective in most settings.
7. Routine testing of the entire population carries two risks. First, when the HCV epidemic is concentrated to a specific age or risk group, generalized testing can dilute the testing effort and reduce the number of HCV cases identified. Second, if an epidemic is highly concentrated with a specific risk or demographic group, screening outside of that group can be inefficient and increase cost. Countries with high HCV prevalence across the entire population should implement routine screening, but in most epidemics, routine screening in the entire population is likely not be cost-effective. The specific threshold at which a country should alter its approach to routine testing, however, is a function of multiple factors and cannot be identified more generally.

2. Background

Hepatitis C virus (HCV) is a global public health burden and major cause of morbidity and mortality including liver failure and hepatocellular carcinoma.[1,2] Current global HCV seroprevalence is estimated to be 2.8%, or more than 185 million infected individuals worldwide.[3] Historically, it has been very difficult to treat HCV and most cases of HCV have gone unidentified. The advent of high-efficacy, low-duration therapy, however,[4] generates new enthusiasm for testing for HCV infection, linking infected patients to care, and curing HCV before patients begin to experience the consequences of cirrhosis and end-stage liver disease.

It is not clear, however, exactly who should be targeted for HCV testing. Similar to the conversation around HIV testing, there are several approaches to screening for HCV that may provide high yield and improve outcomes including: 1) targeted testing of the highest-risk groups, 2) routine testing among specific demographic groups that are readily identified and who have a high prevalence of HCV infection, and 3) routine testing throughout the entire population. This review develops a rubric by which to measure and characterize the HCV epidemic within a country, surveys the literature about the cost–effectiveness of screening for HCV in various populations, and discusses how epidemiology within a country should inform decision-making about who to test for HCV.

3. Overview of report

This report does not represent the results of a full systematic review. It is meant to serve as a summary of existing studies on cost–effectiveness of screening and treatment for HCV, with an analytic summary of key considerations. It was envisaged that there was a lack of relevant literature in low- and middle-income countries (LMICs), so existing studies from high-income countries (HICs) are described and their potential uses and limitations, when drawing conclusions are discussed.

4. Summary of global HCV epidemiology

Generally, HCV epidemics around the world are heterogeneous and represent mixtures of three core epidemic components:

4. *Infection related to high-risk behaviours:* In essentially every geographical region, the highest prevalence of HCV infection is among persons who use injection drugs (PWID).[5,6] The prevalence of injection drug use differs between countries and regions, but within those who do inject drugs, HCV prevalence is nearly universally high. Commercial sex workers and prisoners also have increased prevalence (presumably related to both drug use and perhaps sexual transmission),[7,8] as do men who have sex with men, especially those who are HIV infected.[9] In many cohorts of PWID in North America, Europe, and Asia, HCV prevalence ranges from 30% to 75%.
5. *Infection related to past generalized exposures that have since been identified and removed:* This epidemic pattern, in which there is a high prevalence of HCV within a given age group, is commonly referred to as a "birth cohort epidemic".[10] While typically identified as being the infection pattern in North America and Europe, many nations have some element of birth cohort epidemics with their unique HCV epidemiology (Table 1).[11] Birth cohort epidemics reflect an HCV exposure source that was once present and to which a large portion of the population was exposed, but that has since been identified and removed. For example, before it was identified and sequenced, HCV infected the blood supply of many countries in all regions of the world. When the blood supply began to be screened for the presence of HCV, the exposure was removed. As a result, the incidence of HCV fell dramatically among the general population, but there remains a burden of prevalent, chronic HCV among patients who were alive and likely to get a blood transfusion during the time that HCV existed in the blood supply.
6. *Generalized population epidemic:* This pattern is related to a widespread exposure, often iatrogenic, that results in high prevalence (8–10%) across essentially all age groups. Note that the primary difference between a "birth cohort" pattern and a generalized pattern of infection is the duration of time that the generalized exposure existed and whether it has been removed or mitigated. An example of a generalized exposure is the common use of reusable hypodermic syringes and needles in medical settings without adequate sterilization between uses.

Few epidemics fall into one of the above three categories. Rather, most are mixed, and represent some combination of all components (Table 1). The nature of an epidemic

within a specific country determines a great deal about the appropriate approaches to who to screen.

Epidemic scenarios HCV	Definition	Disaggregation	Country example
Generalized	High (>5%)	With birth cohort	(*N*=23) Cameroon, CAR, Armenia, Egypt, Liberia, Gabon, Guinea, Ghana, Guinea-Bissau, Mongolia, Sierre Leone, Uzbekistan, Cape Verde, Chad, Mali, Niger, Nigeria, Pakistan, San Tome et Principe, Senegal, Togo, Burkina Faso, Georgia
		Without birth cohort	
	High intermediate (3–5%)	With birth cohort	(*N*=14) Angola, Bahrain, Congo, Democratic Republic of the Congo, Equatorial Guinea, Estonia, Lebanon, Moldova, Russia, Taiwan, Turkmenistan, Ukraine, United Arab Emirates, Oman
		Without birth cohort	
	Low intermediate (2–3%)	With birth cohort	(*N*=44) American Samoa, Anguilla, Azerbaijan, Benin, Bermuda, British Virgin Isles, Cayman Islands, Cook Islands, Cote d'Ivoire, Falkland Isalnds, Faroe Islands, Gibraltar, Greenland, Holy See, Hong Kong, Iraq, Isle of Man, Jordan, Kazakstan, Kuwait, Kyrgzstan, Latvia, Lichtenstein, Lithuania, Macau, Monaco, Monserrat, Nauru, Niue, Northern Mariana, Islands, Palau, Palestine, Romania, St Helena, St Kitts and Nevis, St Pierre Miquelon, San Marino, St Martin, Tajikistan, Thailand, Tokelau, Turks and Kakos, Tuvalu, Wallis and Futuna
		Without birth cohort	
Mixed	Generalized population prevalence, low, moderate or high with a sizeable risk population (PWID)	High generalized	(*N*=3) Pakistan, Egypt, Uzbekistan
		High intermediate generalized	(*N*=5) Estonia, Kazakstan, Taiwan, Turkmenistan, Ukraine
		Low intermediate generalized	(*N*=8) Hong Kong, Latvia, Lithuania, Palestine, Romania, Thailand, Tajikistan, Syria
		Low (1–2%) with PWID	(*N*=46) Albania, Algeria, Argentina, Australia, Belarus, Bhutan, Bosnia, Brazil, Cambodia, Chile, China, Colombia, Costa Rica, Croatia, El Salvador, Greece, Honduras, Israel, Italy, Japan, Kenya, Former Yugoslav Republic of Macedonia, Malaysia, Mauritius, Mexico, Montenegro, Morocco, Myanmar, Nepal, New Zealand, Nicaragua, Panama, Paraguay, Portugal, Puerto Rico, Serbia, Slovakia, Slovenia, South Sudan, Spain, Switzerland, United States, United States Virgin Islands, Uruguay, Viet Nam, Yemen

		Low (1–2 %) without PWID	(*N*=8) Botswana, Ethiopia, The Gambia, Guadalope, Lesotho, Rwanda, Saudi Arabia, Singapore
			(*N*=21) Andora, Antigua Comorres, Curacao, Democratic People's Republic of Korea, Dominica, French Guinea, French Polynesia, Guatemala, Martinique, Mauritania, Mayotte, Namibia, New Caledonia, Reunion, Seychelles, South Africa, Sri Lanka, Swaziland, Western Sahara, Zimbabwe
Concentrated	Generalized population prevalence <1% with high-risk groups		(*N*=68) Afghanistan, Aruba, Austria, Bahamas, Bangladesh, Barbados, Belgium, Belize, Bolivia, Brunei, Bulgaria, Burundi, Canada, Cuba, Cyprus, Czech Republic, Denmark, Djibouti, Dominican Republic, Ecuador, Eritrea, Fiji, Finland, France, Germany, Grenada, Guyana, Haiti, Hungary, Iceland, India, Indonesia, Iran, Ireland, Jamaica, Kiribati, Korea Republic of, Laos, Libya, Luxembourg, Madagascar, Maldives, Marshall Islands, Micronesia, Mozambique, Netherlands, Norway, Papua New Guinea, Peru, Philippines, Poland, Qatar, St. Lucia, Samoa, Solomon Islands, Somalia, Surinam, Sweden, Tanzania, Timor-Leste, Tonga, Trinidad & Tobago, Tunisia, Turkey, Uganda, United Kingdom, Vanuatu, Zambia

5. Summary of the literature – Who to screen? What is the evidence base from modelling of the impact and cost–effectiveness of different screening approaches using different prevalence thresholds?

Testing in high-risk groups, including persons who inject drugs, MSM, prisoners, HIV-infected persons, and commercial sex workers

5.1. Persons who inject drugs

Multiple analyses in many geographical regions concur that routine testing for HCV in venues with a high prevalence of persons who inject drugs is cost–effective, even when the studies assume very poor follow-up rates and limited access to therapy.[12,13] Further, dynamic HCV transmission models suggest that aggressive diagnosis and treatment among current drug users could reduce the incidence of HCV – "cure as prevention".[14,15] With typical prevalence estimates of 40%, but ranging as high as 75% in some cohorts, routine screening for HCV is almost certainly cost–effective. It is essential to consider the HCV cascade of care when screening recent or current drug users. Modelling studies demonstrate that even 100%

effective HCV therapy has almost no impact on population-level outcomes without efforts to significantly improve the number of HCV-infected patients who initiate therapy.[16]

5.2. Men who have sex with men

Men who have sex with men (MSM) are also at an increased risk of HCV incidence, particularly if they are also HIV-positive. Since the mid-2000s, outbreaks have surfaced in the US, Europe and Australia among HIV-positive MSM.[17,18] Cost–effectiveness modelling has found testing using liver function tests in combination with HCV Ab testing to be cost–effective in the HIV-positive MSM population.[19] Preliminary studies have suggested that core-antigen testing has the potential to be cost–effective in this population as well.[20] The results of these studies are dependent on appropriate linkage to effective therapy and retention in care. These studies do not fully account for either the reduction in secondary transmission or the possibility of reinfection in high-risk groups.

5.3. Prisoners

Prisons likely have high HCV prevalence as the result of a high prevalence of persons who inject drugs in prisons. One challenge to HCV testing in prisons is that new treatments for HCV are costly and many prisoners do not have access to new therapies. A UK-based study, however, found that HCV case detection, using dried blood spot testing, was cost–effective, even when the model assumed low rates of HCV treatment initiation.[21] A second study concurs that screening in prisons can be cost–effective, but this study concluded that targeting screening to those prisoners with a history of injection drug use improves cost–effectiveness[22] A later study by several of the same authors found that routine screening of all prisoners is not cost–effective, although that study found significant uncertainty in the results. If the prison population had more advanced disease at the time of screening, or if the rate of HCV disease progression is faster than estimated in the base case, then routine screening in prisons can be cost–effective.[23]

5.4. HIV-infected persons

Screening for incident and acute HCV in HIV-infected MSM is likely cost–effective.[19] Although nearly every guideline for HCV care recommends HCV screening at enrolment in care, we were not able to find a cost-effectiveness analysis that answers the specific question of the cost-effectiveness of HCV testing at enrollment in HCV care. Because the prevalence of HCV is known to be high in HIV-infected persons, the pace of progression of fibrosis in HIV/HCV-

coinfected patients is high, and new therapies to treat HCV are effective in HIV/HCV coinfection, testing for HCV at enrolment in HCV is almost certainly cost–effective.

5.5. Sex workers

We were not able to find a study that addresses the cost–effectiveness of HCV testing in sex workers. Because many sex workers are also PWID or non-injection drug users, the prevalence of HCV in this group is likely high. It is not clear at this time, however, if it is cost–effective to routinely screen all sex workers, compared to an approach that targets testing to sex workers who report a history of injection drug use.

5.6. Testing among easily identified age or demographic groups known to have high HCV prevalence ("birth-cohort testing")

Whenever there is an easily identified demographic group that has high HCV prevalence (for example, all individuals born in a certain time period) it is likely cost–effective to routinely test for HCV within that cohort. Several cost–effectiveness studies in the US estimate incremental cost–effectiveness ratios of "birth cohort" screening that are below commonly cited willingness to pay thresholds for resource-rich countries.[10, 24–5] Each of these studies compared "birth cohort testing" to the current standard of care, and shared the same qualitative conclusions. Similarly, one study from Portugal found that birth cohort testing was cost–effective in that country.[26] Notably, based on Portugal's local HCV epidemiology, the cohort to test is not identical to that in the US. Routine screening is preferred to targeted screening because providers are often not skilled at identifying high-risk behaviours, and because for many patients in such an epidemic, the "risk" to target is simply being a member of a high-prevalence age cohort (i.e. there are no specific behavioural risks to identify).

5.7. Testing among the general population without attempt to identify high-risk behaviours or characteristics ("routine testing")

At this time, no jurisdiction of which we are aware recommends routine testing for all individuals regardless of demographics or specific behavioural risk. The data about population screening typically come from HICs such as the US and UK, and such studies find that routine testing in the general population is not cost–effective. For example, one cost–effectiveness analysis, conducted in the US context, found that when the general population prevalence of

HCV infection exceeded 0.53%, the incremental cost–effectiveness ratio of routine universal screening compared to targeted screening was far below commonly cited US willingness to pay thresholds.[27] When compared to "birth cohort testing," however, universal testing resulted in worse outcomes and higher costs than the birth cohort approach. This analysis raises the spectre that in countries whose HCV epidemic is largely concentrated to a specific birth cohort or demographics group, attempting to identify cases by routine testing of the entire population can dilute the testing effort and result in fewer cases of HCV being identified.

Similarly, a recent study in the US context investigated the cost–effectiveness of two approaches to testing for HCV in average-risk, asymptomatic adults accessing primary care: a) HCV EIA followed by quantitative RNA for those with positive EIA results, and b) quantitative RNA for all patients. Neither strategy was cost–effective.[28]

An older study, conducted in the UK, also found that although screening high-risk groups in primary care settings was cost–effective, extending screening beyond high-risk individuals was not.[29] Importantly, however, that study pre-dates the existence of effective, antiviral therapy targeting HCV. Higher efficacy of therapy could improve cost–effectiveness conclusions.

Similarly, a study conducted in Japan found that routine testing of the population was cost–effective compared to "no screening."[30] That paper, however, did not consider a birth cohort approach, and the conclusions therefore are not certain.

Another analysis, conducted in Italy, used Markov modelling to compare "testing" to "no testing" among patients who had undergone surgery. They found that testing was not cost–effective in this group.[31] Notably, individuals who have undergone surgery are more likely to have had exposure to blood products, and therefore likely have a higher HCV prevalence then the general population. If screening among these patients was not cost–effective, screening in even lower prevalence groups, such as the general population, will also not be cost–effective.

There is one recent study, conducted in Canada, that found that one-time testing of patients outside the "birth cohort" of those aged 65 years or older would be cost–effective by Canadian standards.[32] It is difficult from that manuscript to determine the epidemiological assumptions that led to this finding, which differs from most US-based studies. One assumption that could have influenced the results was that early-stage HCV had a low quality of life, which tends to make screening and treating HCV more cost–effective.

Similarly, a modelling study based on ten years of retrospective data at a London antenatal clinic found that routine testing for HCV for pregnant women was cost–effective, even at baseline prevalence levels as low as 0.1%.[33] This contradicts the findings of a 2005 paper based in the US, which found that screening of asymptomatic pregnant women in the US, even when coupled with elective caesarean delivery to minimize antenatal transmission risk, was not cost–effective.[34] It also contradicts a paper based in the Netherlands that found that adding routine one-time testing for HCV in antenatal clinics would not be cost–effective.[35] These disparate findings may be influenced by estimates of fibrosis progression, discounting rates, and health-care costs in each country.

Importantly, all of the above studies reflect the epidemiology of HCV in HICs. One recent paper explicitly studied the cost–effectiveness in Egypt of one-time, routine screening for HCV followed by treatment with either pegylated interferon and ribavirin (PEG-RBV) or PEG-RBV plus an HCV protease inhibitor.[36] Given the very high prevalence of disease, screening was always cost–effective, and often cost-saving. It is important to consider, however, that assumptions about linkage to HCV care and availability of treatment after diagnosis impact cost–effectiveness conclusions. If general population screening will likely identify many cases of HCV, but those who are infected have limited options for treatment, screening may not be cost–effective.

6. Drivers of cost–effectiveness

The main benefit of testing is identifying cases of HCV before they lead to the sequelae of end-stage liver disease; the resource implications of testing broadly are important. First, the cost of testing itself is not trivial. Second, if the testing strategy (i.e. the laboratory protocol one uses to identify HCV exposure and test for HCV viraemia) results in a large number of false-positive tests, the cost of unnecessary HCV therapy could be very large. At the same time, trying to "over target" testing to only the highest-risk groups can be detrimental to public health. Many high-risk behaviours are stigmatized and underreported, and health-care workers are not always skilled at identifying high-risk behaviours. Balancing these considerations is a challenge, and requires country-level determination of best approaches. General themes that should inform the screening approach include the following:

1. *HCV prevalence* – screening provides increasing value as prevalence rises. In one US based study, screening was cost–effective (compared to no screening) at a US willingness to pay threshold down to prevalence of 0.53%.[27] Importantly, however, choice of comparator impacts the incremental cost–effectiveness ratio of routine population testing. When routine testing was compared to "birth cohort testing" in that same paper, routine screening diluted the screening effort in the cohort with the highest prevalence of HCV and therefore resulted in fewer cases of HCV identified and higher cost than "birth cohort testing." However, in any population subgroup that has HCV prevalence >1%, it is likely that some form of testing is cost–effective. The question in such scenarios is whether to routinely screen, or to attempt to identify risk and target screening to that group.
2. *Degree of concentration of the epidemic* – to the extent that an epidemic is concentrated to a specific risk or demographic group, targeting screening to that group becomes more cost–effective. This dynamic is most directly at play when considering "birth cohort testing." Being a member of a birth cohort is easily determined and generally carries no stigma. Thus, targeting testing to birth cohorts is feasible and often cost–effective. In countries with a strong birth cohort dynamic, birth cohort screening is likely preferred. To the extent that epidemics are concentrated among high-risk groups such as PWID, however, targeted testing is more challenging. Because HCV risk behaviours are stigmatized and underreported, trying to identify high-risk individuals is difficult and prone to under-testing high-risk patients.

3. *Treatment rates* – screening clearly becomes less cost–effective when identified patients cannot link to effective therapy. US-based analyses typically assume availability of interferon-free regimens to cure HCV. If such treatments are not available, or only available to a limited proportion of identified cases, then the incremental cost–effectiveness ratio of screening increases.
4. *Assumptions about the HCV cascade of care* – similar to treatment rates, loss to follow up has an important impact on cost–effectiveness conclusions. As the proportion of patients with identified HCV infection who successfully link to HCV care decreases, the incremental cost–effectiveness ratio of screening also goes up.
5. *Cost of testing* – the cost of testing may impact the cost–effectiveness of one testing strategy compared to another (i.e. which tests to use and in what order), but it has little impact on the cost–effectiveness conclusions about *who* to screen. In one study conducted in the US, ranging the cost of testing by as much as 50% in either direction had no impact on cost–effectiveness conclusions.[37]
6. *Efficacy of HCV therapy* – the cost–effectiveness of HCV testing depends in part on the efficacy of HCV treatment. This dynamic is easily demonstrated by a hypothetical scenario, in which patients with identified HCV do not receive any therapy (efficacy = 0%). In such a case, the incremental benefit of screening would be zero, and the incremental cost–effectiveness ratio would approach infinity (no value).
7. *Cost of HCV therapy* – as the cost of treatment increases, the incremental cost–effectiveness ratio also increases. This is not surprising, as the cost of therapy has no impact on clinical outcomes (denominator of the cost–effectiveness ratio), but does increase cost (the numerator of the cost–effectiveness ratio). For example, in one US study, the incremental cost–effectiveness ratio of "birth cohort testing" compared to no testing increased more than 100% when one assumed treatment with pegylated interferon, ribavirin, and an HCV protease inhibitor compared to pegylated interferon and ribavirin alone.
8. *Estimates of quality of life with early-stage HCV* – if early-stage HCV has a large impact on quality of life, then testing (via any approach) becomes more cost–effective. If early-stage HCV has little impact on quality of life, then the benefits of testing accrue only to the minority of patients who become cirrhotic, and only in the distant future when those patients begin to experience complications of end-stage liver disease. In contrast, if early-stage HCV has an immediate impact on quality of life, then every patient with identified and cured HCV accrues lifetime benefits that greatly increase the benefits of testing.
9. *HCV fibrosis progression rates* – most of the sequelae of chronic HCV infection and essentially all HCV-attributable mortality, accrue only when a patient has reached cirrhosis. The time from HCV infection to development of cirrhosis is highly variable and can be as long as 25 years. Some patients never become cirrhotic. Faster rates of fibrosis progression tend to make testing for HCV more cost–effective, because faster fibrosis progression results in a larger proportion of the population experiencing sequelae of HCV.

7. What further research needs to be done to fill this information gap

It is important to collect accurate epidemiological data to better inform decision-making around HCV testing. A formal cost–effectiveness analysis that compares "targeted" vs "birth cohort" vs "routine" testing requires estimates of the prevalence of high-risk behaviours, stratified by age, the prevalence of HCV among those with high- and low-risk behaviours, stratified by age, and the age structure of the population. Further, it is important to know the cost of both HCV therapy in a country, as well as the costs associated with untreated HCV and end-stage liver disease. In addition, more implementation research in LMICs is needed to determine the degree to which providers can accurately identify and test high-risk patients when employing a targeted approach, as well as estimate of linkage to HCV care and the HCV cascade.

8. Conclusions – who should be tested for HCV?

- Testing in high-risk groups such as PWID, MSM, prisoners, HIV-infected persons, and commercial sex workers is likely cost–effective. Testing in settings with a high prevalence of high-risk patients is almost certainly cost–effective in all locations. It is important, however, to ensure adequate follow up after diagnosis.
- The best approach to testing outside of high-risk risk groups depends a great deal on a country's unique HCV epidemiology. Most countries have at least some component of "birth cohort" epidemic, and "birth cohort" testing is likely cost–effective in most settings.
- Routine testing of the entire population carries two risks. First, when the HCV epidemic is concentrated to a specific age or risk group, generalized testing can dilute the testing effort and reduce the number of HCV cases identified. Second, if an epidemic is highly concentrated within a specific risk or demographic group, screening outside of that group can be inefficient and increase costs. Countries with high HCV prevalence across the entire population should implement routine screening, but in most epidemics, routine screening in the entire population may not be cost–effective. The specific threshold at which a country should alter its approach to routine testing, however, is a function of multiple factors and cannot be identified more generally.

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Annex 5.3

PICO 1 - How to test (HBV)
Diagnostic accuracy of tests to detect hepatitis B surface antigen: a meta-analysis and review of the literature

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1. Executive summary

Background: Rapid diagnostic tests are potentially useful tools for the diagnosis of hepatitis B surface antigen (HBsAg) globally, particularly in low-resource areas. Expansion for global use depends on their performance characteristics clinically in the field, ultimately with the aim being to reach low-resource settings and offer cost–efficient screening as an alternative to laboratory tests.

Objectives: The purpose of this review was to identify quantitative evidence on the clinical sensitivity[1] and specificity of available in vitro diagnostics (hereafter referred to as assays) used to detect hepatitis B antibody, synthesize the evidence, and inform models.

Methods: Two reviewers independently assessed the quality and extracted data for estimating accuracy. Meta-analysis was performed. We further performed stratified estimates based on individual products, HIV status, specimen type, study setting and design.

Results: Thirty-three studies were included using an EIA reference standard. The overall pooled clinical sensitivity and specificity of rapid HBsAg tests were 90.0% (95% CI: 89.1, 90.8) and 99.5% (95% CI: 99.4, 99.5), respectively, compared to laboratory-based immunoassay reference standards. Pooled specificity was comparable and less heterogeneous.

Pooled sensitivity in studies of HIV-positive was lower than in known HIV-negative patients; 72.3% (95% CI: 67.9, 76.4) compared to 92.6% (95% CI: 89.8, 94.8), respectively. Pooled sensitivity and specificity in blood donors were 91.6% (95% CI: 90.1, 92.9) and 99.5% (95% CI: 99.5, 99.9), respectively.

Samples using whole blood specimens (venous or capillary) were 91.7% (95% CI: 89.1, 93.9) and 99.9% (95% CI: 99.8, 99.9) sensitive and specific compared to serum.

Results were comparable for studies performed prior to the past ten years as those performed since 2005. Estimates of assay sensitivity demonstrated significant heterogeneity not entirely corrected by sub-analysis, although studies using whole blood specimens (venous or capillary), and the same reference standard (CMIA) were more robust (tau-squared <1 in all cases).

The overall pooled clinical sensitivity and specificity of laboratory-based HBsAg tests were comparable, with 88.9% (95% CI: 87.0, 90.6) and 98.4% (95% CI: 97.8, 98.8) sensitivity and specificity, respectively, compared to immunoassay state-of-the-art chemiluminescent microparticle enzyme immunoassays.

Conclusions: Assays for HBsAg detection such as rapid diagnostic tests (RDTs), including those performed on serum/plasma and capillary whole blood specimens, have good sensitivity and excellent specificity compared to a reference standard comprising laboratory-based methods of HBsAg detection. Improvement in sensitivity, or development of innovative testing strategies could potentially enhance their use as first-line screening globally. Caution in HIV-

[1] Unless otherwise specified, sensitivity and specificity refer to clinical and not analytical sensitivity for tests.

positive individuals is important, while the reassuring accuracy of capillary whole blood specimens compared to plasma/serum further facilitates use in settings where phlebotomy is not available.

2. Background

An estimated 240 million individuals worldwide[1] are chronically infected with hepatitis B virus (HBV) and there are an estimated four million acute HBV infections each year. Twenty per cent to 30% of those with chronic hepatitis B infection will develop cirrhosis[2] or hepatocellular carcinoma,[3] leading to approximately 650 000 deaths each year.[4] However, most individuals with chronic HBV infection are not aware of their serostatus, contributing to delayed diagnosis and complications from advanced disease.[5] HBV testing is critically important in order to refer infected individuals to HBV treatment and care, to refer uninfected individuals for vaccination, and to mobilize prevention and control efforts.

In March 2015, the World Health Organization (WHO) published the first guidelines for the prevention, care, and treatment of individuals with chronic HBV infection.[5] These guidelines focused on assessment for treatment eligibility, initiation of first-line therapies, switching and monitoring. These initial guidelines did not include testing recommendations, and in particular which tests to use. Given the large burden of HBV in low- and middle-income settings where there are limited or no existing HBV testing guidelines, there is a substantial need for HBV testing guidelines.

Chronic HBV infection is defined as persistence of hepatitis B surface antigen (HBSAg) for at least six months. However, interpretation of HBV serologies is complex. The serologies most frequently used for HBV testing include HBsAg, total anti-HBc, and anti-HBs. HBV screening includes both the one-test (e.g. HBsAg) and two-test strategies (e.g. HBsAg followed by hepatitia B core antibody [HBcAb] or nucleic acid testing [NAT]).

Detection of HBsAg can include rapid diagnostic tests (RDTs) or immunoassays. Rapid tests developed for screening include solid-phase assays, flow-through, agglutination and lateral-flow. The majority, however, are immunochromatographic assays. Immunoassays use different methods for detection of HBsAg using polyclonal or monoclonal anti-HBs antibodies. Labelling to measure antigen–antibody complexes can include radioactive compounds, enzymes with a change in colour in solution, or substances emitting light.

Advances in HBV detection technology create new opportunities for enhancing screening, referral, and treatment. Previous systematic reviews on hepatitis B infection have focused on immunological responses,[6] surveillance of cirrhosis,[7] and treatment.[8] Existing systematic reviews[9–11] on hepatitis B testing focused on point-of-care (POC) tests and included tests with unclear reference standards or those not appropriate for assessing operational diagnostic accuracy in the field.

3. Objectives

The purpose of this review was to identify quantitative evidence on the sensitivity and specificity of assays used to detect hepatitis B antibody, synthesize the evidence and inform models.

To our knowledge, this is the first study exclusively comparing the clinical performance of both RDTs and laboratory-based immunoassays, in addition to addressing the question of accuracy in the context of HIV specifically.

PICO 1	Among persons identified for hepatitis B testing, what is the diagnostic accuracy of available assays for detecting HBsAg?
P	Persons identified for HBV testing
I	Rapid diagnostic tests or immunoassays for HBsAg detection
C	Reference standard comprising a laboratory-based enzyme immunoassay (EIA)[2] (one or more HBsAg enzyme immunoassays, with or without neutralization to confirm)
O	Diagnostic accuracy (sensitivity, specificity, TN, TP, FN, and FP; positive predictive value, negative predictive value)

As a subanalysis, we also analysed data for studies comparing the accuracy of HBsAg assays against a nucleic-acid amplification test (NAT) reference standard. This is important given the importance of reducing transmission during the seroconversion period and in the diagnosis of occult hepatitis B, where HBsAg may not be detectable and which is more common in HIV coinfection. [Results in Annex 9.2]

4. Methods

Search strategy and identification of studies

Literature search strategies were developed by a medical librarian with expertise in systematic review searching. Our search algorithm consisted of the following components: hepatitis B, diagnostic tests and diagnostic accuracy (*see* Annex 1). We searched MEDLINE (OVID interface, 1946 onwards), EMBASE (OVID interface, 1947 onwards), the Cochrane Central Register of Controlled Trials (Wiley interface, current issue), Science Citation Index Expanded (Web of Science interface, 1970 onwards), Conference Proceedings Citation Index-Science (Web of Science interface, 1990 onwards), SCOPUS (1960 onwards), Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS) (BIREME interface) and WHO Global Index Medicus. The search was supplemented by searching for ongoing studies in WHO's International Clinical Trials Registry.

In addition to searching databases, we contacted individual researchers, experts working in the field and authors of major trials to address whether any relevant manuscripts are in preparation or in press. The references of published articles found in the above

[2] For convenience we shall refer to all laboratory-based immunoassays for HBsAg detection (ELISA, MEIA, ECLIA, CMIA) as EIAs as most have some form of enzymatic amplification.

databases were searched for additional pertinent articles. The review was registered in PROSPERO and reported in accordance with PRISMA guidelines.

Study selection proceeded in three stages. First, titles/abstracts were screened by a single reviewer (AA/HK) according to standard inclusion and exclusion criteria. Second, all articles identified for full manuscript review were obtained and assessed independently by the two reviewers (AA and HK) against inclusion criteria. Papers were accepted or rejected and reasons for rejection were specified. Third, discrepancies were resolved by discussion between review authors, with several studies resolved by a third independent reviewer (RP).

Selection criteria

Types of studies

We included case–control, cross-sectional, cohort studies and randomized trials with a primary purpose of evaluating HBsAg tests published until May 2015. We excluded: conference abstracts, comments or review papers; studies with primary aims other than evaluation of both sensitivity and specificity of HBsAg detection; studies related to disease prevalence, drug resistance, genotyping, sequencing, or non-diagnostic purposes; studies that focus on detection of anti-HBsAg (antibody to hepatitis B antigen); articles in languages other than English.

Participants

We included studies using original data from patient specimens in defined populations. We included all age groups, settings, countries and specimen types, notably whole blood (venous and capillary), plasma or serum. Saliva specimens were considered, but no suitable studies were identified. We excluded studies using commercial reference panels or clinical panels not sourced by authors given applicability and bias concerns from unknown sampling in unclear populations.

Index tests

Studies utilizing commercially available HBsAg tests were eligible for inclusion. We excluded: in-house developed tests; laboratory-based immunoassays which are no longer commercially available. We did not, however, exclude rapid tests based on current commercial availability, in keeping with the methodology in recent systematic reviews. We did however sub-categorize more recent studies between 2005 and 2015.

Reference standard

The reference standard for definite diagnosis of hepatitis B is complicated, given the different viral kinetics of HBsAg and HBV DNA. We included studies using an established commercially available immunoassay as a reference standard for HBsAg detection. Studies using a NAT reference standard were included as a supplementary secondary analysis. For studies comparing immunoassays, we only included studies using chemiluminescent microparticle

immunoassays (CMIAs) as the reference standard, given the generally accepted higher analytical sensitivity of these assays. For studies of rapid tests, in keeping with previous systematic reviews, we did not limit based on type of immunoassays.

Data extraction

Information on the following variables were independently extracted by the two review authors (AA, KH): first author, total sample size, country (and city) of sampling, specimen type (oral fluid, capillary [finger-prick] whole blood, venous whole blood, etc.), eligibility criteria, reference standard, manufacturer, raw cell numbers (true positives, false negatives, false positives, true negatives), HIV coinfection, sources of funding, reported conflicts of interest.

Assessment of methodological quality

Study quality was evaluated using the QUADAS-2 tool[12] and the STARD checklist.[13] QUADAS includes domains to evaluate bias in the following categories: risk of bias (patient selection, index test, reference standard, flow and timing); applicability concerns (patient selection, index test, reference standard).

GRADE summary of finding tables

The GRADE system was used to rate the strength of evidence of each body evidence as high, moderate, low, or very low on the basis of aggregate quality, consistency, precision, directness, and reporting bias. We did not downgrade directness because the outcome evaluated was diagnostic accuracy (an intermediate outcome), as the PICO focuses on diagnostic accuracy. Summary of finding tables were prepared. We did not perform a formal assessment of publication bias.

Data analysis and synthesis

Standard methods for meta-analysis of diagnostic accuracy were used. For each study we calculated sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and 95% confidence intervals (CI). Likelihood ratios are metrics and are calculated using a combination of sensitivity and specificity. Positive likelihood ratio (PLR) is the ratio of sensitivity (1−specificity), whereas the negative likelihood ratio (NLR) is the ratio of specificity (1−sensitivity). When a diagnostic test has no discriminating capability, both likelihood ratios equal 1.

We conducted meta-analysis pooling data using the DerSimonian–Laird bivariate random effect model (REM) to calculate pooled sensitivity, specificity and other related indices. The REM was more suitable than the fixed-effects model (FEM) given significant heterogeneity in studies found, as it takes into account variability both within studies (random error) and between studies (heterogeneity). Statistical heterogeneity was measured using the random-effect variance (tau-squared).

Meta-regression was performed to investigate the source of heterogeneity within included studies (inverse variance weighted). We analysed sub-groups according to the following characteristics: study year (2005–2015); geographical area; individual tests; sample

type; patient type (blood donors); patient HIV status. We did not formally test for publication bias. Where meta-regression was not possible for covariates, we performed descriptive statistics.

Meta-analysis of the collected data was conducted using the software: Meta-Disc© version 1.4.7. Statistical analysis was performed using Meta-Disc 1.4 for Windows (XI Cochrane Colloquium, Barcelona, Spain). QUADAS-2 analysis was performed using Microsoft Excel.

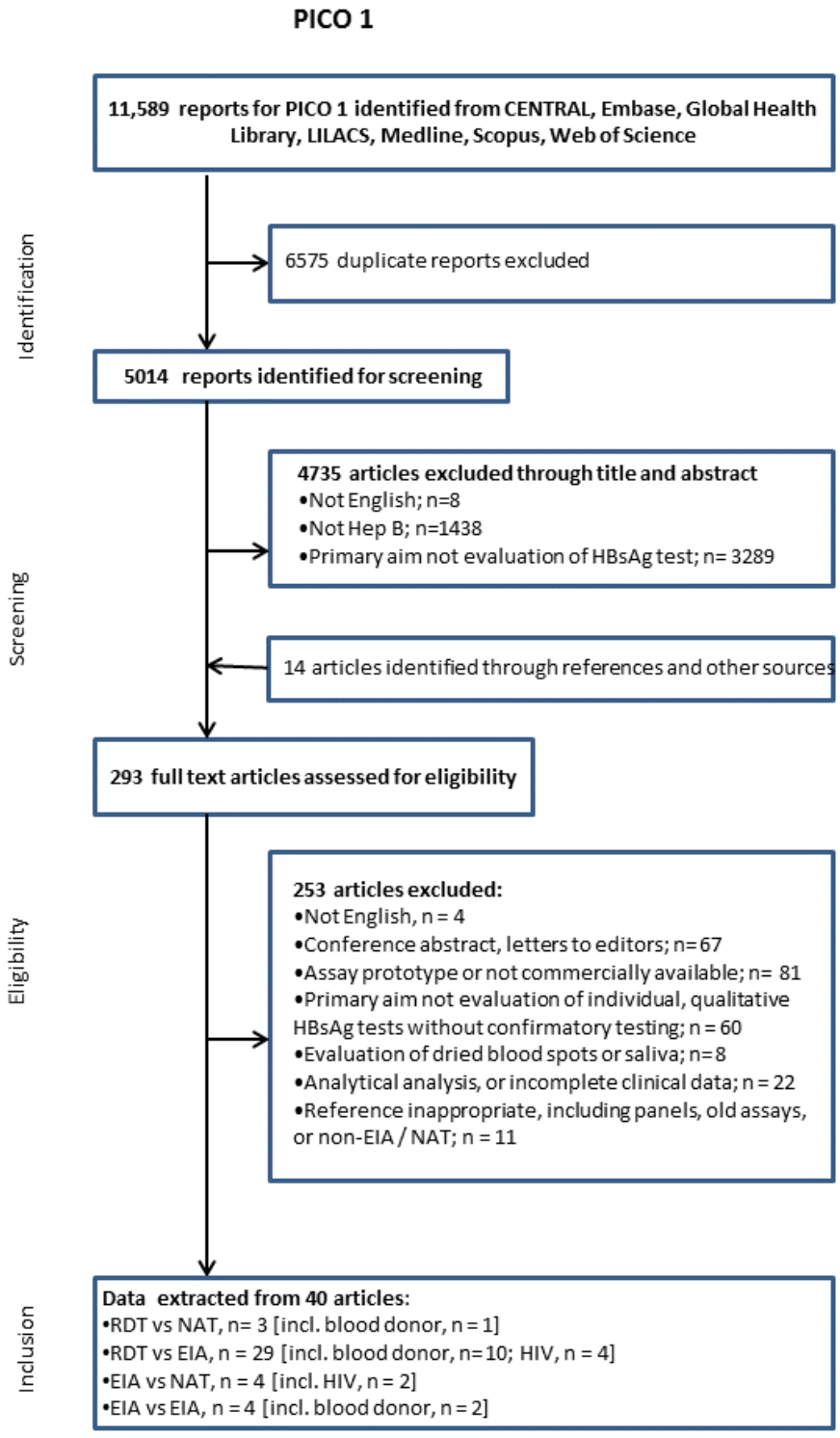
5. Results

Study selection

A total of 11 589 citations were identified and 6575 duplicates were removed. Each of the 5014 titles was examined according to pre-specified inclusion and exclusion criteria. A total of 33 research studies were included in the final primary analysis (Fig. 1), with studies comparing both rapid diagnostic tests (RDTs)[14–42] and enzyme immunoassays[43–46] against an immunoassay reference standard. Of these, 19 studies[14–31, 47] were also included in a recent systematic review by Khuroo et al.;[11] eight papers from that study were excluded as they were conference abstracts or letters to editors,[48–50] foreign language articles,[51, 52] or evaluated reference panels.[53] Two reports by WHO and the International Consortium for Blood Safety (ICBS) were also excluded as they were not published in peer-reviewed journals and were case–control studies constructed using reference panels from populations of affected individuals. Our search identified 11 additional articles[32–42] comparing RDTs against the EIA reference standard not found in the previous review. Six articles exclusively assessed accuracy in cohorts of HIV-positive individuals.[20, 36–38, 54, 55]

Studies evaluating laboratory-based immunoassays for HBsAg detection as the index test all used state of the art CMIAs as the reference standard. Seven studies were included in the supplementary analysis assessing diagnostic accuracy of HBsAg assays against a nucleic-acid based reference standard.[47, 54–59]

Fig. 1. PRISMA flow diagram outlining study selection examining diagnostic accuracy of HBsAg assays in our systematic review



Study characteristics

Immunoassay reference standard [Table 1a; 1b]

Overall, the 33 included articles originated in 23 countries: Australia,[35] Western Europe[16, 18], Western Africa;[15, 25, 28, 34, 36, 38–40] Eastern Africa;[19, 20, 26, 30, 41] South Africa;[38] South-eastern Asia;[24, 32] Eastern Asia;[25, 27, 31, 43] Southern Asia[14, 17, 21, 22, 29, 42] and the United States.[23] Thirty-three RDTs were evaluated using serum (fresh or frozen), plasma, venous and capillary whole blood. Sample sizes varied from 25 to 3928 individuals (mean, 631). Sixty-three data points were generated from the articles. All had bivariate data for analysis; 38% of data points were generated from case–control studies, while the remainder were from cross-sectional cohort studies. Prevalence of hepatitis B in populations tested ranged from 1.9% to 84%.

Populations studied included: general screening of healthy volunteers and blood donors; screening of at-risk populations such as pregnant women, incarcerated adults; and patients from hepatitis cohorts, including confirmed hepatitis B.

The majority of studies were performed in laboratory settings, with some studies performed in the field. A mixture of serum, plasma, capillary and whole blood was used for RDTs, with serum or plasma used for EIAs. Seven studies assessed performance using capillary or venous whole blood specifically.[15, 16, 20, 24, 28, 37, 40]

In terms of tests used, only the Determine HBsAg and BinaxNOW had more than 3 data points.

NAT reference standard [Table 8; Annex 9.2]

Overall, the 7 articles originating in 4 countries (Iran, Nigeria, Uganda, South Africa), contributed 18 data points and included serum or plasma samples from 3304 individuals, with sample size ranging from 74 to 950. All studies were either cross-sectional, cohort or case–control studies. All had data for bivariate analysis.

Table 1a. Study characteristics – EIA vs EIA

Study [Author, Year]	Location [Country, City]	Sample size	Study design	Setting	Sample	Assay under evaluation [Type, Brand][3]	Reference standard [Type, Brand][3]
Liu, 2014	China	250	CC	Hospital patients; outpatients (preselected based on CMIA quantitative results)	Serum	ECLIA, Cobas ELISA, Wantai	CMIA, Architect HBsAg
Peng, 2011	China	498	CC	Hospital patients (preselected based on S/CO from KHB screen)	Serum	ELISA, KHB	CMIA, Architect HBsAg
Geretti, 2010	Ghana, Kumasi	838	CS – CSQ	HIV clinic (1/3 on lamivudine)	Serum	CMIA, Architect HBsAg CMIA, Liaison Ultra EIA, Murex v3	CMIA, Architect/ Liaison EIA, Murex v3*
Ol, 2009	Cambodia	120	CS – CSQ	Blood donors (rural community)	Serum	ELISA, Monolisa	CMIA, Architect HBsAg
Viet, 2012	Vietnam	119	CS – CSQ	Blood donors (rural community)	Serum	EIA, Monolisa Ultra	CMIA, Architect HBsAg

*Reactive all three assays OR reactive in one assay with neutralisation

CMIA: chemiluminescent microparticle enzyme immunoassay; ECLIA: electrochemiluminescent immunoassay; EIA: enzyme immunoassay; ELISA: enzyme-linked immunosorbent assay; CC: case–control; CS: cross-sectional; CSQ: consecutive patients

[3] Abbreviated names for table clarity – full product names in Annex

Table 1b. Study characteristics – RDT vs EIA

Study [Author, Year]	Location [Country, City]	Sample size	Study design	Setting	Sample	RDT under evaluation [Type, Brand]	Reference test [Type, Brand]
Mvere, 1996	Zimbabwe	206	CS	Blood bank	S	Dipstick (PATH) SimpliRed	EIA, Auszyme
Sato, 1996	Japan	462	CC	Hospital	S	Dainascreen Serodia	EIA, Auszyme
Abraham, 1998	India, Vellore	50	CC – Panel	Hospital patients (Multiply transfused; chronic liver disease; preop and antenatal patients)	S	QuickChaser Virucheck	EIA, Auszyme or Hepanostika
		400	CS –Screen				
Oh, 1999	Korea	250	CC – Panel	Blood donor panel	S	Genedia Serodia	EIA, Cobas Core
Kaur, 2000	India	2754	CS – CSQ	Hospital surgery patients; blood donors; patients ruling out HBV	S	Hepacard	EIA, Ortho 3rd generation
Lien, 2000	Viet Nam	328	CC	High-risk volunteers; pregnant women; patients with other infectious diseases (including 10 with HIV); preselected HBsAg pos (101), HBsAg neg (99)	SP	Dainascreen Determine Serodia	EIA, Monolisa MEIA for discordant
Raj, 2001	India, Vellore	999	CS	Hospital laboratory samples (emergency preop screening; antenatal women in labour; haemodialysis; urgent donor screening)	S	Hepacard	EIA, Auszyme MEIA, AxSYM v2
Clement, 2002	Belgium	942	CC	Hospital - patients with biopsy-proven HBV; healthy volunteers from a vaccine evaluation trial; blood donors	WB, S	BinaxNOW	MEIA, AxSYM v2
Lau, 2003	USA	1011	CS – CSQ	Hepatology clinics	S fresh	Binax NOW	EIA, ETI-MAK2
		827	CS	Incarcerated offenders	S frozen		
		625	CS – CSQ	Chinese community health fair (random patients); known HBV-positive patients (liver clinic)	WB		
Akanmu, 2006	Nigeria, Lagos	101	CS – CSQ	Blood donors (male)	WB	Binax NOW	ELISA, Monolisa

		36		Chronic liver disease			
Nyirendra, 2008	Malawi	194	CS – CSQ	Hospital Hospital patients including 152 HIV+	P	Determine	EIA, Bioelisa Neutralisation (positives)
Lin, 2008	China	671	CC	Blood donors (500); Clinical specimens HBsAg+ (171)	S	Determine DRW	EIA, Hepanostika Ultra
	Guinea	579	CC	Blood donors (491); Stored positives (88)	SP		
Randrianirina, 2008	Madagascar	200	CC	Not specified	S	Cypress Determine Hexagon Virucheck	EIA, AxSYM
Ola, 2009	Nigeria	25	CS - CSQ	Medical clinic	WB	AMRAD GWHB	ELISA, Wellcozyme Kit
		55		Blood donors	S	Biotec Latex	
Khan, 2010	Pakistan	57	CC	NS	S	Accurate Onecheck	ELISA, 2nd generation
Davies, 2010	Malawi	75	CS – CSQ	HIV-positive adults (ART naive)	S	Determine	EIA, Biokit Neutralization (positives)
Bjoerkvoll, 2010	Cambodia	1200	CS – CSQ	General screen – blood donors (rural)	S	ACON	EIA, Monolisa Ultra*
	Viet Nam	1200					
Geretti, 2010	Ghana, Kumasi	838	CS – CSQ	HIV clinic (1/3 on lamivudine)	S	Determine VIKIA	CMIA, Architect/ Liason EIA, Murex v3
Hoffman, 2012	South Africa	973	CS – CSQ	HIV-positive adults (ART naïve) – antenatal or primary care	WB (cap)	Determine	ELISA, AxSYM
Bottero, 2013	France, Paris	2472	CS – CSQ	General screening (health-care centres) [general population prevention, screening, vaccination]	WB (ven)	Determine	ELISA, Monolisa Ultra Neutralization (positives)
		3922				QUICK PROFILE	
		3928				VIKIA	
Chameera, 2013	Sri Lanka	50	CS	Hospital (surgical, other)	S	Cortez	EIA, Surase B-96 (TMB)

						Onsite	
Franzeck, 2013	Tanzania, Ifakara	272	CS – CSQ	HIV clinic (ART naive)	WB (ven) P	Determine	EIA, Murex v3 Neutralization (positives)
Chevaliez, 2014	Unclear	558	CC	Chronic hep B (known mutants, blood donors); HBsAg negative (mix, including HIV, 34; HCV, 48)	SP	DRW v2.0	CMI, Architect
		408	CS	Acute hepatitis			
		802	CS	Pregnant - pregnant women at delivery			
Erhabor, 2014	Nigeria, Sokoto	130	CC	Blood donors	SP	ACON	ELISA, HBsAg Ultra
Gish, 2014	Australia, Melbourne	297	CS – CSQ	At risk health fairs, outreach; Vietnamese (72%)	S	Nanosign	EIA, Quest Diagnostics
Honge, 2014	Bissau	438	CS – CSQ	HIV clinic - mixed ART/ naive	S	VEDA LAB	CLIA, Architect
Liu, 2014	China	250	CC	Hospital patients; outpatients (preselected based on CMIA quantitative results)	S	Intec One Step	CMIA, Architect
Mutocheluh, 2014	Ghana	150	CS – CSQ	Blood donors	P	Abon Acull-Tell Core TM Rapid care Wondfo	ELISA, Human Gesellschaft
Upretti, 2014	Nepal	347	CS – CSQ	Children – pre- and post vaccination; mothers (8)	S	SD Bioline	EIA, Surase B-96 (TMB)
Njai, 2015	Gambia	178	CS	Hepatitis patients CHB carriers (study 3), incl 3 coinfected HIV (treatment naive)	S	Determine	CMIA (quantitative), Architect
		203				Espline	
		773	CS – CSQ	General community screen	WB	Determine	EIA (DBS), AxSym + Neutralisation
		476				VIKIA	

*Validated random sample with CMIA, Abbott

CC: case–control study; CS: cross-sectional study; CSQ: consecutive patients; S: serum; P: plasma; WB (cap): capillary whole blood; WB (ven): venous whole blood

Assessment of the quality of the studies

The methodological quality of included studies[4] is summarized [Fig. 2, Table 2].

Patient selection

We judged 15 studies to be at "high risk of bias". Of these, 10 were case–control studies. Others with a high risk of bias included studies in blood donors and highly selected populations, such as patients with known hepatitis B.

Applicability was judged to be "high risk" in 8 studies, notably those published over ten years ago or with tests which are no longer commercially available.

Index test

We judged 7 studies as high risk of bias, and 14 as unclear, with the most common reason being a lack of reported blinding while reading test results.

Reference standard

We judged 5 studies as high risk of bias, with 17 unclear; the most common reason was a lack of reported blinding interpreting reference tests, or utilisation.

Flow and timing

Bias was predominantly due to lack of reported flow and timing. Although the majority of studies did not specificity the exact time differences between performance of the index and reference assays, we can assume they were low risk as they were on the same sample.

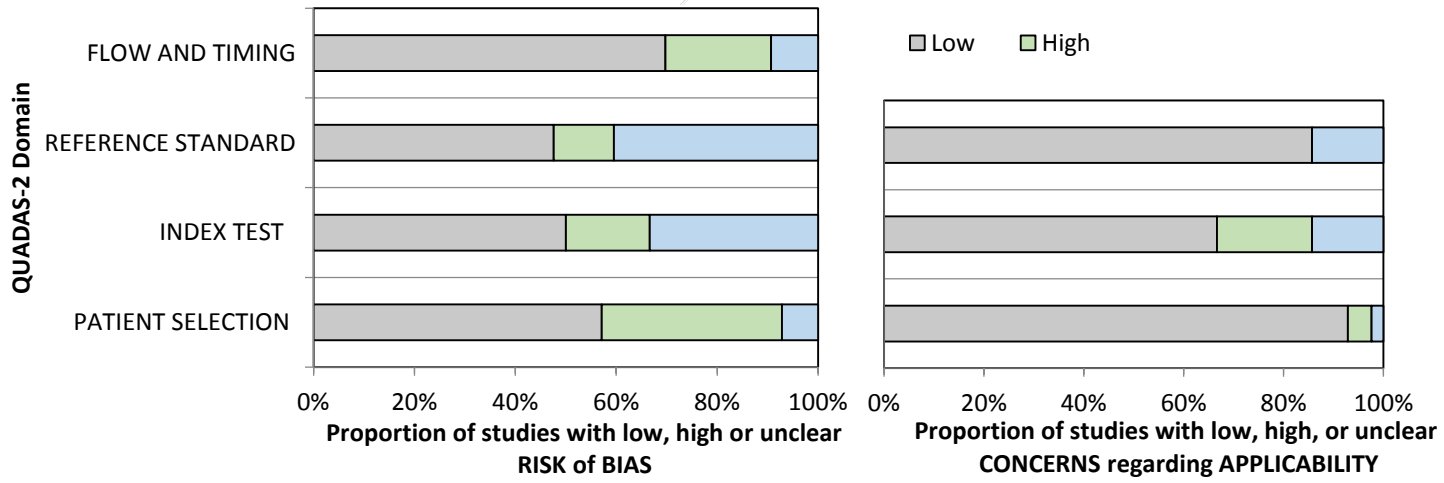
[4] Studies refers to either entire articles or individual sub-studies within a single publication using different patients, methods, index tests or reference standard.

Table 2. Risk of bias and applicability according to QUADAS-2 domains for individual studies

	Risk of bias				Applicability		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Abraham (CC)	High	Low	Low	Low	Low	High	Unclear
Abraham (CS)	Low	Low	Low	Low	Low	High	Unclear
Akanmu (Blood)	Low	Low	Low	Low	Low	Unclear	Low
Akanmu (Liver)	Low	Low	Low	Low	Low	Unclear	Low
Bjoerkvoll	Low	Low	Low	Low	Low	Low	Low
Bottero	Low	High	Low	Low	Low	Low	Low
Chameera	Low	High	Unclear	Low	Low	Low	Low
Chevaliez (CC)	High	Unclear	Unclear	Low	Low	Low	Low
Chevaliez (Hepatitis)	High	Unclear	Unclear	Low	Low	Low	Low
Chevaliez (Pregnant)	High	Unclear	Unclear	Low	Low	Low	Low
Clement	High	Unclear	High	Low	Low	Unclear	Low
Davies	Unclear	Low	Unclear	High	Low	Low	Low
Erhabor	High	Low	Unclear	Low	Low	Low	Low
Franzeck	Low	Low	Low	Low	Low	Low	Low
Geretti	Low	Unclear	Unclear	Unclear	Low	Low	Low
Gish	Low	Unclear	Unclear	Low	Low	Low	Low
Hoffman	Low	Unclear	Low	Unclear	Low	Low	Low
Honge	Low	Low	Unclear	Low	Low	Low	Low
Kaur	Low	Unclear	Unclear	Low	Low	High	Unclear
Khan	High	Low	Low	Low	Low	Low	Low
Lau (Hepatology clinic)	Low	Low	Low	Low	Low	Unclear	Low
Lau (Prison)	Low	Low	Low	Low	Low	Unclear	Low
Lau (Screen + Known)	Low	High	Low	Low	Low	Unclear	Low
Lien	Low	High	Low	Low	Low	Low	Low
Lin	High	Unclear	Unclear	Low	Low	Low	Low
Liu	High	Unclear	Low	High	Low	Low	Low
Mutocheluh	High	Low	Low	Low	Low	Low	Low
Mvere	Low	Low	Low	Low	Low	High	Unclear
Njai (1)	Low	Low	Low	Unclear	Low	Low	Low
Njai (2)	Low	Low	Low	Unclear	Low	Low	Low
Njai (3)	High	Low	Low	Low	Low	Low	Low

	Risk of bias				Applicability		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Nyirendra	Low	Low	Unclear	High	Low	Low	Low
Oh	High	High	High	Low	High	High	Unclear
Ol	Low	Unclear	Unclear	High	Low	Low	Low
Ola BD	Low	High	Unclear	Low	Low	High	Low
Ola Clinic	Low	Low	Unclear	Low	Low	Low	Low
Peng	High	Low	Unclear	High	Low	Low	Low
Raj	Unclear	Unclear	High	High	Low	High	Low
Randrianirina	High	Unclear	High	Low	Low	Low	Low
Sato	Unclear	High	Low	Low	Unclear	High	Unclear
Upretti	High	Low	High	High	High	Low	Low
Viet	Low	Unclear	Unclear	High	Low	Low	Low

Fig. 2. Risk of bias and applicability summary according to QUADAS-2 domains presented as percentages across included studies



Diagnostic accuracy

Overall clinical performance of assays against an immunoassay reference

Pooled test accuracy for RDTs compared to EIAs

A total of 21 studies[14–36, 38–43] contributing 63 data points evaluated 25 brands of RDTs using 15 EIA reference assays, with 36 919 total samples, including serum, plasma, venous and capillary whole blood. Sample sizes ranged from 25 to 3983 (mean 586). Sensitivities ranged from 50% to 100% with overall pooled sensitivity of 90.0% (95% CI: 89.1, 90.8). Specificities ranged from 69% to 100%, with overall pooled specificity of 99.5% (95% CI: 99.4, 99.5). Pooled PLR and NLR were 117.5 (95% CI: 67.7, 204.1) and 0.095 (95% CI 0.067, 0.136), respectively, with tau-square 3.89, 1.72, respectively, suggestive of significant heterogeneity between studies [Fig. 3, Table3].

Pooled test accuracy for EIAs compared to other immunoassays

Five studies[36, 43–46] contributed 8 data points evaluating 8 EIAs with reference to state-of-the-art immunoassays alone, using a total 3751 serum samples, with sample sizes ranging from 119 to 838 (mean, 469). Studies were performed in China, Ghana, Cambodia and Viet Nam. Sensitivities ranged from 73% to 100%% with overall pooled sensitivity of 88.9% (95% CI: 87.0, 90.6). Specificities ranged from 88% to 100%, with overall pooled specificity of 98.4% (95% CI: 97.8, 98.8). Pooled PLR and NLR were 46.76 (95% CI: 12.86, 170.03) and 0.041 (95% CI: 0.013, 0.134), respectively, with tau-square 2.95, 2.46, respectively, suggestive of significant heterogeneity between studies [Fig. 4, Table 3].

Overall clinical performance of assays against a NAT reference

Pooled test accuracy for RDTs compared to NAT

Three articles[47, 57, 59] contributed 9 data points evaluating 7 RDTs with a NAT reference, using a total 1710 serum or plasma samples, with sample sizes ranging from 74 to 950 (mean 190). Of note, 1440 were from the same 240 patients in one case–control study evaluating 6 tests. Only one study (Nna) [57] used plasma. Sensitivities ranged from 38% to 99% with overall pooled sensitivity of 93.3% (95% CI: 91.3, 94.9). Specificities ranged from 94% to 99%, with overall pooled specificity of 98.1% (95% CI: 97.0, 98.9). Pooled PLR and NLR were 39.42 (95% CI: 22.148, 70.185) and 0.051 (95% CI: 0.009, 0.275), respectively, with tau-square 0.2163, 6.264, respectively. [Fig. 5, Table 9; Fig. 8 are in the Annex]

Pooled test accuracy for EIAs compared to NAT

Five articles[54–56, 58, 59] contributed 9 data points evaluating EIAs with a NAT reference, using a total 1594 samples, with sample sizes ranging from 74 to 240 (mean, 177). Sensitivities ranged from 38% to 98%% with overall pooled sensitivity of 75.7% (95% CI: 72.1, 79.1). Specificities ranged from 70% to 98%, with overall pooled specificity of 86.1% (95% CI: 83.8, 88.2). Pooled PLR and NLR were 7.234 (95% CI: 4.441, 11.758) and 0.296 (95%CI: 0.192, 0.458), respectively,

with tau-square 0.3748, 0.3299, respectively suggesting acceptable interstudy heterogeneity. [Fig. 6, Table 9; and Fig. 9 are in the Annex]

Clinical performance of assays in HIV-positive and negative individuals

Pooled test accuracy for RDTs in HIV-positive individuals

Five articles[19, 20, 36–38] contributed 6 data points evaluating 3 RDTs with an EIA reference, using a total 3434 samples from 2566 patients, with sample sizes ranging from 75 to 838. Sensitivities ranged from 62% to 100% with overall pooled sensitivity of 72.3% (95% CI: 67.9, 76.4). Specificities ranged from 99% to 100%, with overall pooled specificity of 99.8% (95% CI: 99.5, 99.9). Pooled PLR and NLR were 192.63 (95% CI: 77.4, 479.17) and 0.288 (95%CI: 0.217, 0.381), respectively, with tau-square 0.3838, 0.0585, respectively [Table 4 and 5; Fig. 11, Annex 9.3].

Pooled test accuracy for RDTs in HIV-negative individuals

One article[40] contributed 4 data points evaluating 3 RDTs with an EIA reference, using a total 1624 samples from 997 patients, with sample sizes ranging from 175 to 773. Sensitivities ranged from 88% to 95% with overall pooled sensitivity of 92.6% (95% CI: 89.8, 94.8). Specificities ranged from 93% to 100%, with overall pooled specificity of 99.6% (95% CI: 99.0, 99.9). Pooled PLR and NLR were 79.449 (95% CI: 11.575, 545.315) and 0.082 (95%CI: 0.053, 0.125), respectively, with tau-square 2.9668, 0.0803 respectively [Table 4 and 5; Fig. 12, Annex 9.3].

Pooled test accuracy for EIAs in HIV-positive individuals

One article[36] contributed 3 data points evaluating 3 EIAs with an EIA reference. 838 samples were tested with each index test. Sensitivities ranged from 97% to 99% with overall pooled sensitivity of 97.9% (95% CI: 96.0, 99.0). Specificities ranged from 99% to 100%, with overall pooled specificity of 99.4% (95% CI: 99.0, 99.7). Pooled PLR and NLR were 167.26 (95% CI: 95.135, 294.07) and 0.022 (95% CI: 0.012, 0.043), respectively, with tau-square <0.005, <0.005, respectively [Table 4; Fig. 14, Annex 9.3.]

Clinical performance of RDTS compared to EIAs in other stratified subgroups

Pooled test accuracy for RDTs using whole blood (capillary or venous)

Seven studies[15, 16, 20, 24, 28, 37, 40] contributed 11 data points evaluating 5 RDTs with an EIA reference, using a total 13731 samples, with sample sizes ranging from 25 to 3928 (mean 722). Sensitivities ranged from 75% to 100% with overall pooled sensitivity of 91.7% (95% CI: 89.1, 93.9). Specificities ranged from 99% to 100%, with overall pooled specificity of 99.9% (95% CI: 99.8, 99.9). Pooled PLR and NLR were 346.64 (95% CI: 157.598, 762.42) and 0.089 (95%CI: 0.058, 0.136), respectively, with tau-square 0.8124, 0.2367, respectively [Table 5; Fig. 18, Annex 9.3.2].

Pooled test accuracy for RDTs in studies using a case-control design

Ten articles[14, 18, 22, 24, 25, 27, 30, 31, 33, 34] contributed 21 data points evaluating 13 RDTs with an EIA reference, using a total 7258 samples, with sample sizes ranging from 50 to 698 (mean 345). Sensitivities ranged from 50% to 100% with overall pooled sensitivity of 96.7% (95% CI: 96.0, 97.3). Specificities ranged from 91% to 100%, with overall pooled specificity of 99.3% (95% CI: 99.0, 99.5). Pooled PLR and NLR were 105.16 (95% CI: 48.038, 230.212) and 0.028 (95%CI: 0.010, 0.076), respectively, with tau-square 2.2261, 4.8632, respectively. Of note, one study[22] had significantly lower sensitivity for both index tests evaluated, with otherwise sensitivities ranging from 90% to 100% in remaining studies [Table 5; Fig. 19, Annex 9.3.3].

Pooled test accuracy for RDTs used in blood donors

Seven articles[25–28, 32, 34, 39] contributed 19 data points evaluating 15 RDTs with an EIA reference, using a total 6881 samples, with sample sizes ranging from 25 to 1200 (mean, 362). Sensitivities ranged from 50% to 100% with overall pooled sensitivity of 91.6% (95% CI: 90.1, 92.9). Specificities ranged from 86% to 100%, with overall pooled specificity of 99.5% (95% CI: 99.3, 99.). Pooled PLR and NLR were 89.219 (95% CI: 32.782, 242.818) and 0.106 (95%CI: 0.055, 0.204), respectively, with tau-square 3.8171, 1.8505, respectively. [Table 5; Fig. 20, Annex 9.3.4]

Pooled test accuracy for RDTs published before and after 2005

Twenty-one articles[15–17, 19, 20, 22, 25, 28, 30, 32–43] contributed 44 data points evaluating 26 RDTs with an EIA reference, using a total 25 261 samples, with sample sizes ranging from 25 to 3928 (mean 574). Sensitivities ranged from 50% to 100 % with overall pooled sensitivity of 86.4% (95% CI: 85.2, 87.5). Specificities ranged from 69% to 100%, with overall pooled specificity of 99.4% (95% CI: 99.2, 99.5). Pooled PLR and NLR were 84.657 (95% CI: 43.553, 164.553) and 0.126 (95%CI: 0.087, 0.183), respectively, with tau-square 4.0986, 1.2712, respectively.

Nine articles published before 2005[14, 18, 21, 23, 24, 26, 27, 29, 31] contributed 19 data points evaluating 10 RDTs with an EIA reference, using a total 25 253 samples, with sample sizes ranging from 25 to 3928 (mean 1122). Sensitivities ranged from 77% to 100% with overall pooled sensitivity of 96.9% (95% CI: 96.0, 97.7). Specificities ranged from 97% to 100%, with overall pooled specificity of 99.7% (95% CI: 99.6, 99.8). Pooled PLR and NLR were 265.5 (95% CI: 106.1, 664.5) and 0.056 (95%CI: 0.033, 0.095), respectively, with tau-square 2.72, 0.91, respectively. [Table 5; Figs 21 and 22, Annex 9.3.4]

Pooled test accuracy for RDTs by brand

Stratifying by test brand did not eliminate statistical heterogeneity. Data for all 50 brands used [Table 6] demonstrate heterogeneous results for sensitivity, with more robust specificity as previously noted.

Determine HBsAg was evaluated in the most studies, with only one published before 2008. Ten articles[16, 19, 20, 24, 25, 30, 36, 37, 40, 41] contributing 12 data points evaluated against an EIA reference, using a total 7553 samples, with sample sizes ranging from 75 to2472. Sensitivities ranged from 56% to 100% with overall pooled sensitivity of 90.8% (95% CI: 88.9, 92.4).

Specificities ranged from 69% to 100%, with overall pooled specificity of 99.1% (95% CI: 98.9, 99.4). Excluding one particularly anomalous study,[41] the lowest sensitivities and specificities would be 69% and 93%, respectively. Pooled PLR and NLR were 239.24 (95% CI: 17.139, 33339.4) and 0.077 (95%CI: 0.035, 0.168), respectively, with tau-square 20.17, 1.556, respectively. [Table 5, 6]

BinaxNOW HBsAg was evaluated in three articles, *(15, 18, 23)* all published before 2007, contributing 6 data points evaluating against an EIA reference, using a total 3550 samples, with sample sizes ranging from 36 to 1011. Sensitivities ranged from 94% to 100% with overall pooled sensitivity of 97.6% (95% CI: 96.2, 98.6). Specificity was 100% in all studies, with overall pooled specificity of 100% (95% CI: 99.7, 100). Pooled PLR and NLR were 221.21 (95% CI: 36.160, 1354.1) and 0.045 (95%CI: 0.016, 0.128), respectively, with tau-square 3.53, 1.20, respectively. [Tables 5, 6]

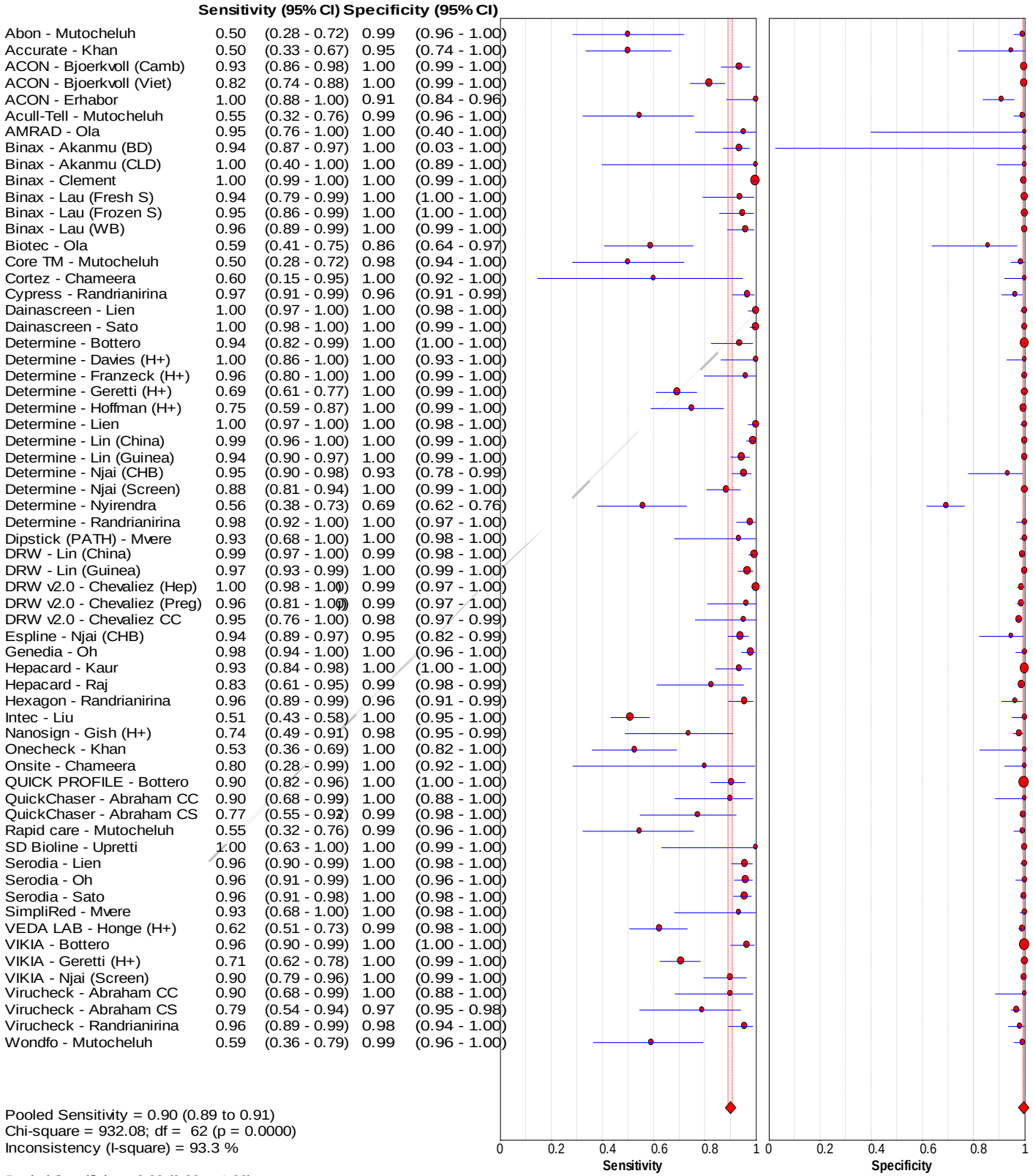
VIKIA HBsAg was also evaluated in three articles, [16, 36, 40] all published after 2010, contributing 3 data points evaluating against an EIA reference, using a total 5242 samples, with sample sizes ranging from 476 to 3928. Sensitivities ranged from 71% to 97% with overall pooled sensitivity of 82.5% (95% CI: 77.5, 86.7). Specificities ranged from 99.8% to 100%, with overall pooled specificity of 99.9% (95% CI: 99.8, 100). Pooled PLR and NLR were 1072.3 (95% CI: 376.082, 3057.2) and 0.108 (95%CI: 0.026, 0.458), respectively, with tau-square <0.005, 1.472, respectively. [Tables 5, 6]

Serodia HBsAg was also evaluated in three articles[24, 27, 31] all published before 2000, contributing 3 data points evaluating against an EIA reference, using a total 1040 samples. Sensitivities ranged from 71% to 97% with overall pooled sensitivity of 82.5% (95% CI: 77.5, 86.7). Specificities ranged from 99.8% to 100%, with overall pooled specificity of 99.9% (95% CI: 99.8, 100). Pooled PLR and NLR were 284.91 (95% CI: 71.42, 1136.6) and 0.045 (95%CI: 0.029, 0.069), respectively, with tau-square <0.005, <0.005, respectively. [Tables 5, 6]

Pooled accuracy of RDTs evaluated against CMIA reference

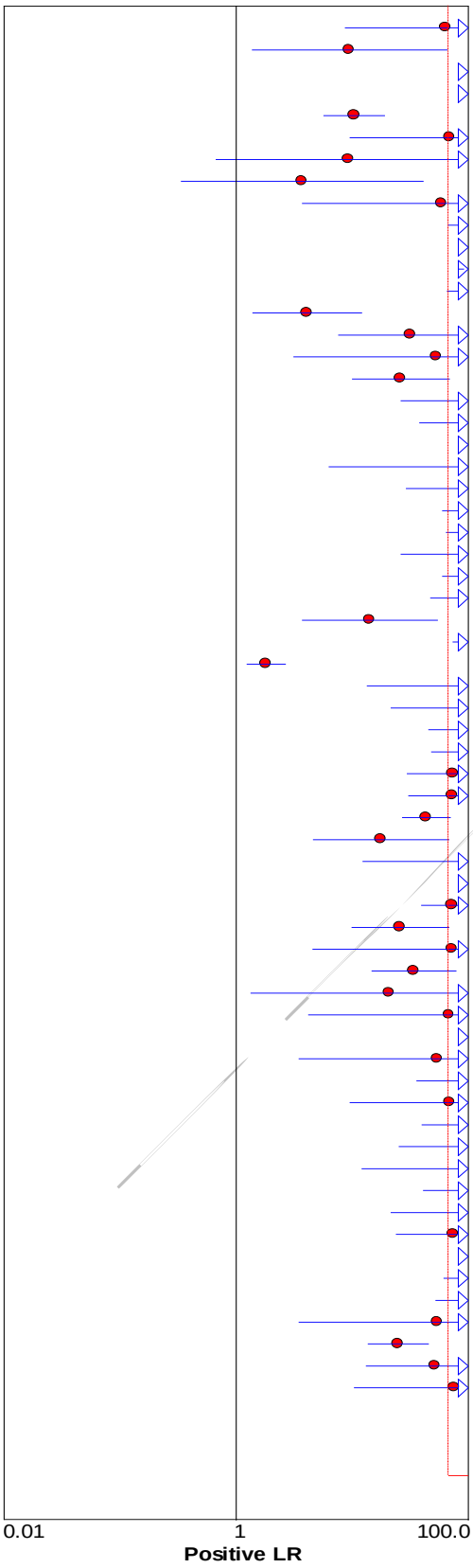
Five articles[33, 36, 38, 40, 43] contributed 9 data points evaluating 6 RDTs against specifically a CMIA reference, using a total 4513 samples, with sample sizes ranging from 178 to 838 (mean 501). Sensitivities ranged from 62% to 100% with overall pooled sensitivity of 80.4% (95% CI: 77.9, 82.6). Specificities ranged from 93% to 100%, with overall pooled specificity of 99.0% (95% CI: 98.6, 99.3). Pooled PLR and NLR were 58.5 (95% CI: 31.3, 109.3) and 0.141 (95%CI: 0.074, 0.268), respectively, with tau-square 0.4375, 0.7337, respectively. [Table 5]

Fig. 3. Forest plots, RDT vs EIA, ordered by [Test, Author]*



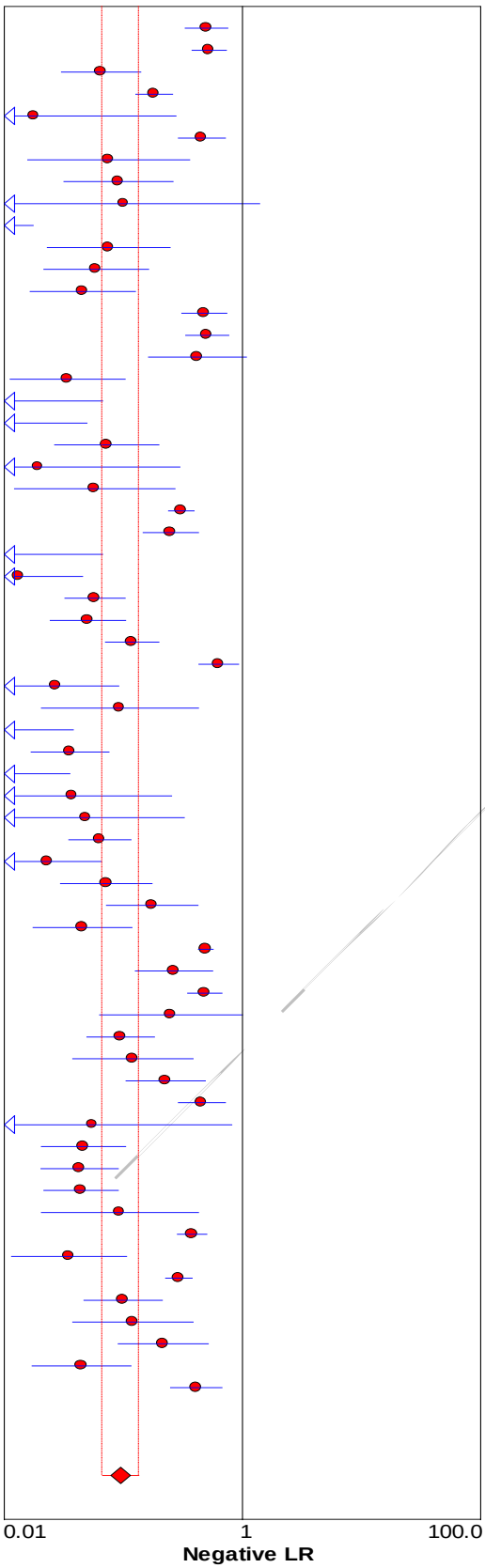
Pooled Sensitivity = 0.90 (0.89 to 0.91)
Chi-square = 932.08; df = 62 (p = 0.0000)
Inconsistency (I-square) = 93.3 %

Pooled Specificity = 0.99 (0.99 to 1.00)
Chi-square = 651.98; df = 62 (p = 0.0000)
Inconsistency (I-square) = 90.5 %



	Positive LR (95% CI)
Abon - Mutocheluh	64.00 (8.69 - 471.26)
Accurate - Khan	9.50 (1.37 - 65.71)
ACON - Bjoerkvoll (Camb)	517.87 (129.54 - 2,070.26)
ACON - Bjoerkvoll (Viet)	434.51 (108.57 - 1,739.03)
ACON - Erhabor	10.46 (5.70 - 19.19)
Acull-Tell - Mutocheluh	69.82 (9.55 - 510.36)
AMRAD - Ola	9.32 (0.67 - 129.54)
Binax - Akanmu (BD)	3.72 (0.34 - 41.10)
Binax - Akanmu (CLD)	59.40 (3.74 - 944.36)
Binax - Clement	268.83 (67.41 - 1,072.16)
Binax - Lau (Fresh S)	1,811.52 (113.19 - 28,991.24)
Binax - Lau (Frozen S)	1,448.64 (90.62 - 23,156.72)
Binax - Lau (WB)	1,051.25 (65.81 - 16,793.27)
Biotec - Ola	4.12 (1.39 - 12.18)
Core TM - Mutocheluh	32.00 (7.60 - 134.68)
Cortez - Chameera	53.67 (3.14 - 917.12)
Cypress - Randrianirina	26.35 (10.06 - 69.00)
Dainascreen - Lien	422.20 (26.49 - 6,728.17)
Dainascreen - Sato	608.08 (38.12 - 9,699.86)
Determine - Bottero	4,498.21 (281.12 - 71,976.88)
Determine - Davies (H+)	100.04 (6.34 - 1,578.57)
Determine - Franzeck (H+)	467.38 (29.27 - 7,463.70)
Determine - Geretti (H+)	966.70 (60.39 - 15,473.86)
Determine - Hoffman (H+)	174.94 (64.74 - 472.74)
Determine - Lien	422.20 (26.49 - 6,728.17)
Determine - Lin (China)	959.01 (60.07 - 15,311.15)
Determine - Lin (Guinea)	753.59 (47.21 - 12,030.12)
Determine - Njai (CHB)	14.29 (3.74 - 54.54)
Determine - Njai (Screen)	1,180.48 (73.85 - 18,870.34)
Determine - Nyirendra	1.82 (1.25 - 2.67)
Determine - Randrianirina	214.02 (13.47 - 3,400.91)
Dipstick (PATH) - Mvere	348.00 (21.75 - 5,568.55)
DRW - Lin (China)	120.60 (45.44 - 320.04)
DRW - Lin (Guinea)	771.27 (48.32 - 12,311.21)
DRW v2.0 - Chevaliez (Hep)	74.50 (29.76 - 186.52)
DRW v2.0 - Chevaliez (Preg)	73.38 (30.62 - 175.83)
DRW v2.0 - Chevaliez CC	43.75 (27.08 - 70.69)
Espline - Njai (CHB)	17.85 (4.63 - 68.81)
Genedia - Oh	197.32 (12.43 - 3,133.39)
Hepacard - Kaur	4,996.94 (312.36 - 79,938.56)
Hepacard - Raj	73.30 (39.56 - 135.82)
Hexagon - Randrianirina	26.05 (9.95 - 68.23)
Intec - Liu	73.20 (4.61 - 1,163.40)
Nanosign - Gish (H+)	34.14 (14.80 - 78.75)
Onecheck - Khan	21.03 (1.34 - 329.93)
Onsite - Chameera	69.00 (4.22 - 1,129.19)
QUICK PROFILE - Bottero	347.25 (186.27 - 647.36)
QuickChaser - Abraham CC	54.62 (3.48 - 857.69)
QuickChaser - Abraham CS	146.05 (35.99 - 592.65)
Rapid care - Mutocheluh	69.82 (9.55 - 510.36)
SD Bioline - Upretti	642.22 (40.07 - 10,293.22)
Serodia - Lien	404.24 (25.36 - 6,443.55)
Serodia - Oh	193.30 (12.17 - 3,069.96)
Serodia - Sato	290.53 (41.05 - 2,056.47)
SimpliRed - Mvere	348.00 (21.75 - 5,568.55)
VEDA LAB - Honge (H+)	75.01 (23.99 - 234.59)
VIKIA - Bottero	1,853.68 (463.49 - 7,413.61)
VIKIA - Geretti (H+)	986.53 (61.64 - 15,789.02)
VIKIA - Njai (Screen)	374.40 (52.77 - 2,656.47)
Virucheck - Abraham CC	54.62 (3.48 - 857.69)
Virucheck - Abraham CS	25.07 (13.71 - 45.82)
Virucheck - Randrianirina	52.10 (13.19 - 205.84)
Wondfo - Mutocheluh	75.64 (10.41 - 549.47)

Random Effects Model
Pooled Positive LR = 117.54 (67.69 to 204.12)
Cochran-Q = 658.59; df = 62 (p = 0.0000)
Inconsistency (I-square) = 90.6 %
Tau-squared = 3.8937



	Negative LR	(95% CI)
Abon - Mutocheluh	0.50	(0.33 - 0.77)
Accurate - Khan	0.53	(0.38 - 0.74)
ACON - Bjoerkvoll (Camb)	0.07	(0.03 - 0.14)
ACON - Bjoerkvoll (Viet)	0.18	(0.13 - 0.26)
ACON - Erhabor	0.02	(0.00 - 0.28)
Acull-Tell - Mutocheluh	0.46	(0.29 - 0.72)
AMRAD - Ola	0.08	(0.02 - 0.36)
Binax - Akanmu (BD)	0.09	(0.03 - 0.26)
Binax - Akanmu (CLD)	0.10	(0.01 - 1.41)
Binax - Clement	0.00	(0.00 - 0.02)
Binax - Lau (Fresh S)	0.08	(0.02 - 0.25)
Binax - Lau (Frozen S)	0.06	(0.02 - 0.16)
Binax - Lau (WB)	0.05	(0.02 - 0.13)
Biotec - Ola	0.48	(0.31 - 0.74)
Core TM - Mutocheluh	0.51	(0.33 - 0.77)
Cortez - Chameera	0.42	(0.16 - 1.09)
Cypress - Randrianirina	0.03	(0.01 - 0.10)
Dainascreen - Lien	0.00	(0.00 - 0.07)
Dainascreen - Sato	0.00	(0.00 - 0.05)
Determine - Bottero	0.07	(0.03 - 0.20)
Determine - Davies (H+)	0.02	(0.00 - 0.30)
Determine - Franzeck (H+)	0.06	(0.01 - 0.27)
Determine - Geretti (H+)	0.31	(0.24 - 0.40)
Determine - Hoffman (H+)	0.25	(0.15 - 0.43)
Determine - Lien	0.00	(0.00 - 0.07)
Determine - Lin (China)	0.01	(0.00 - 0.05)
Determine - Lin (Guinea)	0.06	(0.03 - 0.10)
Determine - Njai (CHB)	0.05	(0.02 - 0.11)
Determine - Njai (Screen)	0.12	(0.07 - 0.20)
Determine - Nyirendra	0.64	(0.43 - 0.94)
Determine - Randrianirina	0.03	(0.01 - 0.09)
Dipstick (PATH) - Mvere	0.09	(0.02 - 0.43)
DRW - Lin (China)	0.01	(0.00 - 0.04)
DRW - Lin (Guinea)	0.04	(0.02 - 0.08)
DRW v2.0 - Chevaliez (Hep)	0.00	(0.00 - 0.04)
DRW v2.0 - Chevaliez (Preg)	0.04	(0.01 - 0.26)
DRW v2.0 - Chevaliez CC	0.05	(0.01 - 0.33)
Espline - Njai (CHB)	0.06	(0.03 - 0.12)
Genedia - Oh	0.02	(0.01 - 0.07)
Hepacard - Kaur	0.07	(0.03 - 0.18)
Hepacard - Raj	0.18	(0.07 - 0.43)
Hexagon - Randrianirina	0.05	(0.02 - 0.12)
Intec - Liu	0.50	(0.43 - 0.58)
Nanosign - Gish (H+)	0.27	(0.13 - 0.57)
Onecheck - Khan	0.49	(0.35 - 0.68)
Onsite - Chameera	0.25	(0.06 - 1.01)
QUICK PROFILE - Bottero	0.10	(0.05 - 0.18)
QuickChaser - Abraham CC	0.12	(0.04 - 0.39)
QuickChaser - Abraham CS	0.23	(0.11 - 0.49)
Rapid care - Mutocheluh	0.46	(0.29 - 0.72)
SD Bioline - Upretti	0.06	(0.00 - 0.82)
Serodia - Lien	0.05	(0.02 - 0.11)
Serodia - Oh	0.04	(0.02 - 0.09)
Serodia - Sato	0.04	(0.02 - 0.09)
SimpliRed - Mvere	0.09	(0.02 - 0.43)
VEDA LAB - Honge (H+)	0.38	(0.28 - 0.51)
VIKIA - Bottero	0.04	(0.01 - 0.11)
VIKIA - Geretti (H+)	0.29	(0.23 - 0.38)
VIKIA - Njai (Screen)	0.10	(0.05 - 0.21)
Virucheck - Abraham CC	0.12	(0.04 - 0.39)
Virucheck - Abraham CS	0.22	(0.09 - 0.52)
Virucheck - Randrianirina	0.04	(0.02 - 0.12)
Wondfo - Mutocheluh	0.41	(0.25 - 0.68)

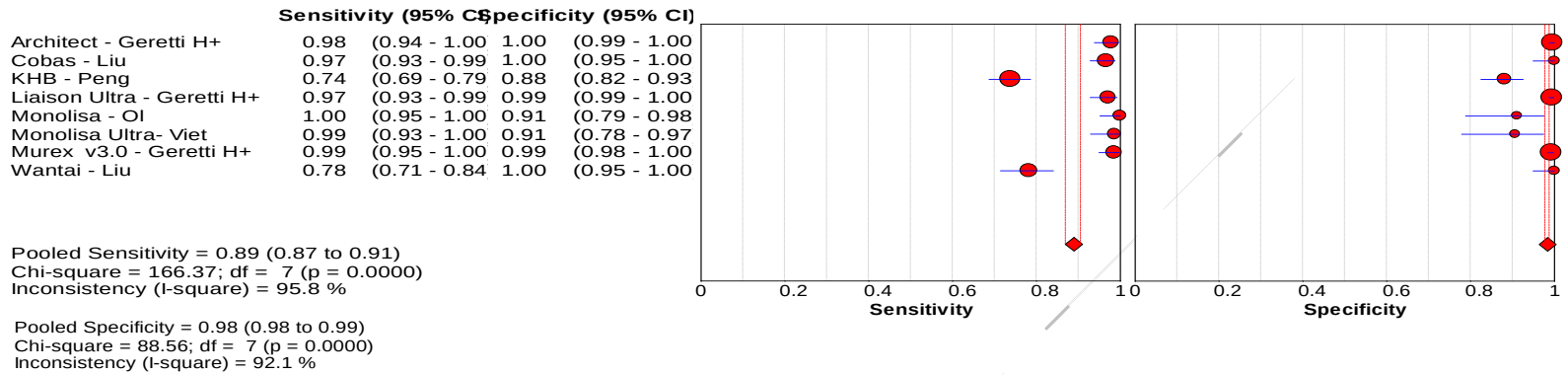
Random Effects Model
Pooled Negative LR = 0.10 (0.07 to 0.14)
Cochran-Q = 1298.04; df = 62 (p = 0.0000)
Inconsistency (I-square) = 95.2 %
Tau-squared = 1.7188

Key brands of RDT and reference standards used in studies

Study	Test brand (manufacturer)	Reference test type, brand (manufacturer)
Mvere, 1996	Dipstick (PATH) SimpliRed	EIA, Auszyme
Sato, 1996	Dainascreen HBsAG Serodia HBsAg	EIA, Auszyme
Abraham, 1998	QuickChaser Virucheck	EIA, Auszyme or Hepanostika
Oh, 1999	Genedia Serodia	EIA, Cobas Core
Kaur, 2000	Hepacard	EIA, Ortho 3rd generation
Lien, 2000	Dainascreen Determine HBsAg Serodia	EIA, Monolisa MEIA for discordant
Raj, 2001	Hepacard	EIA, Auszyme MEIA, AxSYM v2
Clement, 2002	BinaxNOW	MEIA, AxSYM v2
Lau, 2003	BinaxNOW	EIA, ETI-MAK2
Akanmu, 2006	BinaxNOW	ELISA, Monolisa
Bjoerkvoll, 2010	ACON	EIA, Monolisa Ultra*
Lin, 2008	Determine HBsAg DRW HBsAg	EIA, Hepanostika Ultra
Nyirendra, 2008	Determine HBsAG	EIA, Bioelisa Neutralisation (positives)
Randrianirina, 2008	Cypress Determine HBsAg Hexagon Virucheck	EIA, AxSYM
Ola, 2009	AMRAD GWHB Biotec Latex	ELISA, Wellcozyme Kit
Davies, 2010	Determine	EIA, Biokit; Neutralisation (for all positives)
Geretti, 2010	Determine VIKIA	CMIA, Architect/ Liason EIA, Murex v3
Khan, 2010	Accurate Onecheck	ELISA, 2nd generation

Study	Test brand (manufacturer)	Reference test type, brand (manufacturer)
Hoffman, 2012	Determine	ELISA, AxSYM
Bottero, 2013	Determine QUICK PROFILE VIKIA	ELISA, Monolisa Ultra Neutralisation (for all positive)
Franzeck, 2013	Determine HBsAg	EIA, Murex v3 Neutralisation (for all positives)
Chameera, 2013	Cortez Onsite	EIA, Surase B-96 (TMB)
Chevaliez, 2014	DRW v 2 HBsAg	CMIA, Architect
Erhabor, 2014	ACON	ELISA, HBsAg Ultra
Gish, 2014	Nanosign	EIA, Quest Diagnostics
Honge, 2014	VEDA LAB	CLIA, Architect
Liu, 2014	Intec One Step	CMIA, Architect
Upretti, 2014	SD Bioline	EIA, Surase B-96 (TMB)
Mutocheluh, 2014	Abon Acull-Tell Core-TM Rapid care Wondfo	ELISA, Human Gesellschaft
Njai, 2015	Determine HBsAg VIKIA Espline	EIA (DBS), AxSym Neutralisation CMIA (quantitative), Architect

Fig. 4. Forest plots, EIA vs EIA, ordered by [Test, Author]**

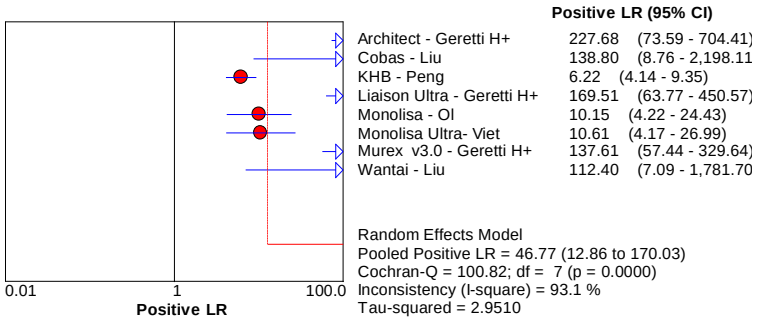


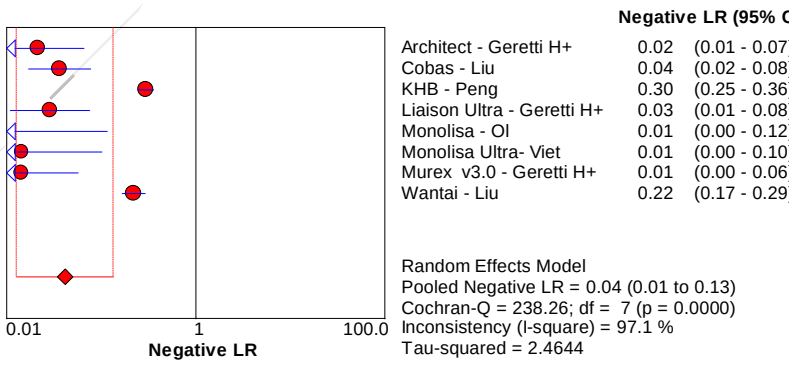
$**H^+$ = HIV positive

Key – Types of EIA and reference standards used in studies

Study	Test type, brand (manufacturer)	Reference
Geretti	EIA, Murex v.3.0 (Abbott) CMIA, Architect (Abbott) CMIA, Liaison Ultra (Diasorin)	CMIA and EIA (agreement) or neutralization
Liu	ELISA, Wantai (Beijing Wantai) ECLIA, Cobas e601 (Roche)	CMIA, Architect (Abbott)

OI	ELISA, Monolisa (bioRad)	CMIA, Architect (Abbott)
Viet	EIA, Monolisa Ultra (bioRad)	CMIA, Architect (Abbott)
Peng	ELISA, KHB (Kehua Bio-engineeering Co)	CMIA, Architect (Abbott)





Abbreviations used in Forest plots

*Camb: Cambodia; Viet: Viet Nam; BD: blood donor study; CLD: chronic liver disease study; Fresh S: fresh serum; Frozen S: frozen serum; WB: whole blood; H^+: HIV positive; CHB: chronic hepatitis B cohort; Screen: general screen cohort; Hep: acute hepatitis cohort; Preg: antenatal cohort; CC: case–control study; CS: cross-sectional study

**H^+ : HIV positive

Table 3. Summary pooled diagnostic accuracy of HBsAg assays using EIA and NAT reference standards

Reference	Index test	Pooled clinical accuracy			Likelihood ratios (REM)[5]		Heterogeneity (Tau-squared)	
		n	*Sen (95% CI)*	*Spec (95% CI)*	*PLR (95% CI)*	*NLR (95% CI)*	*PLR*	*NLR*
EIA	RDT	63	90.0 (89.1–90.8)	99.5 (99.4–99.5)	118 (67.7–204)	0.095 (0.067–0.136)	3.89	1.72
	EIA	8	88.9 (87.0–90.6)	98.4 (97.8–98.8)	46.8 (12.9–170)	0.041 (0.013–0.134)	2.95	2.46
NAT	RDT	9	93.3 (91.3–94.9)	98.1 (97.0–98.9)	39.4 (22.1–70.2)	0.051 (0.009–0.27)	0.22	6.26
	EIA	9	75.7 (72.1–79.1)	86.1 (83.8–88.2)	7.23 (4.44–11.8)	0.296 (0.192—0.458)	0.37	0.33

**n* = number of data points

[5] REM : Random effects model

Table 4. Summary pooled diagnostic accuracy of HBsAg assays in patients with known HIV status

Test type	HIV status	Pooled clinical accuracy			Likelihood ratios (REM)		Heterogeneity (Tau-squared)	
		n	Sen (95% CI)	Spec (95% CI)	PLR (95% CI)	NLR (95% CI)	PLR	NLR
RDT	HIV^{+}[6]	6	72.3 (67.9–76.4)	99.8 (99.5–99.9)	193 (77.4–497)	0.29 (0.22–0.38)	0.384	0.0059
	HIV^{-}	3	92.6 (89.8–94.8)	99.6 (99.0–99.9)	79.5 (11.6–545)	0.08 (0.05–0.13)	2.967	0.080
EIA	HIV^{+}	3	97.9 (96.0–99.0)	99.4 (99.0–99.7)	167 (95.1–294)	0.02 (0.01–0.04)	<0.005	<0.005
	HIV^{-}							

[6] Three studies on ART-naive patients (Hoffman, Davies, Franzeck); two studies from single article (Geretti) in patients who included 1/3 on lamivudine; with one study (Honge) on a mixture

9.1.1.**Table 5. Summary pooled diagnostic accuracy of rapid HBsAg assays stratified by study, patient, index and reference tests**

	Sub-analysis	Pooled clinical accuracy			Likelihood ratios (REM)		Heterogeneity (Tau-squared)	
		n	*Sen (95% CI)*	*Spec (95% CI)*	*LR+ (95% CI)*	*LR- (95% CI)*	*PLR*	*NLR*
Study	Pre 2005	19	96.9 (96.0–97.7)	99.7 (99.6–99.8)	266 (106–665)	0.056 (0.033–0.095)	2.72	0.91
	Post 2005	44	86.4 (85.2–87.5)	99.4 (99.2–99.5)	84.6 (43.6–165)	0.126 (0.087–0.183)	4.10	1.27
	Case–control	21	96.7 (96.0–97.3)	99.3 (99.0–99.5)	105 (48.0–230)	0.028 (0.010–0.076)	2.23	4.86
Patient	Blood donors	19	91.6 (90.1–92.9)	99.5 (99.3–99.7)	89.2 (32.8–243)	0.106 (0.055–0.204)	3.82	1.86
	HIV+	6	72.3 (67.9 –76.4)	99.8 (99.5–99.9)	193 (77.4–497)	0.29 (0.22–0.38)	0.384	0.0059
	HIV–	4	92.6 (89.8–94.8)	99.6 (99.0–99.9)	79.5 (11.6–545)	0.08 (0.05-0.13)	2.97	0.080
Index test	Whole blood	11	91.7 (89.1–93.9)	99.9 (99.8–99.9)	347 (158–762)	0.089 (0.058–0.136)	0.81	0.24
	Determine	12	90.8 (88.9–92.4)	99.1 (98.9–99.4)	239 (17.1–33300)	0.077 (0.035–0.168)	20.2	1.56
	BinaxNOW	6	97.6 (96.2–98.6)	100 (99.7–100)	221 (36.1–1350)	0.045 (0.016–0.128)	3.53	1.20
	VIKIA	3	82.5 (77.5–86.7)	99.9 (99.8–100)	1070 (376–3060)	0.108 (0.026–0.458)	<0.005	1.472
	Serodia	3	82.5 (77.5–86.7)	99.9 (99.8–100)	285 (71.4–1140)	0.045 (0.029–0.069)	<0.005	<0.005
Reference test	CMIA	9	80.4 (77.9–82.6)	99.0 (99.6–99.3)	58.5 (31.3–109)	0.141 (0.074–0.268)	0.44	0.73

n = number of data points

Table 6. Summary pooled diagnostic accuracy of HBsAg assays by brand

		EIA reference		NAT reference	
Type	**Brand name**	***Sen (95% CI)***	***Spec (95% CI)***	***Sen (95% CI)***	***Spec (95% CI)***
RDT	Abon	50.0 (28.2–71.8)	99.2 (95.7–100)		
RDT	Accurate	50.0 (33.4–66.6)	94.7 (74.0–99.9)		
RDT	ACON	88.0 (83.4–91.7)	99.4 (99.0–99.7)	92.9 (87.3–96.5)	99.1 (96.6–99.9)
RDT	Acull–Tell	54.5 (33.2–75.6)	99.2 (95.7–100)		
RDT	AMRAD	95.2 (76.2–99.9)	100 (39.8–100)		
RDT	Atlas			97.5 (92.9–99.5)	97.5 (92.9–99.5)
RDT	BINAX	97.6 (96.2–98.6)	99.9 (99.7–100)		
RDT	Blue Cross			99.2 (95.4–100)	98.3 (94.1–99.8)
RDT	Biotec	58.8 (40.7–75.4)	85.7 (63.7–97.0)		
RDT	Core TM	50.0 (28.2–71.8)	98.4 (94.5–99.8)		
RDT	Cortez	60.0 (14.7–94.7)	100 (92.1–100)	79.7 (73.1–85.3)	97.2 (94.0–99.0)
RDT	Cypress	96.7 (90.7–99.3)	96.3 (90.9–99.0)		
RDT	Dainascreen	100 (98.7–100)	100 (99.3–100)		
RDT	Determine	90.8 (88.9–92.4)	99.1 (98.9–99.4)		
RDT	DIMA			98.3 (94.1–99.8)	99.2 (95.4–100)
RDT	Dipstick (PATH)	93.3 (68.1–99.8)	100 (98.1–100)		
RDT	DRW	98.1 (96.1–99.2)	99.5 (98.8–99.9)		
RDT	DRW v2	99.3 (97.4–99.9)	98.3 (97.5–98.9)		
RDT	Espline	93.9 (89.1–97.1)	94.7 (82.3–99.4)		
RDT	Genedia	98.0 (94.3–99.6)	100 (96.4–100)		
RDT	Hepacard	90.5 (82.1–95.8)	99.7 (99.5–99.9)		
RDT	Hexagon	95.6 (89.1–98.8)	96.4 (90.9–99.0)		
RDT	Intec	50.8 (43.3–58.4)	100 (94.9–100)	99.2 (95.4–100)	97.5 (92.9–99.5)
RDT	Nanosign	73.7 (48.8–90.9)	97.8 (95.4–99.2)		
RDT	Onecheck	52.6 (35.8–69.0)	100 (82.4–100)		
RDT	Onsite	80.0 (28.4–99.5)	100 (92.1–100)		
RDT	Quick Profile	90.5 (82.1–95.8)	99.7 (99.5–99.9)		
RDT	QuickChaser	83.3 (68.6–93.0)	99.5 (98.2–99.9)		
RDT	Rapid Care	54.5 (32.2–75.6)	99.2 (95.7–100)		
RDT	SD Bioline	100 (63.1–100)	100 (98.9–100)		
RDT	Serodia	95.8 (93.4–97.5)	99.8 (99.1–100)		
RDT	SimpliRed	93.3 (68.1-99.8)	100 (98.1-100)		
RDT	VEDA Lab	62.3 (50.6-73.1)	99.2 (97.6-99.8)		

RDT	VIKIA	82.5 (77.5-86.7)	99.9 (99.8-100)		
RDT	Virucheck	92.3 (86.3-96.2)	97.3 (95.5-98.5)		
RDT	Wondfo	59.1 (36.4-79.3)	99.2 (95.7-100)		
EIA	ADVIA			77.4 (65.0–87.1)	97.9 (92.6–99.7)
EIA	Architect	97.9 (93.9–99.6)	99.6 (98.7–99.9)		
EIA	AxSym			56.6 (44.7–67.9)	86.8 (81.5–90.9)
EIA	AxSym v2			77.4 (67.0–85.8)	75.0 (66.1–82.6)
EIA	Cobas	96.6 (92.8–98.8)	100 (94.9–100)		
EIA	Elecsys			63.4 (55.2–71.0)	95.8 (92.4–98.0)
EIA	KHB	73.8 (68.7–78.6)	88.1 (82.4–92.5)		
EIA	Liaison			100 (97.6–100)	70.0 (63.1–76.3)
EIA	Liaison Ultra	97.1 (92.8–99.2)	99.4 (98.5–99.8)		
EIA	Monolisa	100 (95.2–100)	91.1 (78.8–97.5)		
EIA	Monolisa Ultra	98.7 (92.9–100)	90.7 (77.9–97.4)		
EIA	Murex v3	98.6 (94.9–99.8)	99.3 (98.3–99.8)		
EIA	VIDAS Ultra			69.0 (58.0–78.7)	94.0 (88.0–97.5)
EIA	Wantai	78.2 (71.4–84.0)	100 (94.9–100)		

Pooled results and I^2 (heterogeneity)

Test	Ref	Studies, n	Data, nd	I^2 Sen, %	I^2 Spec, %
ACON	NAT	2	2	96.0	0.0
Cortez	NAT	2	3	97.5	0.0
ADVIA	NAT	1	2	0.0	0.0
AxSym	NAT	1	2	95.8	89.1
Elecsys	NAT	2	2	0.0	49.2
ACON	EIA	2	3	86.8	95.2
Binax	EIA	3	6	77.3	25.3
Dainascreen	EIA	2	2	0.0	0.0
Determine	EIA	10	12	92.9	97.0
DRW	EIA	1	2	76.2	79.2
DRW v2	EIA	1	3	71.5	0.0
Hepacard	EIA	2	2	51.3	96.6
Quickchaser	EIA	1	2	20.7	0.0
Serodia	EIA	3	3	0.0	0.0
VIKIA	EIA	3	3	93.4	7.3
Virucheck	EIA	2	3	61.0	11.9

GRADE

Question: **Should RDTs be used to diagnose HBsAg in HIV-negative individuals?**

Sensitivity	0.88–0.95
Specificity	0.93–1.00

Prevalences	5%	20%

Outcome	No. of studies (no. of patients)	Study design	Factors that may decrease quality of evidence					Effect per 1000 patients/year		Test accuracy QoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of **5**%	pre-test probability of **20**%	
True positives (patients with HBsAg)	4 studies 997 patients	Cross-sectional (cohort type accuracy study)	Serious[1]	Not serious[2]	Serious[3]	Serious[4]	None	44–48	176–190	⊕◯◯◯ Very low[1234]
False negatives (patients incorrectly classified as not having HBsAg)								2–6	10–24	
True negatives (patients without HBsAg)	4 studies 997 patients	Cross-sectional (cohort type accuracy study)	Serious[1]	Not serious[2]	Serious[3]	Not serious[5]	None	884–950	744–800	⊕⊕◯◯ Low[1235]
False positives (patients incorrectly classified as having HBsAg)								0–66	0 –56	

1. Downgraded by one for risk of bias: all studies were prospective cohort studies[40], although one was assessed as high risk of bias because patients were pre-selected based from known chronic hepatitis B patients.
2. Although study was not specifically designed in HIV-negative patients, clear testing and results were included.
3. Downgraded by one for inconsistency: unexplained heterogeneity may arise from differences between studies in specimen condition (serum, whole blood), specimen processing (field vs laboratory), reference tests (CMIA; EIA on dried blood spots) and study population (e.g. known chronic hepatitis B patients, general community screen).
4. Downgraded by one for imprecision: confidence intervals extend below 90% accuracy, with tau-squared for PLR >1 (indicating substantial heterogeneity).

Question: **Should RDTs be used to diagnose HBsAg in HIV-positive individuals?**

Sensitivity	0.72 (95% CI: 0.68–0.76)

Prevalences	5%	20%

Specificity	1.00 (95% CI: 0.99–1.00)

Outcome	No. of studies (no. of patients)	Study design	Factors that may decrease quality of evidence					Effect per 1000 patients/year		Test accuracy QoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of **5**%	pre-test probability of **20**%	
True positives (patients with HBsAg)	5 studies 2566 patients	Cross-sectional (cohort type accuracy study)	Serious[1]	Not serious[2]	Serious[3]	Serious[4]	None	36 (34–38)	145 (136–153)	⨁◯◯◯ Very low[1 2 3 4]
False negatives (patients incorrectly classified as not having HBsAg)								14 (12–16)	55 (47–64)	
True negatives (patients without HBsAg)	5 studies 2566 patients	Cross-sectional (cohort type accuracy study)	Serious[1]	Not serious[2]	Not serious[5]	Not serious[6]	None	948 (945–949)	798 (796–799)	⨁⨁⨁◯ Moderate[1 2 5 6]
False positives (patients incorrectly classified as having HBsAg)								2 (1–5)	2 (1–4)	

1. Downgraded by one for risk of bias: all studies were prospective cohort studies of consecutive patients. Studies used different specimens (serum, 2; capillary whole blood, 1; venous whole blood, 1), reference standards (CMIA, EIA confirmed by neutralization), and had patients with different ART status (four studies ART naive).
2. Not downgraded for indirectness: all studies performed in cohorts of consecutive patients in Tanzania[20], Ghana[36], Malawi[19], South Africa[37] and Bissau[38].
3. Downgraded by one for inconsistency with sensitivities ranging from 62% to 100%: unexplained heterogeneity may arise from differences between studies in specimen type, specimen processing and study population. Two Studies[19, 20] had very high sensitivities (100%, 96%) while remainder[36–38] had low sensitivities (range 62-70%). Tau-squared <1 for studies
4. Downgraded by one for imprecision: confidence intervals 67.9–76.4%. Two studies[19, 20] had very high sensitivities (100%, 96%) while remainder[36–38] had low sensitivities (range 62–70%).
5. Not downgraded for inconsistency: specificities ranged from 99% to 100%, with tau-squared <1
6. Not downgraded for imprecision: narrow confidence interval

Question: **Should Determine HBsAg be used to diagnose HBsAg in a global setting?**

Sensitivity	0.91 (95% CI: 0.89 to 0.92)
Specificity	0.99 (95% CI: 0.99 to 0.99)

Prevalences	5%	20%

Outcome	No. of studies (no. of patients)	Study design	Factors that may decrease quality of evidence					Effect per 1000 patients/year		Test accuracy QoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of **5%**	pre-test probability of **20%**	
True positives (patients with HBsAg)	12 studies 7552 patients	Cohort & case–control type studies[1]	Serious	Not serious	Very serious[2]	Not serious	None	45 (44–46)	182 (178–185)	⨁◯◯◯ Very low[2]
False negatives (patients incorrectly classified as not having HBsAg)								5 (4–6)	18 (15–22)	
True negatives (patients without HBsAg)	12 studies 7552 patients	Cohort & case–control type studies	Serious	Not serious	Serious[3]	Not serious	None	941 (940–944)	793 (791–795)	⨁⨁◯◯ Low[3]
False positives (patients incorrectly classified as having HBsAg)								9 (6–10)	7 (5–9)	

1. Lin[25], Lien[24] and Randrianirina[30] used a case–control design
2. Significant heterogeneity across studies for sensitivity; tau-squared 20.2
3. Heterogeneity exists, but with lower clinical impact; tau-squared 1.56

6. Discussion

Study findings

Diagnostic accuracy of HBsAg assays using immunoassay reference

Overall, the diagnostic accuracy of 33 RDTs and 8 EIAs were assessed against an EIA reference standard. Total numbers of patients included 36,131 (RDT vs EIA, figure 3) and 3751 (EIA vs EIA, figure 4). Both RDTs and EIAs had similar sensitivity and specificity compared to an EIA reference standard (Table 3).

Clinical sensitivity estimates for both RDTs and EIAs were characterized by statistical heterogeneity, whereas specificity estimates were less heterogeneous (Figures 3 and 4). This applied across brands (Table 6). Heterogeneity can be caused by the use of different reference standards assays, clinical subgroups within the study population, age (children versus adults), patient status and stage of disease.

Compared to previous systematic reviews, the pooled clinical sensitivity 90.0% (95% CI: 89.1–90.8) and specificity 99.5% (95% CI: 99.4–99.5) is slightly inferior for RDTs compared to an EIA reference standard (Table 3). In particular Results were very heterogeneous in terms of sensitivity (Table 7). Khuroo et al.[11] reported 96.7% sensitivity (95% CI: 95.3, 97.7) and 99.7% specificity (95% CI: 98.6, 99.9). Studies included conference abstracts and studies using reference panels. Shivkumar et al.[60] reported a pooled sensitivity 98.2% (95% CI: 94.7, 99.9) and pooled specificity 99.9% (95% CI: 99.3, 100).

Table 7. Summary pooled diagnostic accuracy of HBsAg assays compared to previous reviews.

Review, year	Index Test		Clinical Accuracy	
		n	Sen (95% CI)	Spec (95% CI)
PICO 1, 2015	RDT	63	90.0 (89.1–90.8)	99.5 (99.4–99.5)
	EIA	8	88.9 (87.0–90.6)	98.4 (97.8–98.8)
Hwang, 2008	RDT		98.1 (97.7–98.5)	99.6 (99.2–99.9)
Shivkumar, 2012	RDT		94.8 (90.1–98.2)	99.5 (99.0–100)
Khuroo, 2014	RDT		97.1 (96.1–7.9)	99.9 (99.8–00)

*Sen : sensitivity; Spec : specificity; CI : confidence interval; RDT : rapid diagnostic test; EIA : enzyme immunoassay; LR^+ : positive likelihood ratio; LR^- : negative likelihood ratio; REM : random effects model

When comparing EIAs to newer CMIA (chemiluminescent assays), two standard ELISA/ EIA based assays manufactured in markets with transitioning economies appeared to perform poorly compared to other assays.[4] Of note, data exists for one assay (KHB) using different signal cut-off ratio's (S/CO), with improved sensitivity but worse specificity when using the grey zone (S/CO 0.2–0.99); sensitivity 96.2%, specificity 70.6% compared to sensitivity of 73.8% and specificity of 88.1%.[45]

Results for EIAs are more specific but less sensitive when used in conjunction with manufacturers' neutralisation assays. In one study,[56] Liason HBsAg (Diasorin) had sensitivity 100% and specificity 70% compared to NAT, with improved overall accuracy using with a neutralisation assay (sensitivity 96%, specificity 100%). This shows the critical need to use the neutralization step to confirm any HBsAg reactivity observed upon initial testing.

Diagnostic accuracy of HBsAg assays using NAT reference

The specificity of RDTs and EIA was lower when using a NAT reference; this is understandable as the serological assays detect HBsAg whereas NAT detects HBV DNA. The viral kinetics of HBV DNA and HBsAg are not identical. One limitation of HBV DNA as a diagnostic assay would be that those on anti-viral treatment, including antiretroviral therapy for HIV (containing lamivudine or tefonovir) might be non-detectable HBV DNA in the presence of HBsAg.

The pooled clinical sensitivity of RDTs was 93.3% (95% CI 91.3–94.9) and significantly higher than EIAs 75.7% (95% CI 72.1–79.1) using a NAT reference. It is important to note that study characteristics varied, with one laboratory based case-control study with pre-selected patients showing particularly good results for RDTs vs NAT and providing 6 data points in this analysis.[47] The remaining studies in this sub-analysis were all performed in field settings in resource-limited settings, with poor sensitivity (38–60%).

Sub-analyses

HIV

Our results showed that RDTs may be less sensitive in HIV-positive patients. There was still heterogeneity in terms of results, with one otherwise good quality review finding that Determine was 96 % sensitive (95% CI: 80,100) and 100% specific (95% CI: 99,100) in this cohort.[20]

The difficulty of accurate diagnosis in HIV patients is possibly explained by an increased incidence of hepatitis B and in particular occult hepatitis B in this cohort. In Sudanese HIV-positive ART naïve patients, 27% had detectable HBV DNA, with occult hepatitis B in 15%.[61] Among 495 treatment naïve, HIV-infected adults in Cote-d'Ivoire, 13% were HBsAg positive, 42% isolated anti-HBc positive, and 10% occult hepatitis B only detected by NAT.[62] Median HBV DNA level was lower in those with occult HBV compared to those with CHB.

Immune pressure has also been hypothesized to contribute, with Geretti et al. noting that discrepant results for RDTs were all mutants in their study. The overlapping surface and polymerase genes in the HBV genome could imply that RT inhibitors (e.g. lamivudine) can lead to the emergence of variants carrying mutations of both the polymerase and surface genes, hence avoiding detection by standard HBsAg assays.

Blood Donors

Pre-transfusion screening of blood donations is a major public health challenge in resource-limited settings, where prevalence rates for TTIs (transfusion-transmissible infections) are significant. Screening of individuals with RDTs pre-donation have been adopted in areas with insufficient laboratory capacity.

Diagnostic accuracy in our review was similar in blood donors compared to the overall pooled estimates of RDTs, with sensitivity 91.6 % (95% CI: 90.1, 92.9) and specificity 99.5% (95% CI 99.3, 99.7). Results were very heterogeneous, with one study in particular having very low sensitivity (~50–60). Two studies comparing EIAs against EIAs in blood donors had higher accuracy, with pooled sensitivity 99.3 % (95% CI: 96.4, 100) and specificity 90.9 % (95% CI 82.9, 96.0).

A recent multinational assessment accuracy of TTI screening in Africa using both RDTs and EIAs on an external quality assessment panel found poor overall sensitivity (75.6%) and specificity (94.5%) for HBsAg detection.[63] This was driven by very poor clinical sensitivity (47.4%) of HBsAg RDTs, which was lower than that for HCV (63.7%) and HIV (72.4%) in this population. This can be explained by their lower analytical sensitivity, difficulties in transport and quality assurance, in addition to often studies being performed on smaller scales. In a Nigerian blood donor study, 10% of 113 HBsAg-negative repeat donors using RDTs were found to have quantifiable HBV DNA.[57] These patients either had acute infection or occult chronic infection.

In a recent systematic review of studies evaluating RDTs for infectious disease blood screening in Africa, there was again significant variability in performance.[64] RDTs for HBsAg detection were again identified for suboptimal sensitivities, with questionable suitability, especially in high prevalence regions. High false negatives could be due to operator error, low HBsAg levels, assay degeneration or lot variation.

Whole blood

For rapid diagnostic tests, accuracy using whole blood (capillary and venous) was marginally superior to serum. The accuracy was comparable to that of EIAs using serum; data from the eight studies (eleven data points) is also less heterogenous (Annex 9.3.2). The significantly lower sensitivity of RDTs using plasma is possibly explained by the nature of the studies; one was in a population of blood donors, while the other was initially designed to assess the accuracy of RDTs in determining HBV and HCV prevalence in a Malawian population with high HIV-co-infection rates.[41] The authors of the latter study hypothesised that local operational problems or unexpected technical issues were the reason for poor performance in resource-limited setting. Others have also since suggested that the high HIV-co-infection rate could have contributed, with suppression of HBV replication using lamivudine containing regiments potentially hindering affecting detection by RDT.

Study setting – field

Some heterogeneity is explained by location where RDTs are performed. Two different studies from Malawi, in a predominantly HIV-positive cohort[41] or entirely HIV-positive cohort[19] produced very different results for the same test (Determine HBsAg). Pooled sensitivity 56% (95% CI: 38, 73) and specificity 69% (95% CI: 62, 76) were much lower in the field study compared to the sensitivity 100% (95% CI: 86, 100) and specificity 100% (95% CI: 93, 100) in the study where samples were returned to the UK.

Analytical sensitivity of different assays as a source of heterogeneity

We were unable to explain heterogeneity of results using different assays. Very few rapid tests meet required analytical LOD (0.130 IU/mL) required by regulatory authorities, but because of insufficient data in studies we were unable to stratify using LOD as a source of heterogeneity. This is important as it has been suggested that false-negative HBsAg RDTs are associated with lower HBsAg levels, low viral load, HBsAg mutants, or specific genotypes, in addition to ART exposure where lamivudine and tenofovir are used. *(16, 25, 36, 65)*

In a recent fields studies in the Gambia,[40] the range of serum HBsAg levels quantified by CMIA that showed reactivity with RDTs in the field was 26.5–27, 320 IU/mL, with a statistically significant (*P* = 0.0002) difference in median HBsAg level (875 IU/mL) compared to false negatives using RDT's (median 1.2; range 0.8–25.5 IU/mL). Interestingly significantly more false-negatives were female (P = 0.05) with lower median ALT levels (*P* = 0.01). The laboratory-based study from the same publication in a chronic hepatitis B cohort found a higher range of HBsAg levels (2.8–124,925 IU/mL) in those testing positive with RDTs, with a significant difference in median HBsAg levels (7, 482 vs 0.40 IU/mL; *P* <0.0001) and median ALT (*P* = 0.01) between true-positives and false negatives. The lower limit of detection may be explained by differences in methodology, given the setting (laboratory vs field), reference test (CMIA vs ELISA) and sample type (dried blood spots vs serum). This suggests that subjects with false negatives have lower HBsAg levels and inactive disease compared to true-positives, minimising the impact of reduced sensitivity. Unfortunately, in the single study identified also assessing LSM, 17% (4/23) subjects with false negative results had evidence of fibrosis and would require antiviral therapy.

Another study (Bottero et al)[16] also found significantly lower median HBsAg in false negatives vs true positives. [19.5 vs 2351 IU/mL; p=0.0001], with only 4 false negative having HBsAg >10 IU/mL. HBV DNA was usually below 200 IU/mL. False positives occurred in vaccinate patients (*n*=7), and one patient with resolved infection and anti-HBs titre. Interestingly ALL false negatives were HBcAb positive.

Data exists from large studies of analytical sensitivity using reference, seroconversion, mutant panels.[53, 65, 66] These include specimens from individuals with low antigenaemia, such as early infection. Unfortunately, studies of analytical sensitivity of EIAs are conflicting. One study suggested that 9 out of 10 EIAs were able to detect HBsAg levels as low as 0.2 IU/mL irrespective of genotype.[67] Another comparing newer EIAs (Advia Centaur; Monolisa Ultra; Liasion; Vidas Ultra) using reference and mutant panels found a lower limit of detection <350IU/mL, but with varying sensitivity for mutant detection (37.1%–91.4%).[68] The authors hypothesised that the lack of detection was due to epitope recognition of the anti-HBs assay reagents in the capture phase and in conjugates. Another study assessing 13 different assays with mutant panels found a range of LOD (0.011-0.096 IU/mL) and sensitivity (63%–98%) in mutants. Another study found comparable analytical sensitivity between four EIAs but significant differences in detection of mutants between assays.[69] One blood donor study in China found a significant difference in sensitivities and mutant detection capabilities amongst assays used by blood banks, with the urgent recommendation of a list of high sensitivity assays for blood bank screening.[70]

In blood donors, studies have found some correlation between HBsAg levels (IU/mL) and NAT (copies/mL).[71] The obvious benefit of more sensitive assays with lower limits of detection would be improved detection of those with occult hepatitis B or in the early window period of sero-conversion.

It has been suggested that utilizing "grey zones" in EIAs could improve sensitivity and allow combination of tests to develop of economic testing strategies.[45] Sensitivity improved from 76–88% to 96–97%, with a further increase to 99% when combining the use of two EIAs.

Studies looking at RDTs using clinical panels have found sometimes conflicting results. One study found equivalent specificities but significant differences in assay sensitivity between Uni-Gold™ HBsAg and Determine HBsAg.[72]

Interestingly, studies in Cambodia and Viet Nam by the same group produced different results for sensitivity, suggesting uncontrolled variables, such as prozone effect and genotype variations. The prozone effect may explain why some true positives turned out negative with rapid tests. Given that specificity is excellent but sensitivity is low suggests that this is genuine poor performance.

Study strengths and limitations

Significant strengths of this meta-analysis include the global evidence base, rigorous pre-specified protocol incorporating numerous major scientific databases, in addition to review of the related literature, notably occult hepatitis B and the impact of NAT. We included studies performed in a range of settings, with a diverse population. We only included studies with bivariate data, to minimise bias, measuring clinical sensitivity which are more applicable. We also included evaluations of both RDTs and EIAs, and as such are able to provide a more comprehensive meta-analysis. Comparing RDTs with EIAs, we were able to identify an additional 11 studies not found in previous reviews. Incorporation bias was unlikely as all participants received both index and confirmatory tests independently. As all studies administered the same reference standard to all patients, which reduces risk of verification bias. Our study also excluded articles deemed to be high risk of bias or less applicable, such as conference abstracts or reference panels; reference panels, included in previous reviews, have higher accuracy than that of tests used in the field but are not as useful in guiding policy. Accuracy on seroconversion panels do not necessarily reflect the antibody or antigen spectrum in the populations studied.

Our study, did, however have a number of limitations. First, we only included studies in English, which potentially introduces publication (language) bias. We will identify relevant studies in non-English languages from reference lists to address whether this contributed to a substantial bias or not. Second, a significant proportion of studies were case–control in design or used preselected cohorts, which would bias results. For example, in well-conducted studies from the Gambia,[40] those in the community setting had a smaller range of HBsAg levels (26.5–27, 320 IU/mL) than the study conducted in chronic hepatitis B patients (2.8–124,925 IU/mL). This is one reason for the reduced sensitivity of the same test (Determine HBsAg) in the screening cohort (88%; 95% CI: 81, 94) compared to the chronic hepatitis cohort (95%; 95% CI:

90, 98) from the same community. Third, some analyses were based on a small number of studies, which included few positive samples.

There are number of technical and patient factors that could impact accuracy, which cannot be addressed based on the currently available literature. Specific to hepatitis B diagnosis, we were unable to correlate the heterogeneity of sensitivity with different stages, severity and genotypes infection. This was due to insufficient information in studies, principally additional serology such as HBeAg, anti-HBc IgM, anti-HBc total and anti-HBs antibodies. Genetic information has long been suspected to impact on diagnostic accuracy,[67–70, 73, 74] although a recent study of analytical performance found no difference in the detection of mutants.[70, 73–75] It should be noted that this study was conducted by authors with significant conflicts of interest. Mutants themselves are also rapidly evolving, such that the prevalence and type of specific mutants cannot be determined based on historical data, making studies difficult to organize. Finally, occult hepatitis should also be considered. The addition of NAT would be useful to stratify patients' results, but the lack of sufficient of quality studies (Annex 9.2) is testament to the challenges in conducting advanced laboratory based studies in areas of high disease prevalence. Reference standards are also imprecise, resulting in overdiagnosis of clinically insignificant disease, and underestimation of diagnostic accuracy of clinically relevant disease. The natural history of hepatitis B, notably progression and infectiousness, is being investigated for correlations of quantitative HBsAg, HbeAg and HBV DNA levels. Low levels of either antigen may not be significant clinically. Short-term spontaneous fluctuations in DNA and HBsAg are recognized in those with chronic hepatitis B and add extra challenges to accurate diagnosis with a "gold standard".[76]

Statistical heterogeneity was an obvious issue as is often observed in diagnostic accuracy reviews. Although we performed stratified analyses to identify potential sources, none fully explained the heterogeneity observed. Firstly, studies evaluated different RDTs, with rapid changes in technology for both EIAs and RDTs meaning that analytical sensitivity is variable among the assays evaluated. Although we pooled based on some RDT brands (Determine HBsAg, BinaxNOW) there were insufficient studies and this pooling did not entirely account for heterogeneity. Another potential confounder is changes in manufacturing processes, including components used to manufacture assays Determine, as one example, has been produced by Abbott, Inverness Medical and Alere Medical Co. Ltd; as the test has been commercially available for over 10 years, there will undoubtedly be minor product changes.

7. Conclusion

WHO has emphasized the importance of timely global testing, prevention and treatment of hepatitis B, with predictions of an increasing prominence as a cause of death globally in years to come. Although RDTs have limitations, many of which can be addressed through improved training and quality assurance systems, they are frequently the only viable option for infectious screening in resource-limited settings. Therefore, additional studies and specific guidelines regarding the use of RDTs in the context of blood safety and patient screening are needed. In terms of global uptake, lower costs of these assays and ease of use across a variety of endemic settings is crucial to achieving goals for control of hepatitis. Worldwide, a

significant proportion of countries are unable to afford quality-assured laboratory-based testing with enzyme immunoassays; the use of NAT to further reduce the window period of infection and detect occult hepatitis is beyond reach in many settings at present.

This meta-analysis, along with others, suggests that assays for detection of HBsAg including RDTs and enzyme immunoassays have the potential to contribute significantly to the control of hepatitis B globally in endemic areas, which are often include low resource remote regions. Other benefits of RDTS include easy storage, small sample volumes required with minimal staff training or additional equipment. Unfortunately, with current issues with poor clinical and analytical sensitivity and potential difficulties in detection of occult hepatitis B and mutant variants, a number of cases would be missed. There is also concern that sensitivity is significantly reduced in HIV-positive patients.

There are numerous difficulties in conducting systematic reviews of the performance of in vitro diagnostics, particularly in resource-limited settings. There is a significant variation in terms of quality of studies, most with key parameters missing. Further promotion of current accepted standards to performing and reporting studies of diagnostic accuracy globally can help improve the evidence base currently available. Further high quality studies are desperately needed to assess the accuracy in a variety of settings and support the growing evidence base for RDTs. Specifically, further studies looking at the impact of different geographic locations and mutant phenotypes would be invaluable.

From included studies, excellent robust specificity of all assays is reassuring in terms of ensuring cost–effective initiation of algorithms for further investigation and treatment. Significant heterogeneity and suboptimal sensitivity of RDTs has to be taken into consideration as country control programmes consider the trade-off between affordability, accuracy and accessibility (i.e. ease of use in all levels of the health-care system). The weighting of these three factors are country specific and could be modelled.

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9. Annexes

9.2. 9.1 Search strategy

Ovid Medline search strategy

Searched on 20 April 2015 from 1946 – April week 2 2015.

1 Hepatitis, Viral, Human/ (10382)
2 Hepatitis Viruses/ (1363)
3 Hepatitis Antibodies/ (5082)
4 exp Hepadnaviridae Infections/ (47484)
5 Hepatitis B Antibodies/ (8638)
6 Hepatitis B virus/ (20604)
7 Hepadnaviridae/ (192)
8 Hepatitis B Surface Antigens/ (17007)
9 (heptatitis-b or hep-b or (hepatitis adj5 b) or (hep adj5 b) or hbv).ti,ab. (64488)
10 hbsag.ti,ab. (15146)
11 or/1-10 [HEPATITIS B] (87943)
12 exp Reagent Kits, Diagnostic/ (17747)
13 ((rapid or point of care or near patient or poc or poct or bedside) adj5 (test or tests or testing or detect* or diagnos* or screen* or kit or kits or assay* or device*)).ti,ab. (63080)
14 (radt or radts or rdt or rdts).ti,ab. (909)
15 rapid test*.ti,ab. (3400)
16 exp Enzyme-Linked Immunosorbent Assay/ (127391)
17 Immunoassay/ (23237)
18 Immunoenzyme Techniques/ (64864)
19 (enzyme-linked immunosorbent assay or ELISA).ti,ab. (139374)
20 (enzyme adj2 (immunoassay* or immuno-assay* or immunosorbent)).ti,ab. (83849)
21 ((antigen* or antibod*) adj3 detect*).ti,ab. (59427)
22 or/12-21 [RAPID DIAGNOSTIC TESTS] (394724)
23 exp "Sensitivity and Specificity"/ (435087)
24 (diagnos* accura* or sensitiv* or specific* or valid*).ti,ab. (3016884)
25 roc curve.ti,ab. (10226)
26 positive predictive value.ti,ab. (25496)
27 negative predictive value.ti,ab. (20415)
28 or/23-27 [DIAGNOSTIC ACCURACY] (3235789)
29 11 and 22 and 28 (3103)
30 Humans/ (13846846)
31 Animals/ (5442465)
32 30 and 31 (1513142)
33 31 not 32 [ALL ANIMAL STUDIES WHICH DO NOT INCLUDE COMPARISON WITH HUMANS] (3929323)
34 29 not 33 (2856)

35 limit 34 to english language (2345)

Ovid Embase search strategy

Searched on 20 April 2015 from 1947 – 2015 April 17.

1 hepatitis virus/ (4410)
2 hepatitis antibody/ (2216)
3 exp hepadnaviridae/ (42214)
4 hepatitis B surface antigen/ (27312)
5 (heptatitis-b or hep-b or (hepatitis adj5 b) or (hep adj5 b) or hbv).ti,ab. (94627)
6 hbsag.ti,ab. (22290)
7 or/1-6 [HEPATITIS B] (111321)
8 exp diagnostic kit/ (13384)
9 "point of care testing"/ (5530)
10 ((rapid or point of care or near patient or poc or poct or bedside) adj5 (test or tests or testing or detect* or diagnos* or screen* or kit or kits or assay* or device*)).ti,ab. (88003)
11 (radt or radts or rdt or rdts).ti,ab. (1652)
12 rapid test*.ti,ab. (5049)
13 enzyme linked immunosorbent assay/ (229634)
14 immunoassay/ (48491)
15 enzyme immunoassay/ (36845)
16 enzyme linked immunospot assay/ (6789)
17 enzyme multiplied immunoassay technique/ (768)
18 (enzyme-linked immunosorbent assay or ELISA).ti,ab. (204052)
19 (enzyme adj2 (immunoassay* or immuno-assay* or immunosorbent)).ti,ab. (98704)
20 antigen detection/ (18155)
21 antibody detection/ (34389)
22 ((antigen* or antibod*) adj3 detect*).ti,ab. (76053)
23 or/8-22 [RAPID DIAGNOSTIC TESTS] (525203)
24 "sensitivity and specificity"/ (221828)
25 diagnostic accuracy/ (189329)
26 (diagnos* accura* or sensitiv* or specific* or valid*).ti,ab. (4113104)
27 roc curve.ti,ab. (20232)
28 positive predictive value.ti,ab. (37541)
29 negative predictive value.ti,ab. (31612)
30 or/24-29 [DIAGNOSTIC ACCURACY] (4280338)
31 7 and 23 and 30 (4018)
32 human/ (15785497)
33 animal/ (1646303)
34 32 and 33 (404532)
35 33 not 34 [ALL ANIMAL STUDIES WHICH DO NOT INCLUDE COMPARISON WITH HUMANS] (1241771)

36 31 not 35 (3963)

37 limit 36 to english language (3344)

Web of Science

Search was conducted on the Science Citation Index Expanded (1970–20 April 2015) and the Conference Proceedings Citation Index-Science (1990–20 April 2015).

1 TOPIC: ("hepatitis-b" OR "hep-b" OR (hepatitis near/5 b) OR (hep near/5 b) OR hbv) (79,505)
2 TOPIC: (hbsag) (12,160)
3 #2 OR #1 (81,526)
4 TOPIC: ((rapid near/5 test) or (rapid near/5 tests) or (rapid near/5 testing) or (rapid near/5 detect*) or (rapid near/5 diagnos*) or (rapid near/5 screen*) or (rapid near/5 kit) or (rapid near/5 kits) or (rapid near/5 assay*) or (rapid near/5 device*)) (77,863)
5 TOPIC: (("point of care" near/5 test) or ("point of care" near/5 tests) or ("point of care" near/5 testing) or ("point of care" near/5 detect*) or ("point of care" near/5 diagnos*) or ("point of care" near/5 screen*) or ("point of care" near/5 kit) or ("point of care" near/5 kits) or ("point of care" near/5 assay*) or ("point of care" near/5 device*)) (5,974)
6 TOPIC: (("near patient" near/5 test) or ("near patient" near/5 tests) or ("near patient" near/5 testing) or ("near patient" near/5 detect*) or ("near patient" near/5 diagnos*) or ("near patient" near/5 screen*) or ("near patient" near/5 kit) or ("near patient" near/5 kits) or ("near patient" near/5 assay*) or ("near patient" near/5 device*)) (423)
7 TOPIC: ((poc near/5 test) or (poc near/5 tests) or (poc near/5 testing) or (poc near/5 detect*) or (poc near/5 diagnos*) or (poc near/5 screen*) or (poc near/5 kit) or (poc near/5 kits) or (poc near/5 assay*) or (poc near/5 device*)) (866)
8 TOPIC: ((poct near/5 test) or (poct near/5 tests) or (poct near/5 testing) or (poct near/5 detect*) or (poct near/5 diagnos*) or (poct near/5 screen*) or (poct near/5 kit) or (poct near/5 kits) or (poct near/5 assay*) or (poct near/5 device*)) (522)
9 TOPIC: ((bedside near/5 test) or (bedside near/5 tests) or (bedside near/5 testing) or (bedside near/5 detect*) or (bedside near/5 diagnos*) or (bedside near/5 screen*) or (bedside near/5 kit) or (bedside near/5 kits) or (bedside near/5 assay*) or (bedside near/5 device*)) (2,705)
10 TOPIC: (radt or radts or rdt or rdts) (1,406)
11 TOPIC: ("rapid test*") (3,783)
12 TOPIC: ("enzyme-linked immunosorbent assay" or ELISA) (141,435)
13 TOPIC: ((enzyme near/2 immunoassay*) or (enzyme near/2 immuno-assay*) or (enzyme near/2 immunosorbent)) (85,660)
14 TOPIC: ((antigen* near/3 detect*) or (antibod* near/3 detect*)) (56,976)
15 #14 OR #13 OR #12 OR #11 OR #10 OR #9 OR #8 OR #7 OR #6 OR #5 OR #4 (286,936)
16 TOPIC: ("diagnos* accura*" or sensitiv* or specific* or valid*) (4,557,124)
17 TOPIC: ("roc curve") (12,767)
18 TOPIC: ("positive predictive value") (23,706)
19 TOPIC: ("negative predictive value") (18,947)
20 #19 OR #18 OR #17 OR #16 (4,566,667)
21 #20 AND #15 AND #3 (1,789)
22 #20 AND #15 AND #3 Refined by: LANGUAGES: (ENGLISH) (1,720)

Scopus

Search was conducted on 20 April 2015.

TITLE-ABS-KEY (("heptatitis-b" OR "hep-b" OR (hepatitis W/5 b) OR (hep W/5 b) OR hbv OR hbsag) AND (((rapid OR "point of care" OR "near patient" OR poc OR poct OR bedside) W/5 (tests OR test OR testing OR detect* OR diagnos* OR screen* OR kit OR kits OR assay* OR device*)) OR radt OR radts OR rdt OR rdts OR "rapid test*" OR "enzyme-linked immunosorbent assay" OR elisa OR (enzyme W/2 (immunoassay* OR immuno-assay* OR immunosorbent)) OR ((antibod* OR anigen*) W/3 detect*)) AND ("diagnos* accura*" OR sensitiv* OR specific* OR valid* OR "roc curve" OR "positive predictive value" OR "negative predictive value")) AND (LIMIT-TO (LANGUAGE, "English")) (3,605)

Cochrane Central Register of Controlled Trials, Wiley

The search was run on 20 April 2015.

1 MeSH descriptor: [Hepatitis, Viral, Human] this term only
2 MeSH descriptor: [Hepatitis Viruses] this term only
3 MeSH descriptor: [Hepatitis Antibodies] this term only
4 MeSH descriptor: [Hepadnaviridae Infections] explode all trees
5 MeSH descriptor: [Hepatitis B Antibodies] this term only
6 MeSH descriptor: [Hepatitis B virus] this term only
7 MeSH descriptor: [Hepadnaviridae] this term only
8 MeSH descriptor: [Hepatitis B Surface Antigens] explode all trees
9 "hepatitis-b":ti,ab,kw (Word variations have been searched)
10 "hep-b":ti,ab,kw (Word variations have been searched)
11 hepatitis near/5 b:ti,ab,kw (Word variations have been searched)
12 hep near/5 b:ti,ab,kw (Word variations have been searched)
13 hbv:ti,ab,kw (Word variations have been searched)
14 hbsag:ti,ab,kw (Word variations have been searched)
15 #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14
16 MeSH descriptor: [Reagent Kits, Diagnostic] explode all trees
17 (rapid or "point of care" or "near patient" or poc or poct or bedside) near/5 (test or tests or testing or detect* or diagnos* or screen* or kit or kits or assay* or device*):ti,ab,kw (Word variations have been searched)
18 radt or radts or rdt or rdts:ti,ab,kw (Word variations have been searched)
19 "rapid test*":ti,ab,kw (Word variations have been searched)
20 MeSH descriptor: [Enzyme-Linked Immunosorbent Assay] explode all trees
21 enzyme near/2 (immunoassay* or immuno-assay* or immunosorbent):ti,ab,kw (Word variations have been searched)
22 (antigen* or antibod*) near/3 detect*:ti,ab,kw (Word variations have been searched)
23 MeSH descriptor: [Immunoassay] this term only
24 MeSH descriptor: [Immunoenzyme Techniques] this term only

25 "enzyme-linked immunosorbent assay" or ELISA:ti,ab,kw (Word variations have been searched)
26 #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25
27 MeSH descriptor: [Sensitivity and Specificity] explode all trees
28 diagnos* accura* or sensitiv* or specific* or valid*:ti,ab,kw (Word variations have been searched)
29 "roc curve":ti,ab,kw (Word variations have been searched)
30 "positive predictive value":ti,ab,kw (Word variations have been searched)
31 "negative predictive value":ti,ab,kw (Word variations have been searched)
32 #27 or #28 or #29 or #30 or #31
33 #15 and #26 and #32

The search found 64 trials.

Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS) (BIREME interface)

LILACS was searched on 20 April 2015

("hepatitis b" or "hep b" or "hbv" or "hbsag") and ("rapid test$" or "point of care test$" or "near patient test$" or "poc test$" or poct or "bedside test$" or "rapid detect$" or "point of care detect$" or "near patient detect$" or "poc detect$" or "bedside detect$" or "rapid diagnos$" or "point of care diagnos$" or "near patient diagnos$" or "poc diagnos$" or "bedside diagnos$" or "rapid screen$" or "point of care screen$" or "near patient screen$" or "poc screen$" or "bedside screen$" or "rapid kit$" or "point of care kit$" or "near patient kit$" or "poc kit$" or "bedside kit$" or "rapid assay$" or "point of care assay$" or "near patient assay$" or "poc assay$" or "bedside assay$" or "rapid device$" or "point of care device$" or "near patient device$" or "poc device$" or "bedside device$" or radt or radts or rdt or rdts or "enzyme-linked immunosorbent assay" or "antigen$ detect$" or "antibod$ detect$" or elisa or immunoassay or immunoenzyme or "immuno-assay") and ("diagnos$ accura$" or sensitiv$ or specific$ or valid$ or "roc curve" or "positive predictive value" or "negative predictive value") (33)

WHO Global Index Medicus

The database was searched on 22 April 2015.

SUBJECT: (("Hepatitis, Viral, Human" OR "Hepatitis Viruses" OR "Hepatitis B virus" OR "Hepatitis Antibodies" OR "Hepadnaviridae Infections" OR "Hepatitis B Antibodies" OR "Hepatitis B Virus" OR "Hepadnaviridae" OR "Hepatitis B Surface Antigens") AND ("Reagent Kits, Diagnostic" OR "Enzyme-Linked Immunosorbent Assay" OR "Immunoassay" OR "Immunoenzyme Techniques") AND ("Sensitivity and Specificity")) (478)

Summary data for studies assessing diagnostic accuracy against a NAT-reference standard

Table 8. Study characteristics – RDT/ EIA vs NAT

Study [Author, Year]	Location [Country, City]	Sample size	Study design	Setting	Sample	Test under evaluation [Type, Brand]	Reference test [Type, Brand]
Ansari, 2007	Iran, Urumieh	240	CC	Hospital patients	S	RDT, ACON RDT, Atlas RDT, Blue Cross RDT, Cortez RDT, DIMA RDT, Intec	qPCR
Khadem-Ansari, 2014	Iran, Urumieh	350	CC – CSQ	Hospital patients – referred as ?HBV	S	ChLIA, Liaison	Rt-PCR
Lukhwareni, 2009	South Africa	192	CC	HIV cohort – pre ART	S	ChLIA, Elecsys	qPCR
Mphahlele, 2006	South Africa	167 (HIV^+) 128 (HIV^-)	CC	HIV cohort	S	EIA, AxSYM	Nested PCR
Nna, 2014	Nigeria	113	CS	Blood donors (repeat)	P	RDT, ACON	Nested PCR; qPCR for positive
Olinger, 2007	Nigeria, Ibadan	200	CS	Hospital patients – liver disease, HIV	S	MEIA, AxSYM v2 ChLIA, Elecsys ELFA, VIDAS Ultra	rtPCR and nested PCR
Seremba, 2010	Uganda	74 (HIV-) 83 (HIV+)	CS – CSQ	Hospital patients - ED, including HIV	S	RDT, Cortez EIA, ADVIA	PCR

qPCR: quantitative PCR; rtPCR: realtime PCR; ChLIA: chemiluminescent immunoassay; CMIA: chemiluminescent microparticle enzyme immunoassay; ECLIA: electrochemiluminescent immunoassay; EIA: enzyme immunoassay; ELFA: enzyme-linked fluorescent assay; ELISA: enzyme-linked immunosorbent assay; MEIA: microparticle enzyme immunoassay; RDT: rapid diagnostic test; rtPCR: real-time PCR; CC: case–control; CS: cross-sectional; CSQ: consecutive patients; LB: lab-based study

Table 9. Summary pooled diagnostic accuracy of HBsAg assays compared to NAT reference

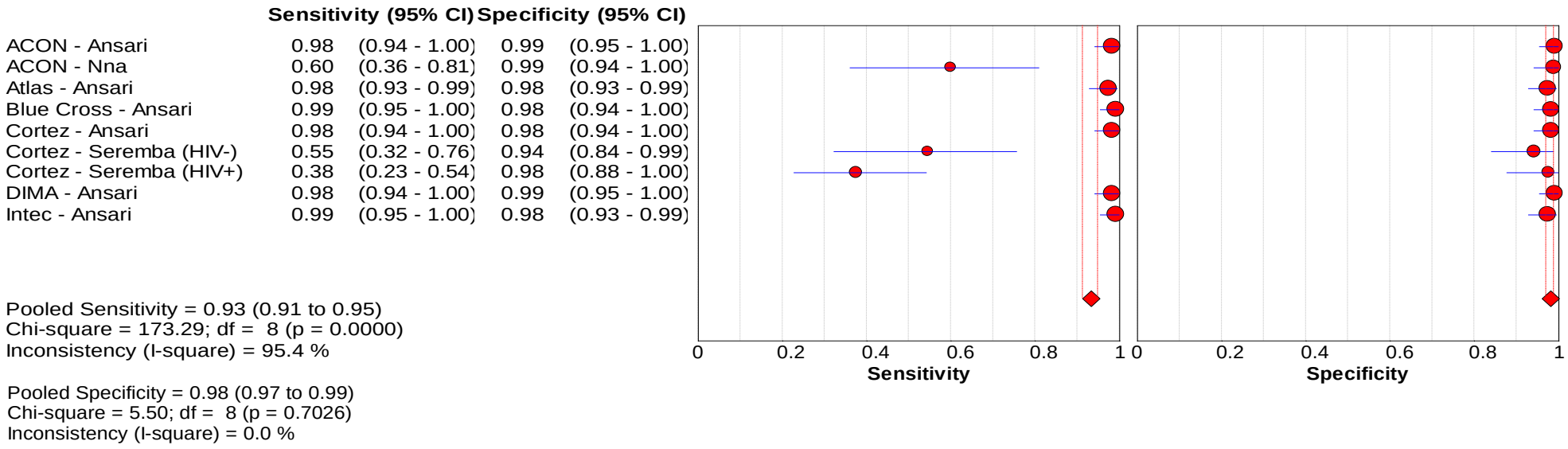
Test type	NAT reference	
	Sen (95% CI)	**Spec (95% CI)**
RDT	93.3 (91.3–94.9)	98.1 (97.0–98.9)
EIA	75.7 (72.1–79.1)	86.1 (83.8–88.2)

*Sen: sensitivity; Spec: specificity; CI: confidence interval; RDT: rapid diagnostic test; EIA: enzyme immunoassay

Table 10. Summary pooled diagnostic accuracy of HBsAg assays

Test type	HIV status	NAT reference		
		n	**Sen (95% CI)**	**Spec (95% CI)**
RDT	HIV^+	1	37.5 (22.7–54.2)	97.7 (87.7–99.9)
	HIV^-	2	57.1 (41.0–72.3)	97.2 (93.1–99.2)
EIA	HIV^+	3	57.9 (49.8–65.6)	95.8 (92.7–97.8)
	HIV^-	2	83.3 (69.8–92.5)	85.7 (79.2–90.8)

Fig. 5. Forest plots, RDT vs NAT, ordered by [Test, Author]

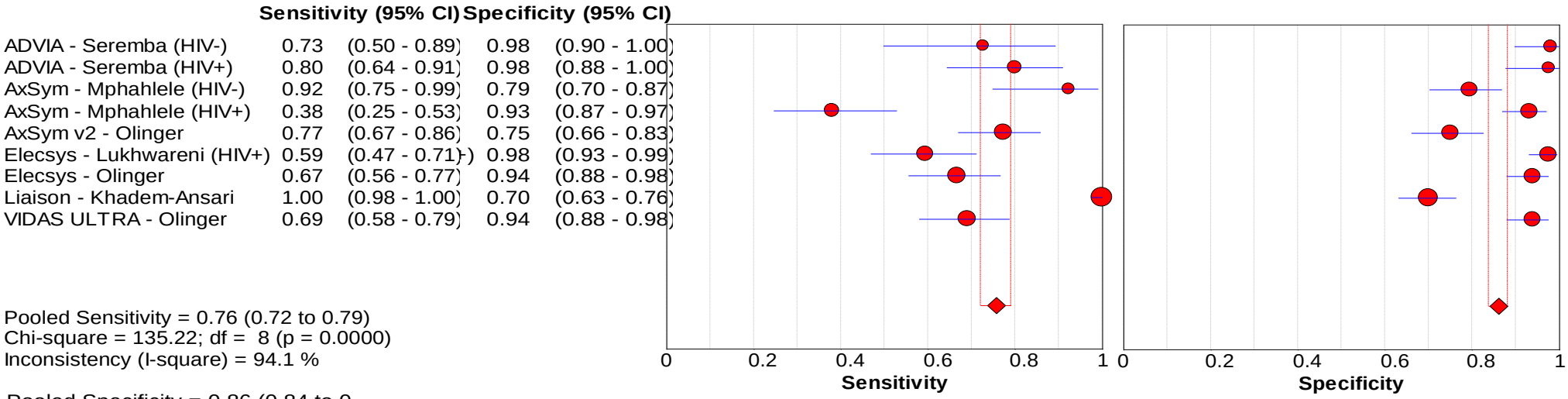


Key – Types of RDT and reference standards used in studies

Study	Test brand (manufacturer)	Reference test type, brand (manufacturer)
Ansari	ACON (Acon laboratories) Atlas (William James House) Blue Cross (Blue Cross Inc.) Cortez (Cortez diagnostics) DIMA (Geseeschaft fur Diagnostika mbH) Intex (Intec Products Inc.)	QPCR Roto-GENE 3000 Research (Corbet real time PCR) and kit artus (Hamburg)

Nna	ACON (Acon laboratories)	Nested PCR QPCR for positive
Seremba	Cortez (Cortez diagnostics)	b-DNA (Versant); PCR, Amplicor for discrepant

Fig. 6. Forest plots, EIA vs NAT, ordered by [Test, Author]



Key – Types of EIA and reference standards used in studies

Study	Test type, brand (manufacturer)	Reference test type, brand (manufacturer)
Seremba	EIA, ADVIA Centaur (Siemens)	b-DNA (Versant); PCR, Amplicor for discrepant
Mphahlele	EIA, AxSym (Abbott)	Nested PCR (in house); positive quantified with COBAS Amplicor ()
Olinger	MEIA, AxSym v2 (Abbott) ELFA, VIDAS Ultra (Biomérieux) ChLIA, Elecsys (Roche)	RT-PCR
Lukhwareni	ChLIA, Elecsys (Roche)	Nested PCR, High Pure Viral Nucleic Acid assay (Roche)

		Q-PCR, COBAS TaqMan HBV Test 48 assay ()
Khadem- Ansari	ChLIA, Liasison (Diasorin)	RT-PCR, Robogene (Corbett)

Summary Receiver Operating Characteristic (SROC) curves

Fig. 7. SROC curves for studies comparing RDTs with EIAs

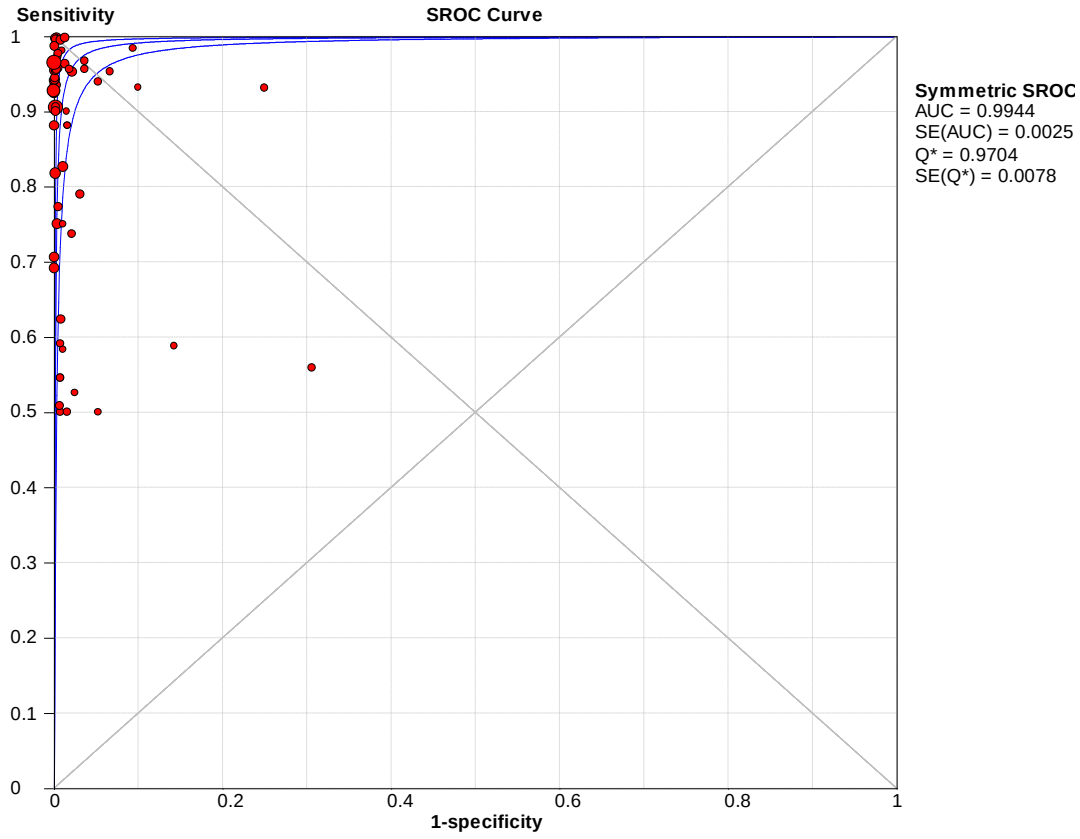


Fig. 8. SROC curves for studies comparing RDTs with NATs

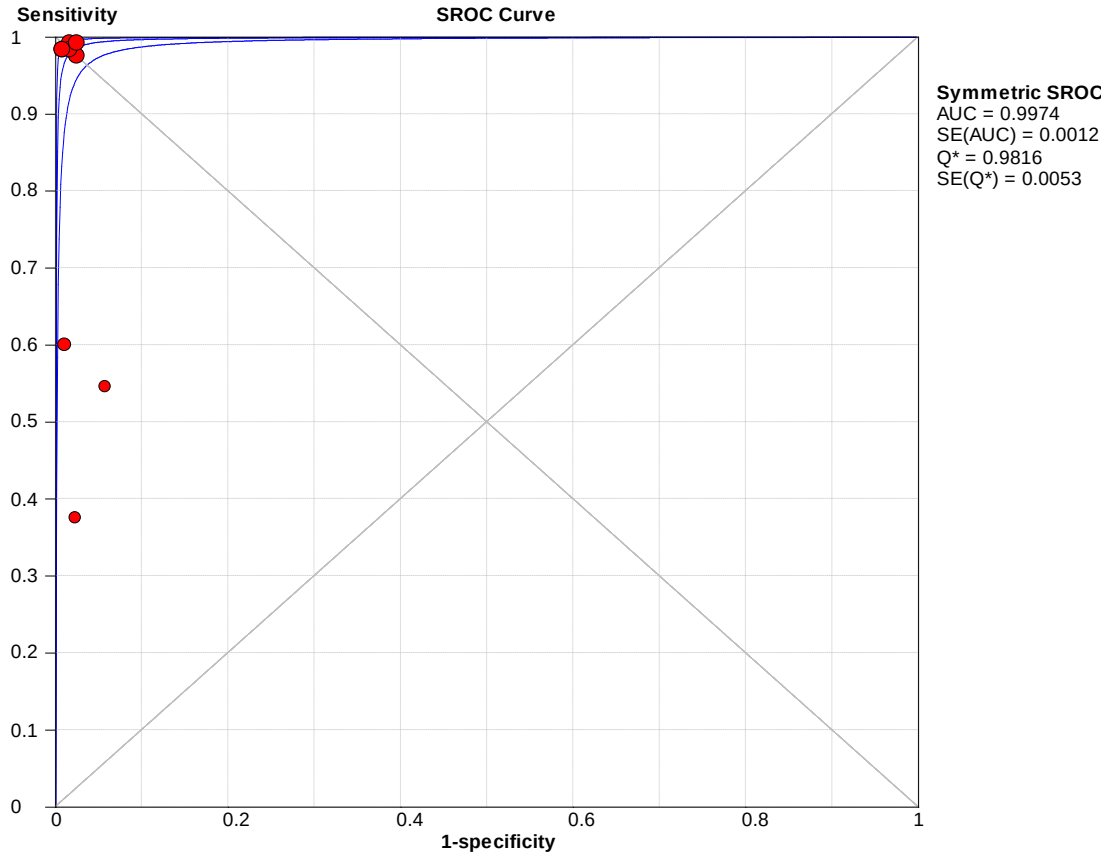


Fig. 9. SROC curves for studies comparing EIAs with EIAs

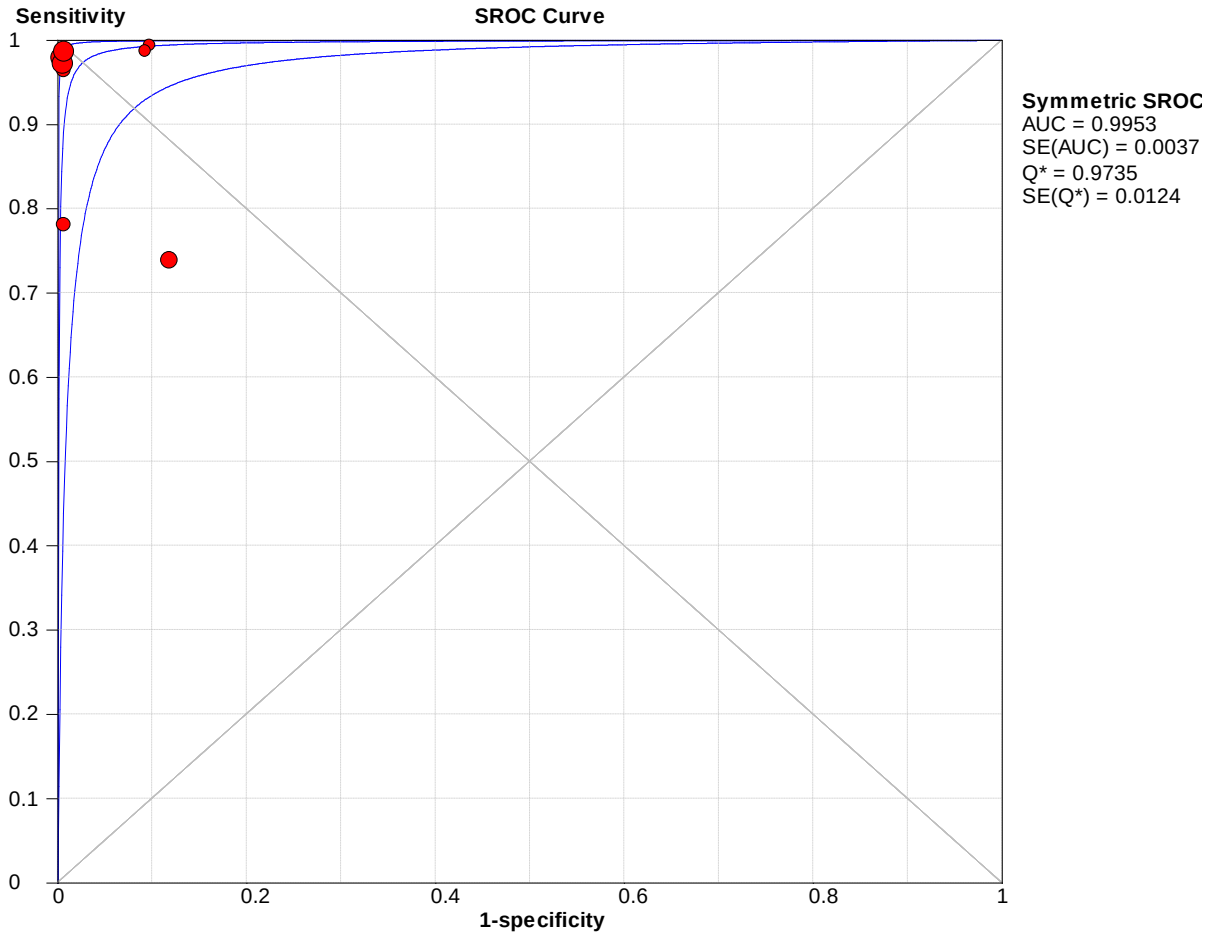
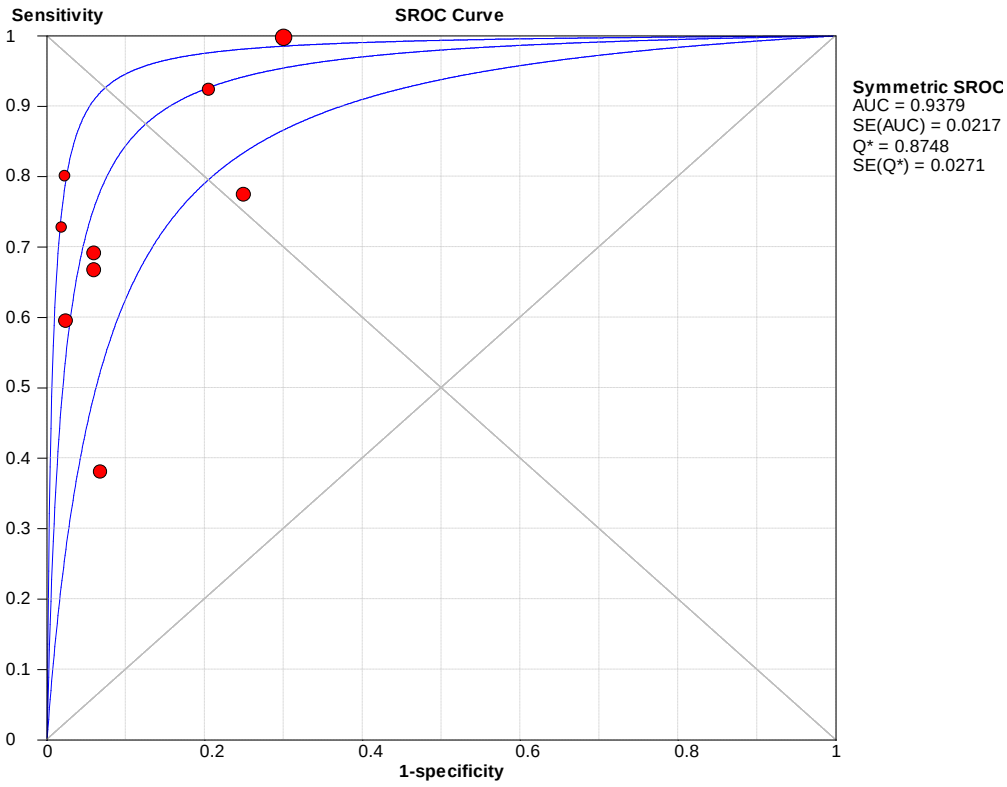


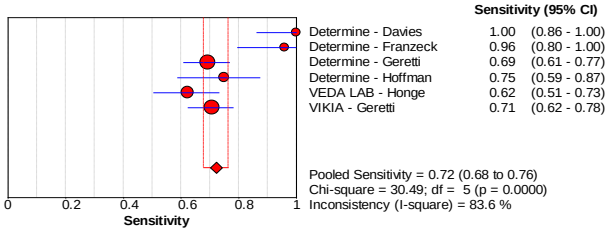
Fig. 10. SROC curves for studies comparing EIAs with NAT



Forest plots of sub-analyses

Forest plots, analysed by HIV status

Fig. 11. Forest plots of RDTs vs EIAs in HIV-positive patients



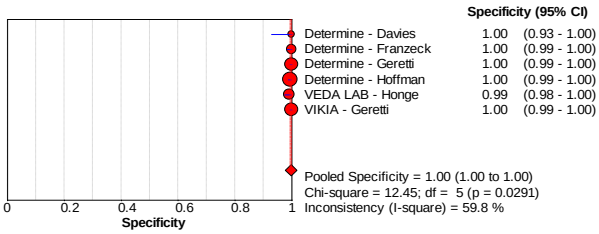


Fig. 13. Forest plots, RDTs vs EIAs in HIV-negative patients

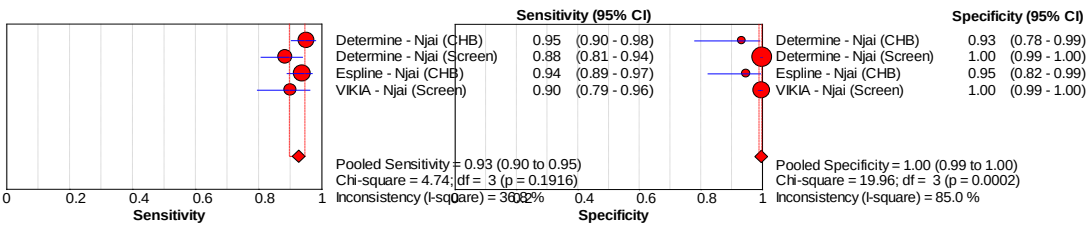


Fig. 14. Forest plots, EIAs vs EIAs in HIV-positive patients

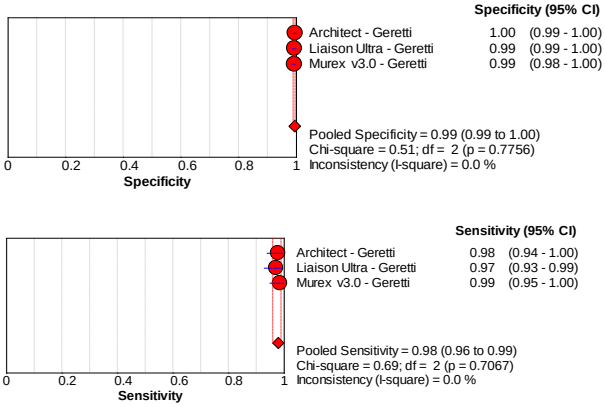


Fig. 15. Forest plots, EIAs vs NAT in HIV-positive patients

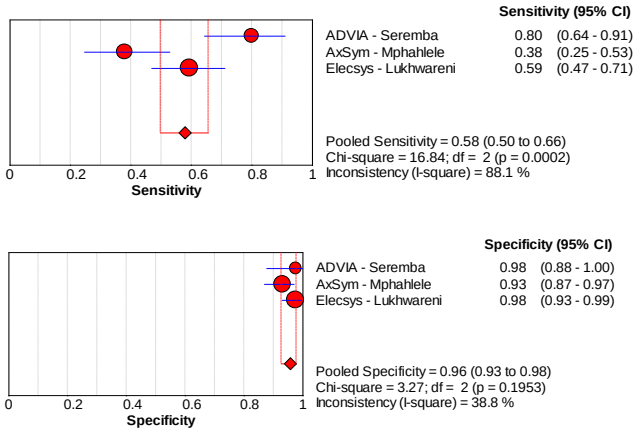


Fig. 16. Forest plots, EIAs vs NAT in HIV-negative patients

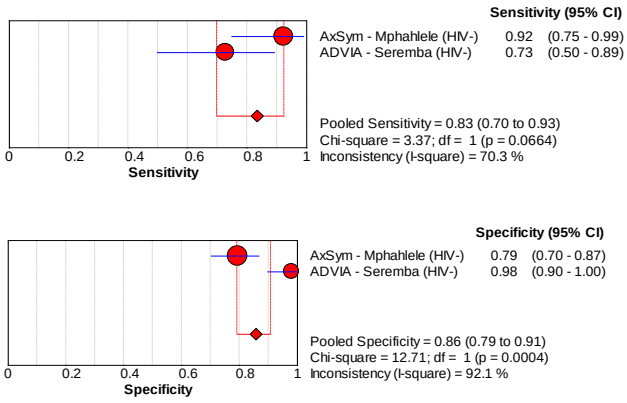
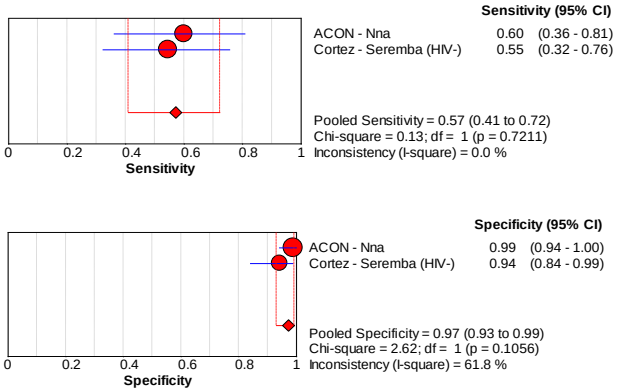
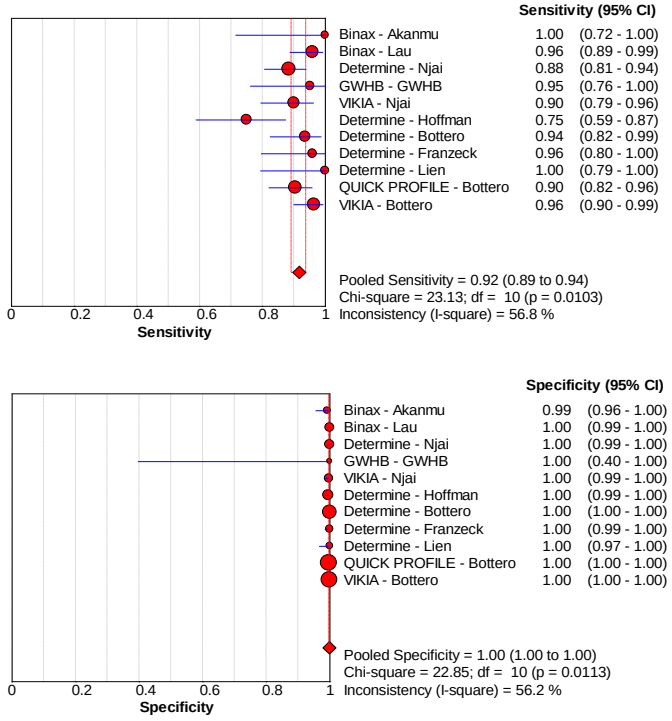


Fig. 17. Forest plots, RDTs vs NAT in HIV-negative patients



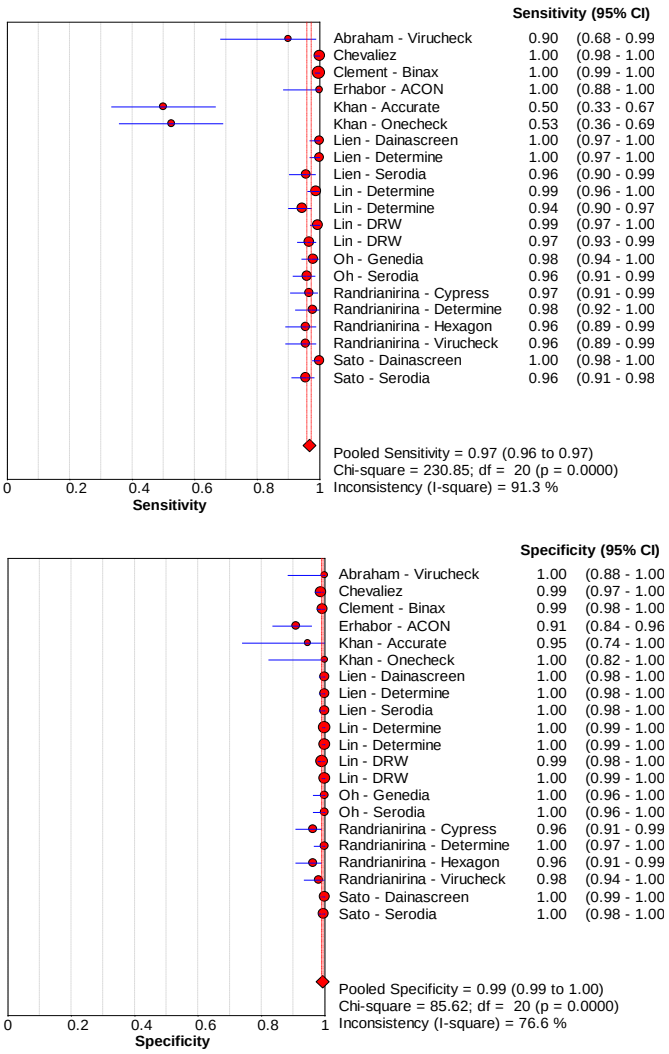
Forest plots, analysed by sample type

Fig. 18. Forest plots, RDTs vs EIA in whole blood



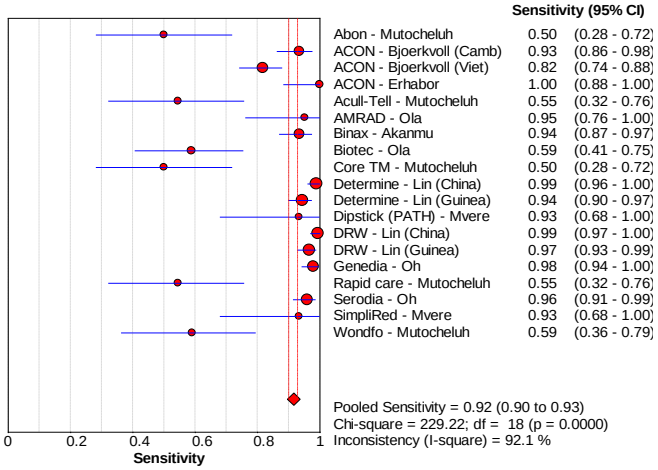
Forest plots, analysed by study design

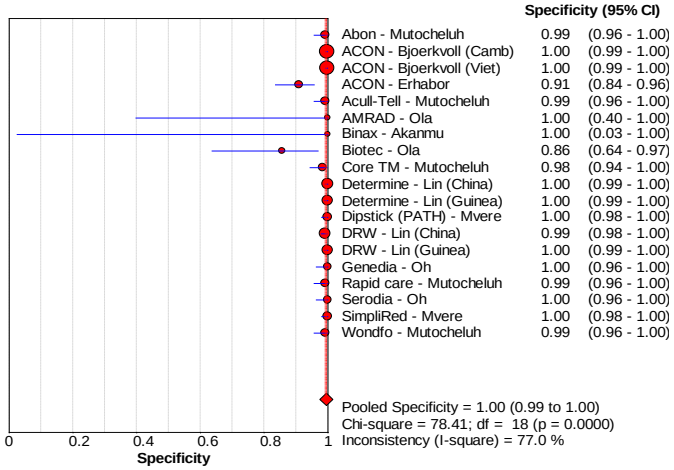
Fig. 19. Forest plots, RDTs vs EIA in case–control studies



9.3.4 Forest plots, analysed by study setting

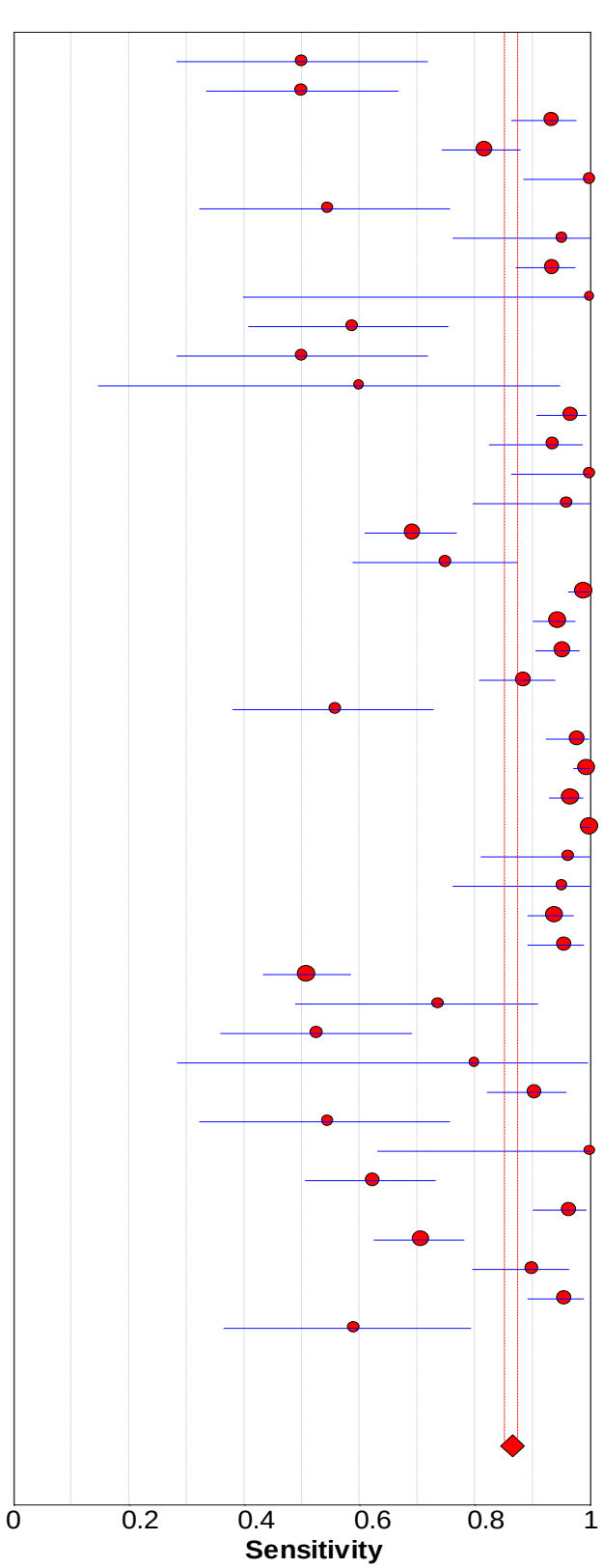
Fig. 20. Forest plots, RDTs vs EIA in blood donors





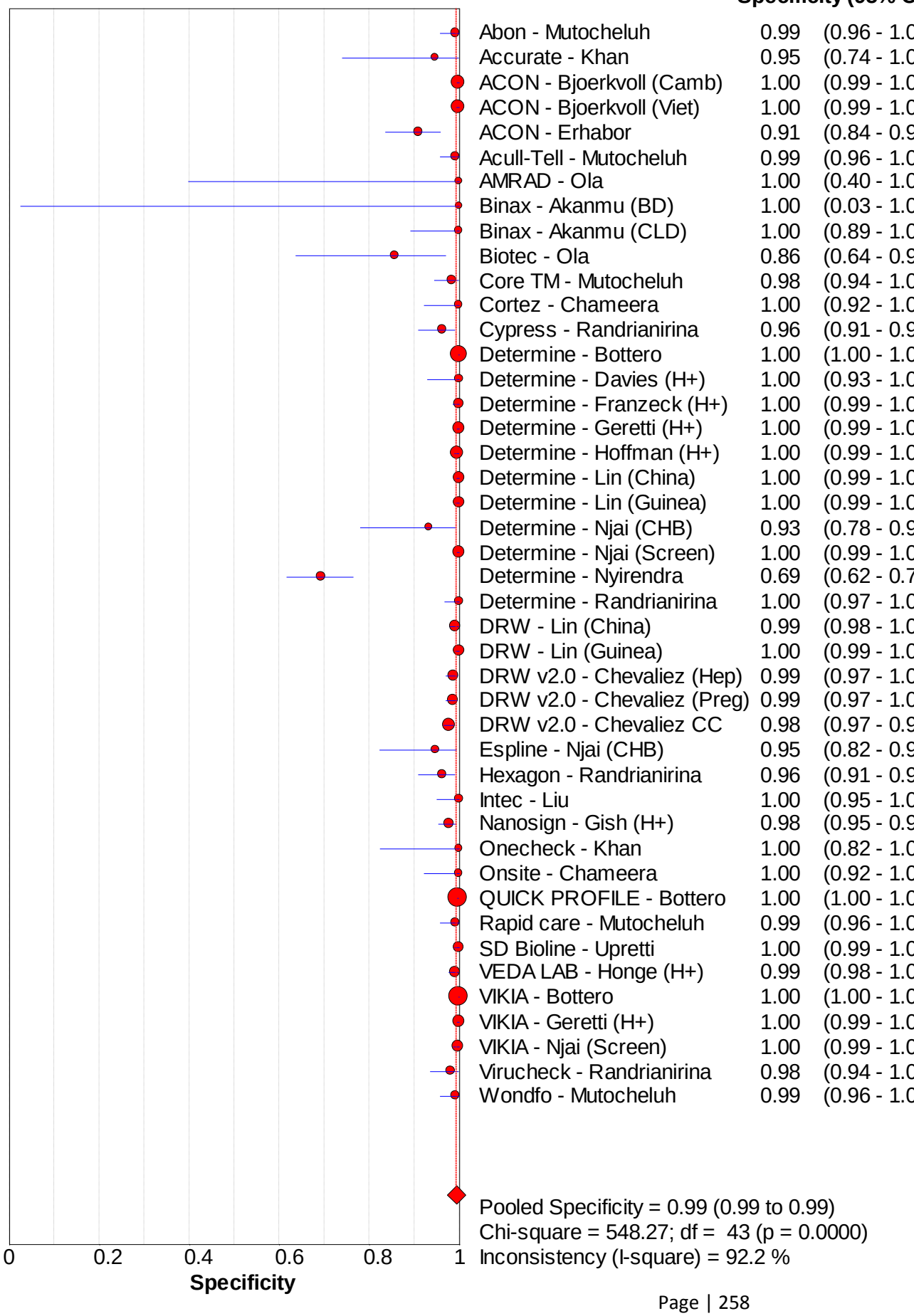
Forest plots, analysed by study year

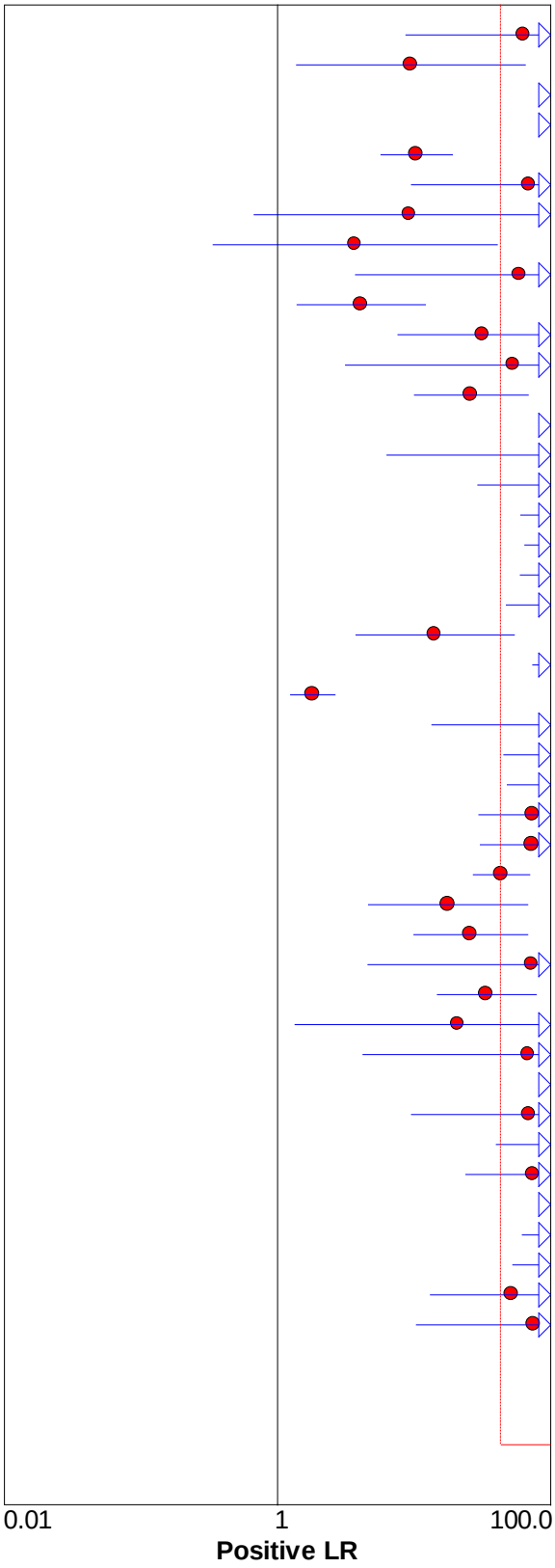
Fig. 21. Forest plots, RDTs vs EIA for studies after 2005



	Sensitivity	(95% CI)
Abon - Mutocheluh	0.50	(0.28 - 0.72)
Accurate - Khan	0.50	(0.33 - 0.67)
ACON - Bjoerkvoll (Camb)	0.93	(0.86 - 0.98)
ACON - Bjoerkvoll (Viet)	0.82	(0.74 - 0.88)
ACON - Erhabor	1.00	(0.88 - 1.00)
Acull-Tell - Mutocheluh	0.55	(0.32 - 0.76)
AMRAD - Ola	0.95	(0.76 - 1.00)
Binax - Akanmu (BD)	0.94	(0.87 - 0.97)
Binax - Akanmu (CLD)	1.00	(0.40 - 1.00)
Biotec - Ola	0.59	(0.41 - 0.75)
Core TM - Mutocheluh	0.50	(0.28 - 0.72)
Cortez - Chameera	0.60	(0.15 - 0.95)
Cypress - Randrianirina	0.97	(0.91 - 0.99)
Determine - Bottero	0.94	(0.82 - 0.99)
Determine - Davies (H+)	1.00	(0.86 - 1.00)
Determine - Franzeck (H+)	0.96	(0.80 - 1.00)
Determine - Geretti (H+)	0.69	(0.61 - 0.77)
Determine - Hoffman (H+)	0.75	(0.59 - 0.87)
Determine - Lin (China)	0.99	(0.96 - 1.00)
Determine - Lin (Guinea)	0.94	(0.90 - 0.97)
Determine - Njai (CHB)	0.95	(0.90 - 0.98)
Determine - Njai (Screen)	0.88	(0.81 - 0.94)
Determine - Nyirendra	0.56	(0.38 - 0.73)
Determine - Randrianirina	0.98	(0.92 - 1.00)
DRW - Lin (China)	0.99	(0.97 - 1.00)
DRW - Lin (Guinea)	0.97	(0.93 - 0.99)
DRW v2.0 - Chevaliez (Hep)	1.00	(0.98 - 1.00)
DRW v2.0 - Chevaliez (Preg)	0.96	(0.81 - 1.00)
DRW v2.0 - Chevaliez CC	0.95	(0.76 - 1.00)
Espline - Njai (CHB)	0.94	(0.89 - 0.97)
Hexagon - Randrianirina	0.96	(0.89 - 0.99)
Intec - Liu	0.51	(0.43 - 0.58)
Nanosign - Gish (H+)	0.74	(0.49 - 0.91)
Onecheck - Khan	0.53	(0.36 - 0.69)
Onsite - Chameera	0.80	(0.28 - 0.99)
QUICK PROFILE - Bottero	0.90	(0.82 - 0.96)
Rapid care - Mutocheluh	0.55	(0.32 - 0.76)
SD Bioline - Upretti	1.00	(0.63 - 1.00)
VEDA LAB - Honge (H+)	0.62	(0.51 - 0.73)
VIKIA - Bottero	0.96	(0.90 - 0.99)
VIKIA - Geretti (H+)	0.71	(0.62 - 0.78)
VIKIA - Njai (Screen)	0.90	(0.79 - 0.96)
Virucheck - Randrianirina	0.96	(0.89 - 0.99)
Wondfo - Mutocheluh	0.59	(0.36 - 0.79)

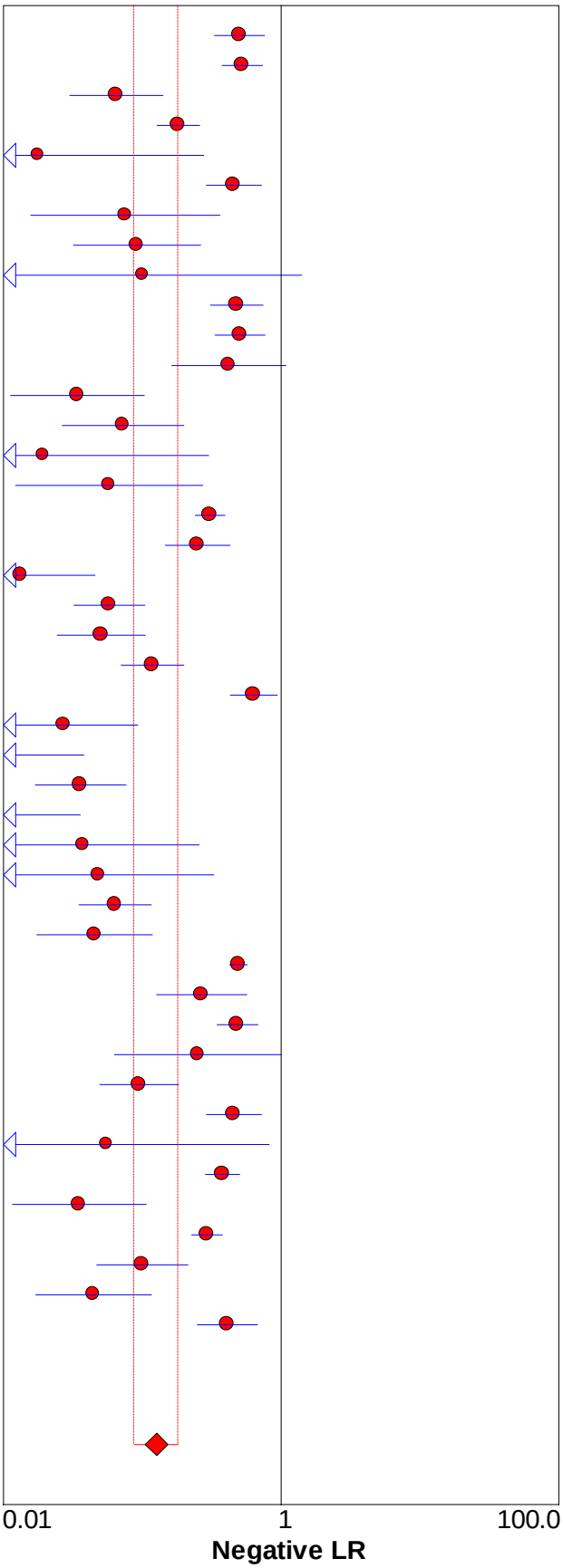
Pooled Sensitivity = 0.86 (0.85 to 0.88)
Chi-square = 680.76; df = 43 (p = 0.0000)
Inconsistency (I-square) = 93.7 %


Specificity (95% C
Abon - Mutocheluh 0.99 (0.96 - 1.0
Accurate - Khan 0.95 (0.74 - 1.0
ACON - Bjoerkvoll (Camb) 1.00 (0.99 - 1.0
ACON - Bjoerkvoll (Viet) 1.00 (0.99 - 1.0
ACON - Erhabor 0.91 (0.84 - 0.9
Acull-Tell - Mutocheluh 0.99 (0.96 - 1.0
AMRAD - Ola 1.00 (0.40 - 1.0
Binax - Akanmu (BD) 1.00 (0.03 - 1.0
Binax - Akanmu (CLD) 1.00 (0.89 - 1.0
Biotec - Ola 0.86 (0.64 - 0.9
Core TM - Mutocheluh 0.98 (0.94 - 1.0
Cortez - Chameera 1.00 (0.92 - 1.0
Cypress - Randrianirina 0.96 (0.91 - 0.9
Determine - Bottero 1.00 (1.00 - 1.0
Determine - Davies (H+) 1.00 (0.93 - 1.0
Determine - Franzeck (H+) 1.00 (0.99 - 1.0
Determine - Geretti (H+) 1.00 (0.99 - 1.0
Determine - Hoffman (H+) 1.00 (0.99 - 1.0
Determine - Lin (China) 1.00 (0.99 - 1.0
Determine - Lin (Guinea) 1.00 (0.99 - 1.0
Determine - Njai (CHB) 0.93 (0.78 - 0.9
Determine - Njai (Screen) 1.00 (0.99 - 1.0
Determine - Nyirendra 0.69 (0.62 - 0.7
Determine - Randrianirina 1.00 (0.97 - 1.0
DRW - Lin (China) 0.99 (0.98 - 1.0
DRW - Lin (Guinea) 1.00 (0.99 - 1.0
DRW v2.0 - Chevaliez (Hep) 0.99 (0.97 - 1.0
DRW v2.0 - Chevaliez (Preg) 0.99 (0.97 - 1.0
DRW v2.0 - Chevaliez CC 0.98 (0.97 - 0.9
Espline - Njai (CHB) 0.95 (0.82 - 0.9
Hexagon - Randrianirina 0.96 (0.91 - 0.9
Intec - Liu 1.00 (0.95 - 1.0
Nanosign - Gish (H+) 0.98 (0.95 - 0.9
Onecheck - Khan 1.00 (0.82 - 1.0
Onsite - Chameera 1.00 (0.92 - 1.0
QUICK PROFILE - Bottero 1.00 (1.00 - 1.0
Rapid care - Mutocheluh 0.99 (0.96 - 1.0
SD Bioline - Upretti 1.00 (0.99 - 1.0
VEDA LAB - Honge (H+) 0.99 (0.98 - 1.0
VIKIA - Bottero 1.00 (1.00 - 1.0
VIKIA - Geretti (H+) 1.00 (0.99 - 1.0
VIKIA - Njai (Screen) 1.00 (0.99 - 1.0
Virucheck - Randrianirina 0.98 (0.94 - 1.0
Wondfo - Mutocheluh 0.99 (0.96 - 1.0
Pooled Specificity = 0.99 (0.99 to 0.99)
Chi-square = 548.27; df = 43 (p = 0.0000)
Inconsistency (I-square) = 92.2 %
0
0.2
0.4
0.6
0.8
1
Specificity



	Positive LR (95% CI)
Abon - Mutocheluh	64.00 (8.69 - 471.26)
Accurate - Khan	9.50 (1.37 - 65.71)
ACON - Bjoerkvoll (Camb)	517.87 (129.54 - 2,070.26)
ACON - Bjoerkvoll (Viet)	434.51 (108.57 - 1,739.03)
ACON - Erhabor	10.46 (5.70 - 19.19)
Acull-Tell - Mutocheluh	69.82 (9.55 - 510.36)
AMRAD - Ola	9.32 (0.67 - 129.54)
Binax - Akanmu (BD)	3.72 (0.34 - 41.10)
Binax - Akanmu (CLD)	59.40 (3.74 - 944.36)
Biotec - Ola	4.12 (1.39 - 12.18)
Core TM - Mutocheluh	32.00 (7.60 - 134.68)
Cortez - Chameera	53.67 (3.14 - 917.12)
Cypress - Randrianirina	26.35 (10.06 - 69.00)
Determine - Bottero	4,498.21 (281.12 - 71,976.8
Determine - Davies (H+)	100.04 (6.34 - 1,578.57)
Determine - Franzeck (H+)	467.38 (29.27 - 7,463.70)
Determine - Geretti (H+)	966.70 (60.39 - 15,473.86)
Determine - Hoffman (H+)	174.94 (64.74 - 472.74)
Determine - Lin (China)	959.01 (60.07 - 15,311.15)
Determine - Lin (Guinea)	753.59 (47.21 - 12,030.12)
Determine - Njai (CHB)	14.29 (3.74 - 54.54)
Determine - Njai (Screen)	1,180.48 (73.85 - 18,870.34
Determine - Nyirendra	1.82 (1.25 - 2.67)
Determine - Randrianirina	214.02 (13.47 - 3,400.91)
DRW - Lin (China)	120.60 (45.44 - 320.04)
DRW - Lin (Guinea)	771.27 (48.32 - 12,311.21)
DRW v2.0 - Chevaliez (Hep)	74.50 (29.76 - 186.52)
DRW v2.0 - Chevaliez (Preg)	73.38 (30.62 - 175.83)
DRW v2.0 - Chevaliez CC	43.75 (27.08 - 70.69)
Espline - Njai (CHB)	17.85 (4.63 - 68.81)
Hexagon - Randrianirina	26.05 (9.95 - 68.23)
Intec - Liu	73.20 (4.61 - 1,163.40)
Nanosign - Gish (H+)	34.14 (14.80 - 78.75)
Onecheck - Khan	21.03 (1.34 - 329.93)
Onsite - Chameera	69.00 (4.22 - 1,129.19)
QUICK PROFILE - Bottero	347.25 (186.27 - 647.36)
Rapid care - Mutocheluh	69.82 (9.55 - 510.36)
SD Bioline - Upretti	642.22 (40.07 - 10,293.22)
VEDA LAB - Honge (H+)	75.01 (23.99 - 234.59)
VIKIA - Bottero	1,853.68 (463.49 - 7,413.61
VIKIA - Geretti (H+)	986.53 (61.64 - 15,789.02)
VIKIA - Njai (Screen)	374.40 (52.77 - 2,656.47)
Virucheck - Randrianirina	52.10 (13.19 - 205.84)
Wondfo - Mutocheluh	75.64 (10.41 - 549.47)

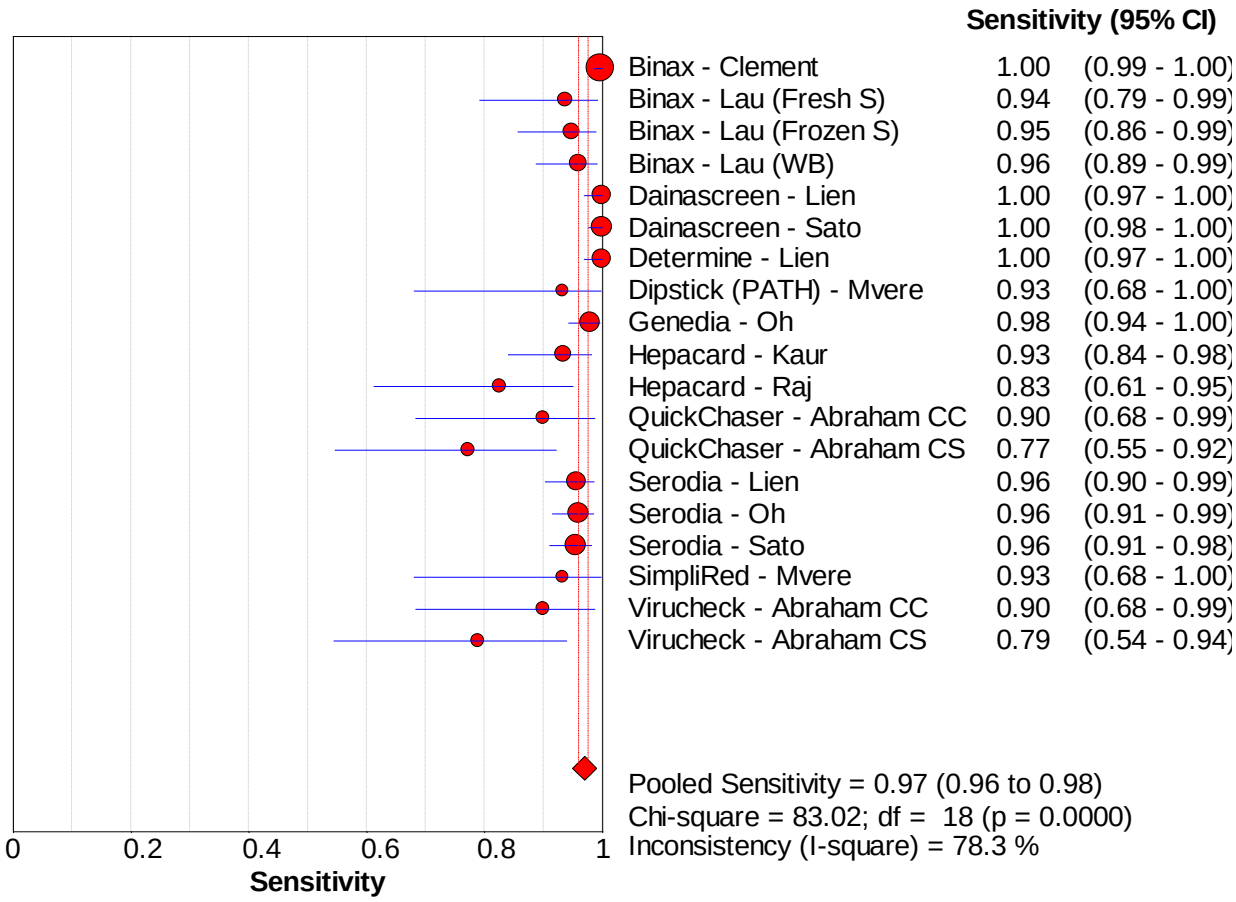
Random Effects Model
Pooled Positive LR = 84.66 (43.55 to 164.55)
Cochran-Q = 534.07; df = 43 (p = 0.0000)
Inconsistency (I-square) = 91.9 %
Tau-squared = 4.0986

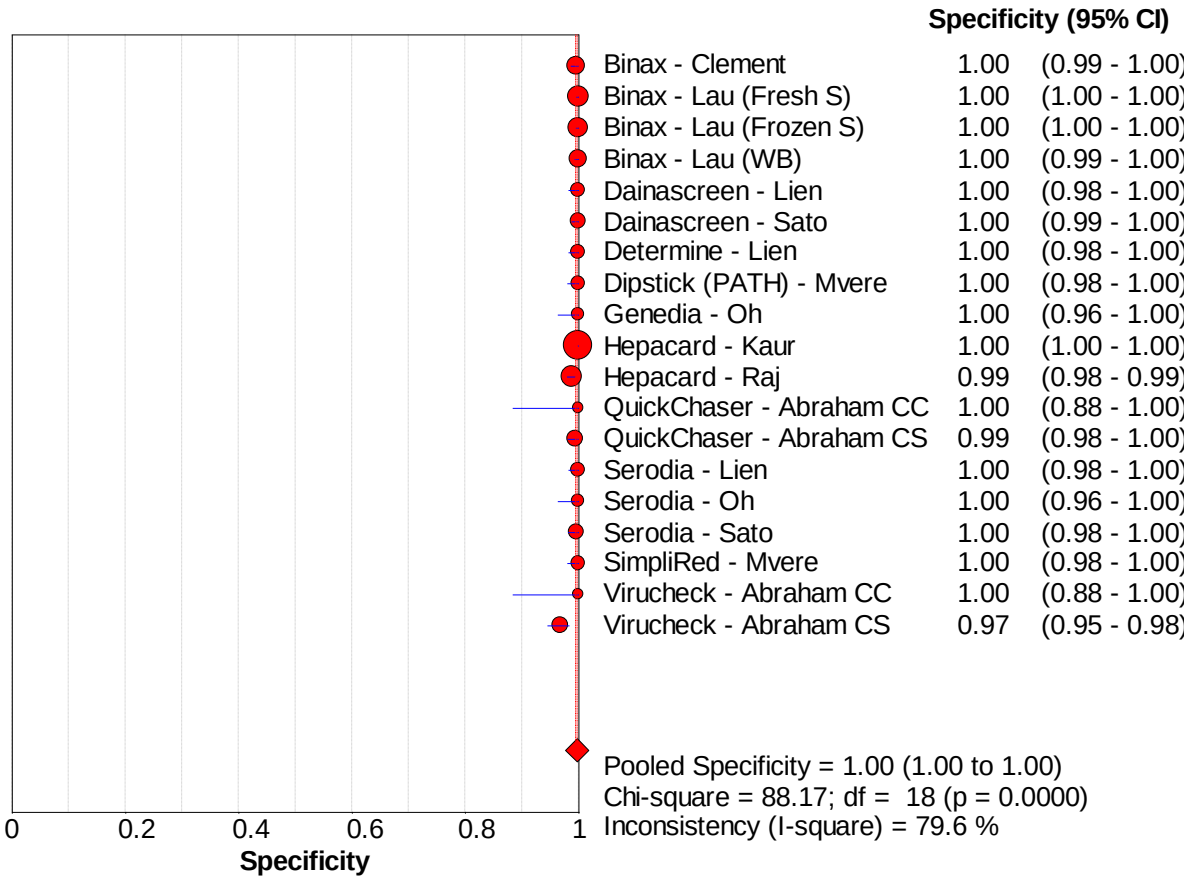


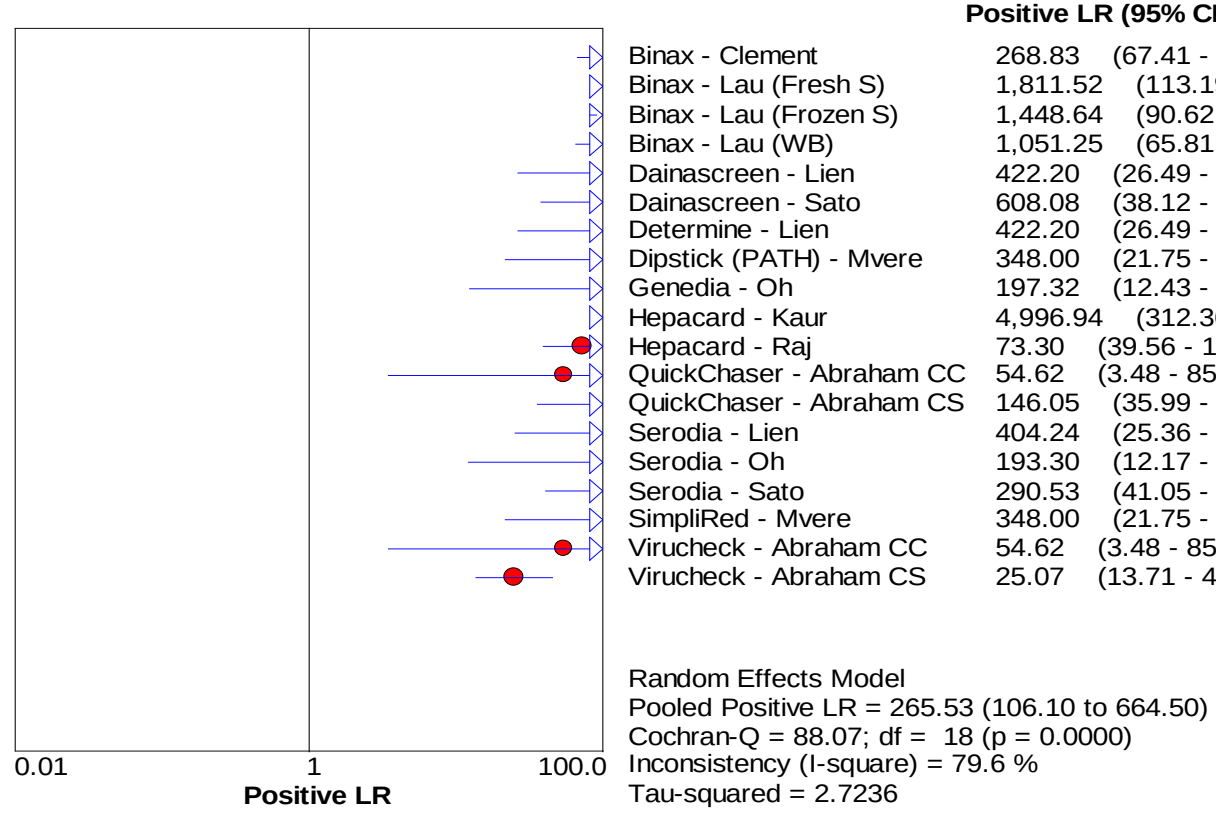
	Negative LR	(95% CI
Abon - Mutocheluh	0.50	(0.33 - 0.77)
Accurate - Khan	0.53	(0.38 - 0.74)
ACON - Bjoerkvoll (Camb)	0.07	(0.03 - 0.14)
ACON - Bjoerkvoll (Viet)	0.18	(0.13 - 0.26)
ACON - Erhabor	0.02	(0.00 - 0.28)
Acull-Tell - Mutocheluh	0.46	(0.29 - 0.72)
AMRAD - Ola	0.08	(0.02 - 0.36)
Binax - Akanmu (BD)	0.09	(0.03 - 0.26)
Binax - Akanmu (CLD)	0.10	(0.01 - 1.41)
Biotec - Ola	0.48	(0.31 - 0.74)
Core TM - Mutocheluh	0.51	(0.33 - 0.77)
Cortez - Chameera	0.42	(0.16 - 1.09)
Cypress - Randrianirina	0.03	(0.01 - 0.10)
Determine - Bottero	0.07	(0.03 - 0.20)
Determine - Davies (H+)	0.02	(0.00 - 0.30)
Determine - Franzeck (H+)	0.06	(0.01 - 0.27)
Determine - Geretti (H+)	0.31	(0.24 - 0.40)
Determine - Hoffman (H+)	0.25	(0.15 - 0.43)
Determine - Lin (China)	0.01	(0.00 - 0.05)
Determine - Lin (Guinea)	0.06	(0.03 - 0.10)
Determine - Njai (CHB)	0.05	(0.02 - 0.11)
Determine - Njai (Screen)	0.12	(0.07 - 0.20)
Determine - Nyirendra	0.64	(0.43 - 0.94)
Determine - Randrianirina	0.03	(0.01 - 0.09)
DRW - Lin (China)	0.01	(0.00 - 0.04)
DRW - Lin (Guinea)	0.04	(0.02 - 0.08)
DRW v2.0 - Chevaliez (Hep)	0.00	(0.00 - 0.04)
DRW v2.0 - Chevaliez (Preg)	0.04	(0.01 - 0.26)
DRW v2.0 - Chevaliez CC	0.05	(0.01 - 0.33)
Espline - Njai (CHB)	0.06	(0.03 - 0.12)
Hexagon - Randrianirina	0.05	(0.02 - 0.12)
Intec - Liu	0.50	(0.43 - 0.58)
Nanosign - Gish (H+)	0.27	(0.13 - 0.57)
Onecheck - Khan	0.49	(0.35 - 0.68)
Onsite - Chameera	0.25	(0.06 - 1.01)
QUICK PROFILE - Bottero	0.10	(0.05 - 0.18)
Rapid care - Mutocheluh	0.46	(0.29 - 0.72)
SD Bioline - Upretti	0.06	(0.00 - 0.82)
VEDA LAB - Honge (H+)	0.38	(0.28 - 0.51)
VIKIA - Bottero	0.04	(0.01 - 0.11)
VIKIA - Geretti (H+)	0.29	(0.23 - 0.38)
VIKIA - Njai (Screen)	0.10	(0.05 - 0.21)
Virucheck - Randrianirina	0.04	(0.02 - 0.12)
Wondfo - Mutocheluh	0.41	(0.25 - 0.68)

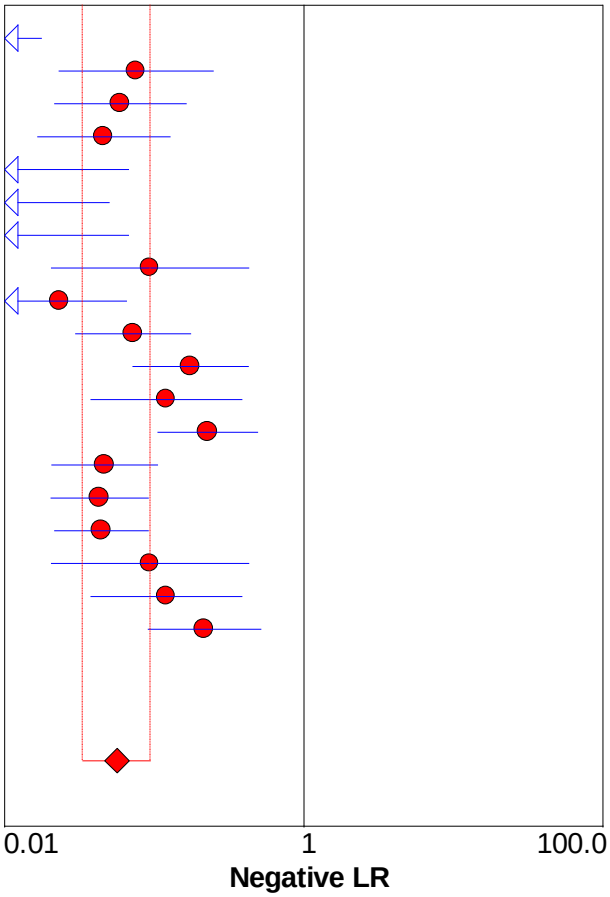
Random Effects Model
Pooled Negative LR = 0.13 (0.09 to 0.18)
Cochran-Q = 866.48; df = 43 (p = 0.0000)
Inconsistency (I-square) = 95.0 %
Tau-squared = 1.2712

Fig. 22. Forest plots, RDTs vs EIA for studies before 2005




Specificity (95% CI)
Binax - Clement 1.00 (0.99 - 1.00)
Binax - Lau (Fresh S) 1.00 (1.00 - 1.00)
Binax - Lau (Frozen S) 1.00 (1.00 - 1.00)
Binax - Lau (WB) 1.00 (0.99 - 1.00)
Dainascreen - Lien 1.00 (0.98 - 1.00)
Dainascreen - Sato 1.00 (0.99 - 1.00)
Determine - Lien 1.00 (0.98 - 1.00)
Dipstick (PATH) - Mvere 1.00 (0.98 - 1.00)
Genedia - Oh 1.00 (0.96 - 1.00)
Hepacard - Kaur 1.00 (1.00 - 1.00)
Hepacard - Raj 0.99 (0.98 - 0.99)
QuickChaser - Abraham CC 1.00 (0.88 - 1.00)
QuickChaser - Abraham CS 0.99 (0.98 - 1.00)
Serodia - Lien 1.00 (0.98 - 1.00)
Serodia - Oh 1.00 (0.96 - 1.00)
Serodia - Sato 1.00 (0.98 - 1.00)
SimpliRed - Mvere 1.00 (0.98 - 1.00)
Virucheck - Abraham CC 1.00 (0.88 - 1.00)
Virucheck - Abraham CS 0.97 (0.95 - 0.98)
Pooled Specificity = 1.00 (1.00 to 1.00)
Chi-square = 88.17; df = 18 (p = 0.0000)
Inconsistency (I-square) = 79.6 %
0
0.2
0.4
0.6
0.8
1
Specificity


Positive LR (95% CI)
Binax - Clement 268.83 (67.41 - 1,072.
Binax - Lau (Fresh S) 1,811.52 (113.19 - 28,
Binax - Lau (Frozen S) 1,448.64 (90.62 - 23,1
Binax - Lau (WB) 1,051.25 (65.81 - 16,7
Dainascreen - Lien 422.20 (26.49 - 6,728.
Dainascreen - Sato 608.08 (38.12 - 9,699.
Determine - Lien 422.20 (26.49 - 6,728.
Dipstick (PATH) - Mvere 348.00 (21.75 - 5,568.
Genedia - Oh 197.32 (12.43 - 3,133.
Hepacard - Kaur 4,996.94 (312.36 - 79,
Hepacard - Raj 73.30 (39.56 - 135.82)
QuickChaser - Abraham CC 54.62 (3.48 - 857.69)
QuickChaser - Abraham CS 146.05 (35.99 - 592.65
Serodia - Lien 404.24 (25.36 - 6,443.
Serodia - Oh 193.30 (12.17 - 3,069.
Serodia - Sato 290.53 (41.05 - 2,056.
SimpliRed - Mvere 348.00 (21.75 - 5,568.
Virucheck - Abraham CC 54.62 (3.48 - 857.69)
Virucheck - Abraham CS 25.07 (13.71 - 45.82)
Random Effects Model
Pooled Positive LR = 265.53 (106.10 to 664.50)
Cochran-Q = 88.07; df = 18 (p = 0.0000)
Inconsistency (I-square) = 79.6 %
Tau-squared = 2.7236
0.01
1
100.0
Positive LR



	Negative LR	(95% CI
Binax - Clement	0.00	(0.00 - 0.02)
Binax - Lau (Fresh S)	0.08	(0.02 - 0.25)
Binax - Lau (Frozen S)	0.06	(0.02 - 0.16)
Binax - Lau (WB)	0.05	(0.02 - 0.13)
Dainascreen - Lien	0.00	(0.00 - 0.07)
Dainascreen - Sato	0.00	(0.00 - 0.05)
Determine - Lien	0.00	(0.00 - 0.07)
Dipstick (PATH) - Mvere	0.09	(0.02 - 0.43)
Genedia - Oh	0.02	(0.01 - 0.07)
Hepacard - Kaur	0.07	(0.03 - 0.18)
Hepacard - Raj	0.18	(0.07 - 0.43)
QuickChaser - Abraham CC	0.12	(0.04 - 0.39)
QuickChaser - Abraham CS	0.23	(0.11 - 0.49)
Serodia - Lien	0.05	(0.02 - 0.11)
Serodia - Oh	0.04	(0.02 - 0.09)
Serodia - Sato	0.04	(0.02 - 0.09)
SimpliRed - Mvere	0.09	(0.02 - 0.43)
Virucheck - Abraham CC	0.12	(0.04 - 0.39)
Virucheck - Abraham CS	0.22	(0.09 - 0.52)

Random Effects Model
Pooled Negative LR = 0.06 (0.03 to 0.09)
Cochran-Q = 74.14; df = 18 (p = 0.0000)
Inconsistency (I-square) = 75.7 %
Tau-squared = 0.9133

9.3. **9.5 List of tests available**

Manufacturer	Test	Test type
Abbott	AxSym HBsAg v2	MEIA
Abbott	Architect HBsAg	CMIA (Quant)
Abbott	Architect HBsAg Qualitative	CMIA
Abbott	Architect HBsAg Qualitative II	CMIA
Abbott	Auszyme Monoclonal	
Abbott	PRISM HBsAg	ChLIA
Adaltis	EIAgen HBsAg Kit	EIA
Siemens	ADVIA Centaur HBsAg	ChLIA
Siemens	ADVIA Centaur HBsAg II	ChLIA

BIOKIT	Bioelisa HBsAg 3.0	
bioMerieux	Hepanostika Uni-Form II	Microelisa
bioMerieux	VIKIA HBsAg Kit	
bioMerieux	VIDAS HBsAg	ELFA
bioMerieux	VIDAS HBsAg Ultra	ELFA
BIO-RAD	Genscreen HBsAg 3.0	
BIO-RAD	MONOLISA HBsAg Ultra	EIA/ ELISA
BIO-RAD	MONOLISA HBsAg Plus	EIA
BIO-RAD	MONOLISA AgHBs (2nd Gen)	
Dade Behring	Enzygnost HBsAg EIA 5.0	EIA
Dade Behring	Enzygnost HBsAg EIA 6.0	EIA
Diasorin	ETI-MAK 4 HBsAg EIA	EIA - ? not available
Diasorin (prev Abbott)	Murex HBsAg Version 3	ELISA
Diasorin	Liason HBsAg	ChLIA
Diasorin	Liason XL MUREX HBsAg	ChLIA (Quant)
General Biologicals Corp.	SURASE B-96, TMB	ELISA
MBS SRL Medical Biological Services	HBsAg One Step	ELISA
Ortho	HBsAg ELISA Test System 3	
Ortho	Vitros ECi HBsAg	
Roche	Cobas Core HBsAg II	EIA
Roche	Elecsys HBsAg	ECLIA
Roche	Elecsys II HBsAg	ECLIA
Siemens Medical Solutions	Immulite 2000 HBsAg	ELISA

Diagnostics		
Beijing Wantai Biological Pharmacy Enterprise Co., Ltd	Hepatitis B Virus Surface Antigen (HBsAg) ELISA	ELISA

Annex 5.4

PICO 2 - How to test (HCV)

Diagnostic accuracy of tests to detect hepatitis C antibody: a meta-analysis and review of the literature

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1. Executive summary

Background: Although direct acting antivirals (DAAs) have led to sustained virological response in greater than 90% of all individuals treated for hepatitis C virus (HCV), most HCV-infected individuals remain undiagnosed and untreated. Enzyme immunoassays have been used to detect exposure to HCV but access to these laboratory-based assays has been a barrier in reaching at-risk populations for testing and treatment. Rapid diagnostic tests (RDTs) to detect HCV antibody (HCV Ab) are commercially available and may be useful in decentralizing HCV screening outside of laboratory settings. The purpose of this work was to review the peer-reviewed literature and determine the diagnostic accuracy of available assays in detecting antibodies to HCV as a biomarker of exposure.

Method: We used the PRISMA guidelines and Cochrane guidance to develop our search protocol. The search strategy was registered in PROSPERO (CRD42015023567). A literature search was conducted focused on hepatitis C, diagnostic tests and diagnostic accuracy among eight databases. Studies were included if they evaluated an assay to determine the sensitivity and specificity of HCV Ab in humans. Reference standards included enzyme immunoassay (EIA), immunoblot (e.g. recombinant immunoblot assay), and/or nucleic acid testing (NAT). Two reviewers independently extracted data and performed a quality assessment of the studies using the QUADAS tool.

Results: A total of 52 studies were included that included 52 273 unique test measurements. Based on five studies, the pooled RDT sensitivity and specificity were 0.98 (95% CI 0.98–1.00) and 1.00 (95% CI 1.00–1.00) compared to an EIA reference standard. High HCV Ab RDT sensitivity and specificity were observed across screening populations (general population, key populations, hospital patients) using different reference standards (EIA, NAT, immunoblot). Limiting the RDT analysis to studies published in the past five or ten years did not change the results. There were insufficient studies to undertake subanalyses based on HIV coinfection. Oral HCV Ab RDTs had excellent sensitivity and specificity compared to blood reference tests, respectively, at 0.94 (95% CI 0.93–0.96) and 1.00 (95% CI 1.00–1.00). Among studies that assessed individual oral RDT tests, the eight studies that examine OraQuick ADVANCE® had a slightly higher sensitivity (0.98, 95% CI 0.97–0.98) compared to the six studies that examined other brands and found a pooled sensitivity of 0.88 (95% CI 0.84-0.92).

Conclusions: RDTs, including oral tests, have excellent sensitivity and specificity compared to laboratory-based methods for HCV antibody detection across a wide range of settings. Although the sensitivity of the HCV Ab RDT decreases in low- and middle-income country (LMIC) contexts, this would still be an important public health tool for screening purposes. Oral HCV Ab RDTs had good sensitivity and specificity compared to blood reference standards and may be particularly useful in contexts where the use of blood-based tests may be challenging.

2. Background

Hepatitis C is a liver disease caused by the hepatitis C virus (HCV) that causes acute and chronic infection.[1, 2] An estimated 130–150 million people have chronic hepatitis C infection worldwide, leading to 350 000–500 000 deaths per year.[1–4] The introduction of direct-acting antivirals (DAAs) has led to sustained virological response (SVR) in more than 90% of all HCV-infected individuals.[5, 6] DAAs are now recommended by WHO and many other HCV treatment guidelines.[1] DAAs will not only improve SVR rates, but also may simplify HCV management algorithms and allow smaller health facilities to manage HCV-infected individuals.[7] Despite the availability of effective treatment, most HCV-infected individuals remain undiagnosed and untreated.[4] As a result, approximately 15–30% of individuals with chronic HCV infection progress to cirrhosis, leading to end-stage liver disease and hepatocellular carcinoma.[1, 2]

In April 2014, WHO published the guidelines for the screening, care and treatment of individuals with HCV infection.[8] These guidelines included recommendations on who to screen for HCV and how to confirm HCV infection, but not which tests are optimal for initial screening. The World Health Assembly has passed several resolutions highlighting the importance of prevention and control of viral hepatitis for global health.

Advances in HCV detection technology create new opportunities for enhancing screening, referral, and treatment. Previous systematic reviews on hepatitis C infection have focused on treatment response,[9,10] clinical complications[11] and epidemiology.[12, 13] Two systematic reviews on hepatitis C testing focused on evaluating point-of-care (POC) tests compared to EIAs and other reference tests.[14, 15] This review extends previous reviews by including new studies, including a subanalysis focused on oral tests, and including studies that evaluated immunoassays using a NAT reference standard.

3. Objectives

The purpose of this review was to identify quantitative evidence on the sensitivity and specificity of rapid diagnostic tests used to detect HCV Ab, synthesize the evidence, and inform models to estimate cost–effectiveness of different strategies for testing.

PICO 2	Among persons identified for hepatitis C testing, what is the diagnostic accuracy of available assays for detecting HCVAb?
P	Persons identified for HCV testing
I	Rapid diagnostic tests and enzyme immunoassays for HCsAg detection
C	1) EIA (with subanalysis based on the last ten years) 2) NAT (nucleic acid testing) 3) Immunoblot or similar assay 4) A combination of 1,2,3 above
O	Diagnostic accuracy (Sensitivity, Specificity, Positive predictive value, Negative predictive value, TN, TP, FN, and FP).

4. Methods

Search strategy and identification of studies

We included observational and randomized control trial (RCT) studies that provide original data from patient specimens from cross-sectional or case–control studies. Literature search strategies were developed by a medical librarian with expertise in designing systematic review searches. Our search algorithm consisted of the following components: hepatitis C, diagnostic tests, and diagnostic accuracy (*see* annex 1). We searched MEDLINE (OVID interface, 1946 onwards), EMBASE (OVID interface, 1947 onwards), the Cochrane Central Register of Controlled Trials (Wiley interface, current issue), Science Citation Index Expanded (Web of Science interface, 1970 onwards), Conference Proceedings Citation Index-Science (Web of Science interface, 1990 onwards), SCOPUS (1960 onwards), Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS) (BIREME interface) and WHO Global Index Medicus. The search was supplemented by searching for ongoing studies in WHO's International Clinical Trials Registry. The literature search was limited to the English language and human subjects. In addition to searching databases, we contacted individual researchers and authors of major trials to address whether any relevant manuscripts are in preparation or in press. The references of published articles found in the above databases were searched for additional pertinent materials.

Study selection proceeded in three stages: (1) titles/abstracts were screened by a single reviewer according to standard inclusion and exclusion criteria; (2) full manuscripts were obtained and assessed against inclusion criteria. Papers were accepted or rejected and reasons for rejection were specified; (3) two independent reviewers assessed each manuscript. Differences were resolved by a third independent reviewer.

Selection criteria

The inclusion criteria included the following: primary purpose is HCV Ab test evaluation, reported sensitivity and specificity of HCV Ab test kits, and studies published before May 2015. Studies that only reported sensitivity or specificity, conference abstracts, comments or review papers, panel studies, and studies that only used reference assay for positive samples were excluded.

Data extraction

Information on the following variables were extracted from each individual study: first author, total sample size, country (and city) of sampling, sample type (oral fluid, finger-prick, venous blood, etc.), POC (defined as being able to give a result within 60 minutes and having the results to guide clinical management in the same encounter), eligibility criteria, reference standard, manufacturer, raw cell numbers (true positives, false negatives, false positives, true negatives), antibody–antigen combo (yes or no), sources of funding, reported conflicts of interest.

Assessment of methodological quality

Study quality was evaluated using the QUADAS-2 tool[16] and the STARD checklist.[17] QUADAS includes domains to evaluate bias in the following categories: risk of bias (patient selection, index test, reference standard, flow and timing); applicability concerns (patient selection, index test, reference standard).

Data analysis and synthesis

Data synthesis

Data were extracted to construct 2 × 2 tables. According to the test results of reference standard, anti-HCV positive and negative were defined. By comparing with reference standard results, the index test results were categorized as a true positive, a false positive, a false negative, or a true negative. Indeterminate test results were not included in pooled analyses.

Statistical analysis

To estimate test accuracy, we calculated sensitivity and specificity for each study and pooled statistics, along with 95% confidence intervals (CIs). We pooled test estimates using the DerSimonian–Laird method, a bivariate random effect model. We did further sub-analysis based on reference standard (EIA alone; NAT or immunoblot; EIA, NAT, or immunoblot), brand, and combination test. We performed all statistical analysis (including heterogeneity) using the software R and RevMan 5.3.

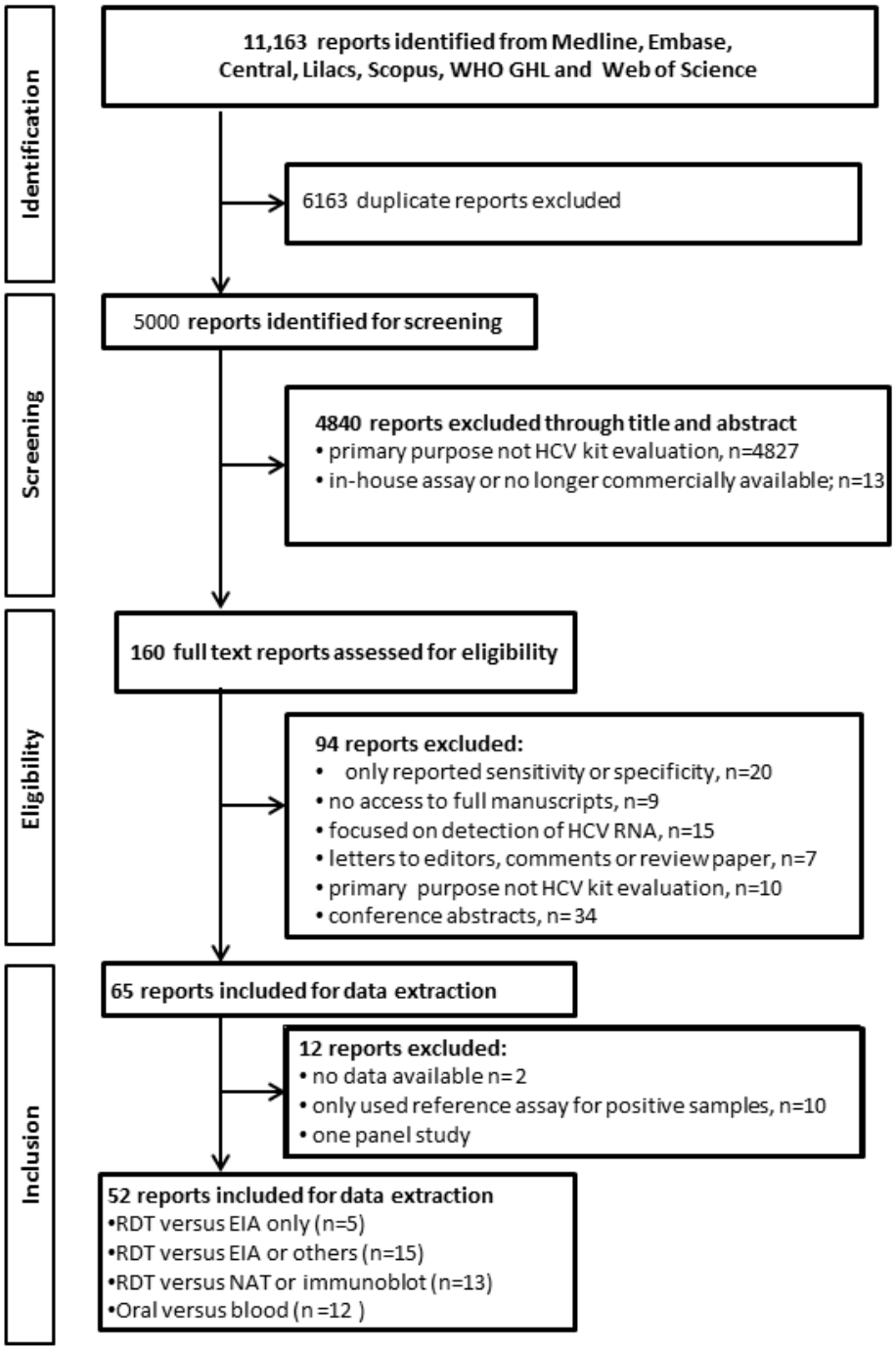
5. Results

Study selection

A total of 11 163 citations were identified and 6163 duplicates were removed. Each of the 5000 titles was examined. A total of 52 research studies were included in the final analysis (Fig. 1 below).[8, 18-68] Of the 52 studies, 32 studies evaluated the accuracy of 30 different RDTs, of which 5 evaluated RDTs compared to EIA alone, 13 compared RDT results to NAT or immunoblot, and 15 focused on evaluating RDT by comparing with the results of EIA or immunoblot or NAT. Twelve studies evaluated the diagnostic accuracy of oral fluid RDTs.

There were insufficient data to undertake a subanalysis based on HIV coinfection or other coinfections.

Fig. 1. PRISMA flow diagram outlining study selection examining diagnostic accuracy of HCV antibody tests



Study characteristics

Of the 52 included studies, 9 were published before 2010, 12 reported evaluation using oral fluid samples, and 34 were reported performance of POC tests (Table 1). Of the 52 studies, 41 different brands of testing kits were evaluated (Table 1).

***Table 1**. Characteristics of studies focused on evaluating diagnostic accuracy of HCV antibody tests*

First author	Year	Settings	Sample type	Manufacturer	Study type	Sample size	POC (Y/N)	Reference standard
Al-Tahish et al.	2013	Egypt	Venous blood	HCV one step test device (ACON Laboratories, USA), Fourth- generation HCV TRI_DOT (J. Mitra Co, India) and ImmunoComb II HCV (Inverness Medical Innovations, USA)	Cross-sectional	100	Y	PCR
Bonacini et al.	2001	USA	Venous blood	Ortho Clinical Diagnostics (Raritan, NJ, USA)	Cross-sectional	222	N	Chiron Immunoblot HCV 3.0 SIA
Buti et al.	2000	Spain	Serum	Not available	Cross-sectional	188	Y	Immunoblot
Caudai et al.	1998	USA	Serum or plasma samples	ELISA 2nd generation Abbott Laboratories, Abbott park, IL, USA)	Cross-sectional	682	N	PCR
Cha et al.	2013	Korea	Oral fluids and serum	OraQuick (OraSure Technologies, PA USA)	Case–control	437	Y	PCR
Croom et al	2006	Austria	Venous blood	Monolisa anti-HCV PLUSVersion 2 EIA (Bio-Rad, France)	Cross-sectional	182	N	EIA
da Rosa et al.	2013	Brazil	Serum	Rapid Test Bioeasy® (Standard Diagnostics, Yongin, Korea) and Imuno-Rapido HCV® (Wama Diagnostica, São Carlos, Brazil).	Cross-sectional	307	Y	Architect HCV, PCR
Daniel et al.	2005	India	Serum	TRI DOT (J. MITRA &Co. Ltd., New Delhi, India)	Cross-sectional	2590	Y	EIA, Immunoblot, PCR

First author	Year	Settings	Sample type	Manufacturer	Study type	Sample size	POC (Y/N)	Reference standard
Denoyel et al.	2004	France and Germany	Serum or plasma samples	AxSYM HCV 3.0 (other information is not available)	Cross-sectional	5700	N	Immunoblot
Dokubo et al.	2014	USA	Blood	HCV Version 3.0 ELISA (Ortho®)	Cross-sectional	132	N	PCR
Drobnik et al.	2011	USA	Oral fluid	OraQuick (OraSure Technologies, PA USA)	Cross-sectional	484	Y	EIA, Immunoblot
Eroglu et al.	2000	Turkey	Plasma specimens	ELISA v3.0(Ortho®)	Cross-sectional	160	N	PCR
Feucht et al.	1995	Germany	Plasma specimens	Abbott HCV second-generation enzyme immunoassay (other information is not available)	Cross-sectional	262	N	Immunoblot
Gao et al.	2014	USA	Serum	OraQuick (OraSure Technologies, PA USA)	Cross-sectional	289	Y	EIA
Hess et al.	2014	USA	Whole blood	DPP HIV-HCV-Syphilis Assay (Chembio Diagnostic Systems, Inc. , Medford, NY)	Cross-sectional	948	Y	EIA
Hui et al.	2002	Hong Kong, China	Whole blood	OraQuick (OraSure Technologies, PA USA)	Cross-sectional	197	Y	EIA
Ibrahim et al.	2015	Saudi Arabia	Oral fluid	OraQuick (OraSure Technologies, PA USA)	Case–control	160	Y	PCR
Ivantes et al.	2010	Brazil	Whole blood	HCV Rapid Test Bioeasy (Bioeasy Diagnostica Ltda, Minas Gerais, Brazil)	Cross-sectional	71	Y	CLIA
Jewett et al.	2012	USA	Oral fluids and serum	Chembio DPP HCV test (Chembio Diagnostic Systems,USA) and Rapid HIV/HCV antibody test (Medmira Laboratories, Canada)	Cross-sectional	407	Y	Immunoblot/NAT
Kant et al.	2012	Germany	Whole blood	Toyo anti-HCV test (Turklab, Izmir, Turkey)	Cross-sectional	185	Y	Architect HCV
Kaur et al.	2000	India	Serum	HCV Bidot (J. Mitra Co., India)	Cross-sectional	2754	Y	EIA 3rd generation
Kim et al.	2013	Republic of	Serum	GENEDIA® HCV Rapid LF (Green Cross medical	Case–control	200	Y	Immunoblot

First author	Year	Settings	Sample type	Manufacturer	Study type	Sample size	POC (Y/N)	Reference standard
		Korea		science corp., Korea)				
Kosack et al.	2014	Germany	Serum	The ImmunoFlow HCV test (Core Diagnostics,United Kingdom)	Cross-sectional	81	Y	Immunoblot
Lakshmi et al.	2007	India	Blood	Beijing United Biomedical, Ortho Clinical Diagnostics, General Biologicals; other information is not avaliable	Cross-sectional	69	N	PCR
Larrat et al.	2012	France	FSB (finger-stick blood) and oral fluid	cEIA: the Monolisa® HCV-Ag-Ab-ULTRA (Bio-Rad, Marnes-la-Coquette, France)	Case–control	201	Y	PCR
Lee et al.	2010	USA	Oral fluid, whole blood	OraQuick (OraSure Technologies, PA USA)	Cross-sectional	572	Y	EIA, Immunoblot
Lee et al.	2011	USA	Serum, plasma, venous blood, finger-stick blood and oral fluid	OraQuick (OraSure Technologies, PA USA)	Cross-sectional	2183	Y	EIA, Immunoblot, PCR
Lee et al.	2011	USA	Oral fluid	OraQuick (OraSure Technologies, PA USA)	Cross-sectional	2180 or 2178	Y	EIA
Maity et al.	2012	India	Serum	J Mitra & Co. Pvt Ltd, SPAN Diagnostics Ltd. and Standard Diagnostics, INC, other information is not available	Case–control	100	Y	EIA
Montebugnoil et al.	1999	Italy	Whole blood	Anti-HCV Ab rapid test (1st IRP 75/537 by Thema Ricerca, WHO Geneva)	Case–control	100	Y	EIA, Immunoblot
Mvere et al.	1996	Zimbabwe	Serum	HCV-SPOT (Genelabs Diagnostics, Singapore)	Cross-sectional	206	Y	EIA 2nd generation, INNO-LIA HCV Ab III

First author	Year	Settings	Sample type	Manufacturer	Study type	Sample size	POC (Y/N)	Reference standard
Nalpas et al.	1992	France	Serum	Ortho Diagnostics, other information is not available	Cross-sectional	62	N	PCR
Njouom et al.	2006	Cameroon	Plasma	ImmunoComb® II HCV assay (Orgenics Ltd, Not reported manufacturer located country); ImmunoComb® II HCV assay (Orgenics Ltd, Not reported manufacturer located country)	Cross-sectional	329	Y	EIA 3rd generation, PCR
Nyirenda et al.	2008	Malawi	Serum	Monoelisa HCV Ag/Ab ultra microplate EIA (Bio-Rad, France)	Cross-sectional	202	Y	EIA
O'Connell et al.	2013	USA	Plasma, whole blood (normal) and whole blood (cold storge)	OraQuick (OraSure Technologies, PA USA); CORE (CORE Diagnostics, United Kingdom); Axiom (Axiom Diagnostics, Burstadt,Germany); FirstVue (AT First Diagnostic, Woodbury,NY, USA) and Instant View Cassette (Alfa Scientific Designs, Poway)	Case–control	674 or 168	Y	EIA, Immunoblot, and when available viral load)
O'Flynn et al.	1997	Ireland, Germany, UK	Plasma and serum	AxSYM (Abbott laboratories, other information is not available)	Case–control	5554, 1421 or 643	N	ABBOTT MATRIX HCV, Chiron Immunoblot HCV 2.0 or 3.0
Park et al.	2012	Korea	Serum	Vitros anti-HCV assay kits (Ortho-Clinical Diagnostics, Buckinghamshire, UK) and Elecsys (Roche Diagnostics GmbHMannheim, Germany)	Cross-sectional	1008	N	Immunoblot HCV 3.0 and Cobas Ampliprep/ Taqman HCV RNA
Poovorawari et al.	1994	Thailand	Serum	HCV-SPOT assay (Genelabs Diagnostics Pty Ltd, Singapore)	Cross-sectional	192	Y	EIA 2nd generation or Immunoblot
Prayson et al.	1993	USA	Serum	C100-3 HCV EIA (Abbott laboratories, other information is not available)	Cross-sectional	123	N	Immunoblot 2.0
Rihn et al.	2000	France	Serum	MATRIX hcv2 (Abbott laboratories, other information is not available)	Cross-sectional	146	N	PCR

First author	Year	Settings	Sample type	Manufacturer	Study type	Sample size	POC (Y/N)	Reference standard
Scalioni Lde et al.	2014	Brazil	Serum, whole blood and oral fluid	WAMA Imuno-Rápido HCV Kit (WAMA Diagnóstica, Brazil); Bioeasy HCV Rapid Test, (Bioeasy Diagnóstica Ltd, Brazil) and OraQuick (OraSure Technologies, PA USA)	Cross-sectional	194 or 172	Y	PCR
Smith et al.	2011	USA	Whole blood, oral fluid	Multiplo Rapid HIV/HCV Antibody Test (MedMira, Canada); Chembio DPP HCV test (Chembio Diagnostic Systems, USA) and OraQuick (OraSure Technologies, PA USA)	Cross-sectional	476, 385, 432, 549 or 266	Y	MEIA/EIA/CLIA, Immunoblot
Smith et al.	2011	USA	Oral fluid and blood	Multiplo Rapid HIV/HCV Antibody Test (MedMira, Canada); Chembio DPP HCV test (Chembio Diagnostic Systems, USA)	Cross-sectional	1081	Y	Chiron Immunoblot HCV 3.0 SIA; Bayer Advia Centaur HCV Chemiluminescent immunoassay
Sommese et al.	2014	Italy	Blood	CMIA assays (Abbott Diagnostics, Wiesbaden, Germany)	Cross-sectional	17894	N	INNO-LIA (Innogenetics, Ghent, Belgium), NAT
Tagny et al.	2014	Cameron	Plasma	HCV Ag/Ab combination assay (Monolisa HCV Ag-Ab Ultra, BioRad, Marnes La Coquette, France)	Cross-sectional	1998	Y	EIA
Vrielink et al.	1996	Netherlands	Blood	Abbott HCV EIA 3.0 (Abbott laboratories, Murex anti-HCV VK47 (Murex Diagnostic) and Ortho HCV 3.0 elisa (Ortho Diagnostic Systems; other information is not avaliable	Cross-sectional	403, 212, 253 03 1055	N	PCR
Vrielink et al.	1995	Netherlands	Blood	Monolisa anti-HCV new antigens (Sanofi Diagnostics Pasteur), Abbott HCV EIA 3.0 (Abbott laboratories); other information is not available	Cross-sectional	403, 212, 253	N	PCR

First author	Year	Settings	Sample type	Manufacturer	Study type	Sample size	POC (Y/N)	Reference standard
Yang et al.	2011	China	Serum	AxSYM HCV 3.0 (Abbott Laboratories), Murex Ag/Ab test (Abbott Laboratories); other information is not available	Case–control	101 or 100	N	HCV RNA test (COBAS AMPLICOR Hepatitis C Virus Test, version 2.0
Yang et al.	2013	China	Serum	Elecsys anti-HCV II (Roche Diagnostics GmbH), Architect anti-HCV (Abbott) and Vitros anti-HCV(Ortho-Clinical Diagnostics), other information is not available	Cross-sectional	859 or 167	N	IMMUNOBLOT 3.0 test or the Realtime HCV RNA assay
Yarri et al.	2006	Israel	Serum and oral fluid	ImmunoComb II HCV (Inverness Medical Innovations, USA)	Cross-sectional	37	Y	PCR
Yoo et al.	2015	South Korea; China; China/Taiwan; Thailand; Australia; Malaysia; Indonesia	Serum	Elecsys® Anti-HCV II assay (Roche Diagnostics GmbH, other information is not avaliable)	Cross-sectional	7726	Y	1 or more of the following comparator assays at 9 centers: ARCHITECTTM Anti-HCV; Serodia®-HCV Particle Agglutination; Vitros® ECi Anti-HCV; Elecsys® Anti-HCV; ADVIA Centaur® HCV; InTec® HCV EIA; or Livzon® Anti-HCV.
Yuen et al.	2001	China	Serum	SM-HCV Rapid Test (SERO-Med Laborspezialita¨ten GmbH, Eichsta ¨tt, Germany)	Case–control	290	Y	EIA, PCR

Assessment of the quality of the studies

All studies used cross-sectional or case–control design. Risk of bias in patient selection, index test, or reference standard was assessed using QUADAS-2 (Table 2). Among the included studies, 21 have at least one category that was considered high risk. Risk of bias in patient selection usually came from a poor description of patient selection and clinical scenario. Bias in the index test was primarily due to a lack of reported blinding while reading test results. Bias in the reference standard was due to the use of multiple reference standards (EIA, NAT, and/or immunoblot). Bias in the flow and timing was primarily due to a lack of reported details.

Table 2. Quality assessment by QUADAS-2 of the included studies

Reports		Bias assessment/Risk of bias				Acceptability concerns		
		Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Al-Tahish et al.	2013	UC	LR	LR	LR	LR	LR	LR
Bonacini et al.	2001	HR	LR	LR	LR	LR	LR	LR
Buti et al.	2000	UC	UC	LR	LR	HR	LR	LR
Caudai et al.	1998	HR	LR	LR	LR	UC	LR	LR
Cha et al.	2013	HR	LR	LR	LR	UC	LR	LR
Croom et al.	2006	LR	LR	LR	UC	LR	LR	LR
da Rosa et al.	2013	HR	UC	LR	LR	HR	UC	LR
Daniel et al.	2005	LR	LR	LR	LR	LR	LR	LR
Denoyel et al.	2004	UC	LR	LR	LR	UC	LR	HR
Drobnik et al.	2011	LR	UC	LR	UC	LR	UC	LR
Eroglu et al.	2000	LR	LR	LR	LR	LR	LR	LR
Feucht et al.	1995	HR	LR	LR	LR	HR	LR	LR
Gao et al.	2014	LR	LR	LR	HR	LR	LR	LR
Hess et al.	2014	LR	HR	LR	LR	LR	HR	LR
Hui et al.	2002	HR	LR	HR	LR	HR	LR	HR
Ivantes et al.	2010	LR	UC	HR	LR	LR	LR	HR
Jewett et al.	2012	LR	LR	LR	LR	LR	LR	LR
Dokuboa et al.	2014	UC	LR	LR	LR	UC	LR	LR
Kant et al.	2012	HR	UC	HR	LR	HR	UC	HR
Kaur et al.	2000	LR	UC	HR	LR	LR	LR	LR
Kim et al.	2013	UC	LR	LR	LR	UC	LR	LR
Kosack et al.	2014	HR	LR	LR	LR	HR	LR	LR
Lakshmi et al.	2007	UC	LR	LR	UC	HR	LR	LR
Larrat et al.	2012	LR	LR	LR	LR	LR	LR	LR
Lee et al.	2010	LR	UC	LR	LR	LR	UC	LR

Lee et al.	2011	HR	UC	LR	LR	LR	LR	LR
Maity et al.	2012	HR	UC	HR	LR	HR	UC	HR
Montebugnoil et al.	1999	HR	LR	LR	LR	HR	LR	LR
Mvere et al.	1996	HR	LR	LR	LR	HR	LR	LR
Nalpas et al.	1992	HR	LR	LR	UC	HR	LR	LR
Njouom et al.	2006	HR	UC	LR	LR	HR	UC	LR
Nyirenda et al.	2008	LR	UC	LR	LR	LR	LR	LR
O'Connell et al.	2013	HR	LR	HR	LR	HR	LR	LR
O'Flynn et al.	1997	UC	LR	LR	UC	LR	LR	LR
Park et al.	2012	UC	LR	LR	UC	LR	LR	LR
Poovorawari et al.	1994	LR	UC	LR	LR	LR	LR	LR
Prayson et al.	1993	UC	LR	LR	UC	UC	LR	LR
Rihn et al.	2000	UC	LR	LR	UC	UC	LR	LR
Scalioni et al.	2014	UC	LR	LR	UC	UC	LR	LR
Smith et al.	2011	LR	LR	LR	LR	LR	LR	LR
Smith et al.	2011	HR	LR	LR	LR	HR	LR	LR
Sommese et al.	2014	LR	LR	LR	LR	LR	LR	LR
Lee et al.	2010_2	LR	LR	LR	LR	LR	LR	LR
Ibrahim et al.	2015	HR	LR	LR	LR	HR	LR	LR
Tagny et al.	2014	LR	UC	HR	LR	LR	UC	HR
Vrielink et al.	1995	UC	LR	LR	LR	UC	LR	LR
Vrielink et al.	1995_2	UC	LR	LR	LR	HR	LR	LR
Yang et al.	2011	UC	LR	LR	LR	UC	LR	LR
Yang et al.	2013	LR	LR	LR	UC	LR	LR	LR
Yarri et al.	2006	HR	LR	LR	LR	HR	LR	LR
Yoo	2015	UC	LR	LR	HR	UC	LR	LR
Yuen et al.	2001	HR	LR	LR	LR	HR	LR	LR

LR: low risk; HR: high risk; UC: unclear risk

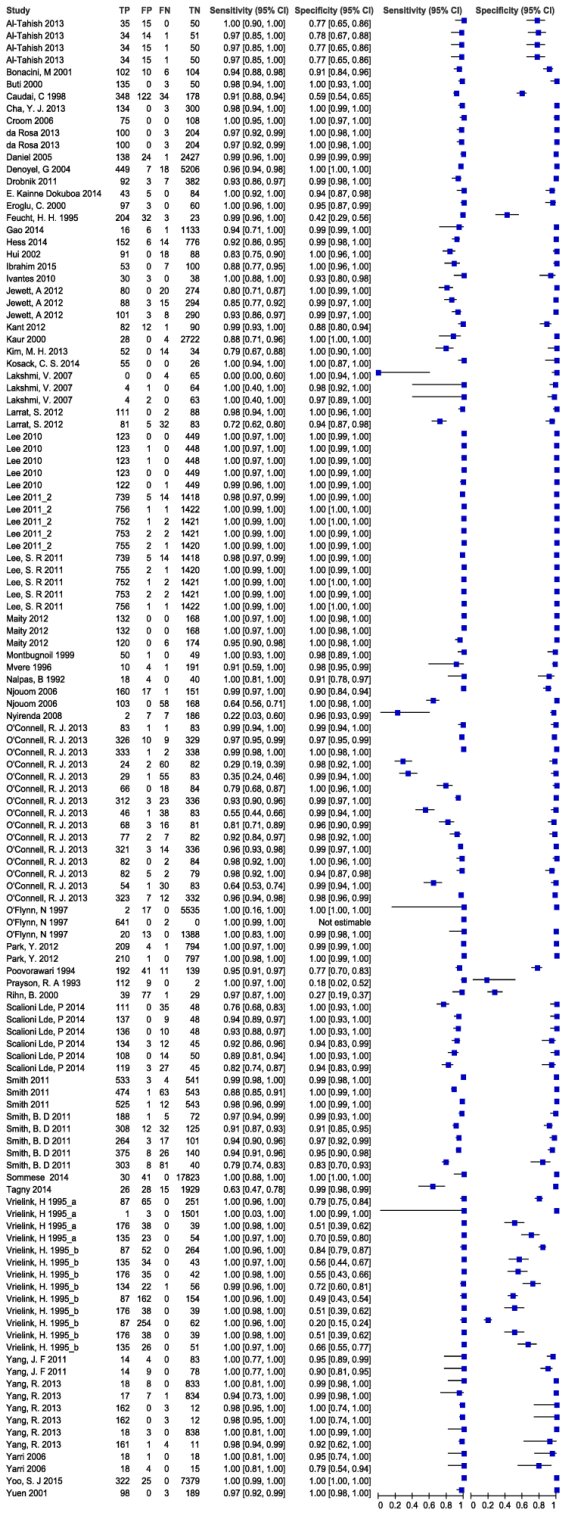
Diagnostic accuracy

Overall clinical performance of assays

The 52 included studies contributed 127 data points from 52 273 unique test measurements. Some studies contributed additional data points by comparing the accuracy of two or more tests, reporting data from multiple study sites, or reporting the accuracy of a test in more than one type of specimen. The sample sizes of the included studies ranged from 37 to 17 894. Sensitivities of included studies ranged from 0.22 to 1.00, and specificities ranged from 0.77 to 1.00. The overall pooled sensitivity and specificity for all tests were 0.97 (95% CI: 0.97–0.98) and 0.99 (95% CI: 0.98–

0.99), respectively. Figure 2 show estimates of sensitivity and specificity from each study.

Fig. 2. Sensitivity and specificity of HCV Ab tests included in the review (n=52)



Manufacturers and accuracy of RDTs among included studies

Overall, 32 studies evaluated the accuracy of 30 different RDTs (Table 3). The most commonly evaluated test kit was the OraQuick ADVANCE® from OraSure Technologies.

An Ag–Ab test by BioRad and a combo HIV-HCV test by MedMira were among the tests evaluated.

Table 3. Manufacturers and accuracy of RDTs among included studies

First author	Manufacturer	Sample size	TP	FP	TN	FN	SE	SP
Montbugnoil et al.	Anti-HCV Ab rapid test (1st IRP 75/537 by Thema Ricerca, WHO Geneva)	100	50	1	49	0	1.00	0.98
O'Connell. et al.	Axiom (Axiom Diagnostics, Burstadt, Germany)	674	326	10	329	9	0.97	0.97
O'Connell et al.	Axiom (Axiom Diagnostics, Burstadt, Germany)	168	77	2	82	7	0.92	0.98
O'Connell et al.	Axiom (Axiom Diagnostics, Burstadt, Germany)	168	82	5	79	2	0.98	0.94
Scalioni Lde, et al.	Bioeasy HCV Rapid Test, (Bioeasy Diagnóstica Ltd, Brazil)	194	137	0	48	9	0.94	1.00
Scalioni Lde et al.	Bioeasy HCV Rapid Test (Bioeasy Diagnóstica Ltd, Brazil)	194	111	0	48	35	0.76	1.00
Scalioni Lde et al.	Bioeasy HCV Rapid Test (Bioeasy Diagnóstica Ltd, Brazil)	194	136	0	48	10	0.93	1.00
Jewett et al.	Chembio DPP HCV Test (Chembio Diagnostic Systems, USA)	407	101	3	290	8	0.93	0.99
Jewett et al.	Chembio DPP HCV test (Chembio Diagnostic Systems,USA)	400	88	3	294	15	0.85	0.99
Smith et al.	Chembio DPP HCV test (Chembio Diagnostic Systems, USA)	476	308	12	125	32	0.91	0.91
Smith et al.	Chembio DPP HCV test (Chembio Diagnostic Systems, USA)	385	264	3	101	17	0.94	0.97
Smith et al.	Chembio DPP HCV test (Chembio Diagnostic Systems, USA)	1081	525	1	543	12	0.98	1.00
O'Connell et al.	CORE (CORE Diagnostics, United Kingdom)	168	29	1	83	55	0.35	0.99
O'Connell, et al.	CORE (CORE Diagnostics, United Kingdom)	168	24	2	82	60	0.29	0.98
O'Connell et al.	CORE (CORE Diagnostics, United Kingdom)	674	323	7	332	12	0.96	0.98
Maity et al.	Diagnostics Ltd (other information is not available)	300	132	0	168	0	1.00	1.00
O'Connell et al.	FirstVue (AT First Diagnostic, Woodbury, NY, USA)	168	66	0	84	18	0.79	1.00
O'Connell et al.	FirstVue (AT First Diagnostic, Woodbury, NY, USA)	168	54	1	83	30	0.64	0.99
O'Connell et al.	FirstVue (AT First Diagnostic, Woodbury, NY, USA)	674	312	3	336	23	0.93	0.99

Al-Tahish et al.	Fourth-generation HCV TRI_DOT (J. Mitra Co, India)	100	34	15	50	1	0.97	0.77
Daniel et al.	Fourth-generation HCV TRI_DOT (J. Mitra Co, India)	2590	138	24	2427	1	0.99	0.99
Kim, M. H. et al.	GENEDIA® HCV Rapid LF (Green Cross medical science corp., Korea)	100	52	0	34	14	0.79	1.00
Kaur et al.	HCV Bidot (J. Mitra Co., India)	2754	28	0	2722	4	0.88	1.00
Al-Tahish et al.	HCV one step test device (ACON Laboratories, USA)	100	34	15	50	1	0.97	0.77
Ivantes et al.	HCV Rapid Test Bioeasy (Bioeasy Diagnostica Ltd, Brazil)	71	30	3	38	0	1.00	0.93
da Rosa et al.	HCV Rapid Test Bioeasy® (Standard Diagnostics, South Korea)	307	100	0	204	3	0.97	1.00
Poovoran et al.	HCV-SPOT assay (Genelabs Diagnostics Pty Ltd, Singapore)	192	41	11	139	1	0.98	0.93
Mvere et al.	HCV-SPOT assay (Genelabs Diagnostics Pty Ltd, Singapore)	206	10	4	191	1	0.91	0.98
Njouom et al.	Hexagon® HCV (Not reported manufacturer located country)	329	160	17	151	1	0.99	0.90
Al-Tahish et al.	ImmunoComb II HCV (Inverness Medical Innovations, USA)	100	34	14	51	1	0.97	0.78
Yarri et al.	ImmunoComb II HCV (Inverness Medical Innovations, USA)	37	18	4	15	0	1.00	0.79
Yarri et al.	ImmunoComb II HCV (Inverness Medical Innovations, USA)	37	18	1	18	0	1.00	0.95
Njouom et al.	ImmunoComb® II HCV assay (Orgenics Ltd, Not reported manufacturer located country)	329	103	0	168	58	0.64	1.00
da Rosa et al.	Imuno-Rapido HCV® (Wama Diagnostica, Brazil).	307	100	0	204	3	0.97	1.00
O'Connell et al.	Instant View Cassette (Alfa Scientific Designs, Poway, CA, USA)	674	321	3	336	14	0.96	0.99
O'Connell et al.	Instant View Cassette (Alfa Scientific Designs, Poway, CA, USA)	168	68	3	81	16	0.81	0.96
O'Connell et al.	Instant View Cassette (Alfa Scientific Designs, Poway, CA, USA)	168	46	1	83	38	0.55	0.99

Maity et al.	J Mitra Co. India (other information is not available)	300	120	0	174	6	0.95	1.00
Jewett et al.	Rapid HIV/HCV antibody test (Medmira Laboratories, Canada)	374	80	0	274	20	0.80	1.00
Nyirenda et al.	Monoelisa HCV Ag/Ab Ultra microplate EIA (Bio-Rad, France)	202	2	7	186	7	0.22	0.96
Tagny et al.	Monolisa HCV Ag-Ab Ultra, (BioRad, France)	1998	26	28	1929	15	0.63	0.99
Smith et al.	Multiplo Rapid HIV/HCV Antibody Test (MedMira, Canada)	1081	474	1	543	63	0.88	1.00
Smith et al.	Multiplo Rapid HIV/HCV Antibody Test (MedMira, Canada)	432	303	8	40	81	0.79	0.83
Cha et al.	OraQuick (OraSure Technologies, PA USA)	437	134	0	300	3	0.98	1.00
Lee et al.	OraQuick (OraSure Technologies, PA USA)	2183	756	1	1422	1	1.00	1.00
Lee et al.	OraQuick (OraSure Technologies, PA USA)	2183	755	2	1420	1	1.00	1.00
Lee et al.	OraQuick (OraSure Technologies, PA USA)	2183	753	2	1421	2	1.00	1.00
Lee et al.	OraQuick (OraSure Technologies, PA USA)	2183	752	1	1421	2	1.00	1.00
Lee et al.	OraQuick (OraSure Technologies, PA USA)	2183	739	5	1418	14	0.98	1.00
O'Connell et al.	OraQuick (OraSure Technologies, PA USA)	674	333	1	338	2	0.99	1.00
O'Connell et al.	OraQuick (OraSure Technologies, PA USA)	168	83	1	83	1	0.99	0.99
O'Connell et al.	OraQuick (OraSure Technologies, PA USA)	168	82	0	84	2	0.98	1.00
Smith et al.	OraQuick (OraSure Technologies, PA USA)	549	375	8	140	26	0.94	0.95
Smith et al.	OraQuick (OraSure Technologies, PA USA)	266	188	1	72	5	0.97	0.99
Lee et al.	OraQuick (OraSure Technologies, PA USA)	572	122	0	449	1	0.99	1.00
Lee et al.	OraQuick (OraSure Technologies, PA USA)	572	123	0	449	0	1.00	1.00
Lee et al.	OraQuick (OraSure Technologies, PA USA)	572	123	0	449	0	1.00	1.00
Lee et al.	OraQuick (OraSure Technologies, PA USA)	572	123	1	448	0	1.00	1.00
Lee et al.	OraQuick (OraSure Technologies, PA USA)	572	123	1	448	0	1.00	1.00

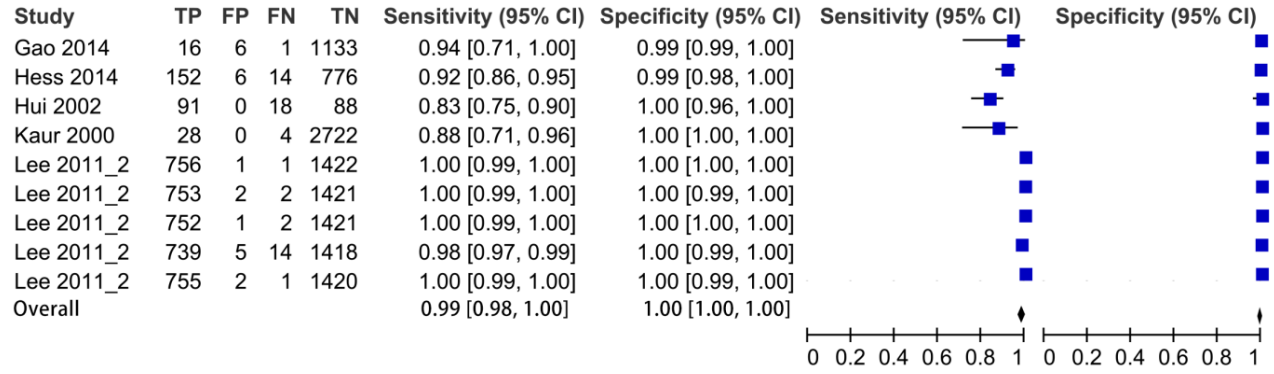
Smith et al.	OraQuick (OraSure Technologies, PA USA)	1081	533	3	541	4	0.99	0.99
Drobnik et al.	OraQuick (OraSure Technologies, PA USA)	484	92	3	382	7	0.93	0.99
Lee et al.	OraQuick (OraSure Technologies, PA USA)	2180	756	1	1422	1	1.00	1.00
Lee et al.	OraQuick (OraSure Technologies, PA USA)	2178	755	2	1420	1	1.00	1.00
Lee et al.	OraQuick (OraSure Technologies, PA USA)	2178	753	2	1421	2	1.00	1.00
Lee et al.	OraQuick (OraSure Technologies, PA USA)	2176	752	1	1421	2	1.00	1.00
Lee et al.	OraQuick (OraSure Technologies, PA USA)	2176	739	5	1418	14	0.98	1.00
Gao et al.	OraQuick (OraSure Technologies, PA USA)	1156	16	6	1133	1	0.94	0.99
Ibrahim	OraQuick (OraSure Technologies, PA USA)	160	53	0	100	7	0.88	1.00
Scalioni Lde_2014	OraQuick (OraSure Technologies, PA USA)	172	108	0	50	14	0.89	1.00
Hess et al.	DPP HIV-HCV-Syphilis Assay (Chembio Diagnostic Systems, Inc. , Medford, NY).	948	152	6	776	14	0.92	0.99
Buti et al.	Not available	188	135	0	50	3	0.98	1.00
Yuen et al.	SM-HCV Rapid Test (SERO-Med Laborspezialita¨ten GmbH, Eichsta ¨tt, Germany)	290	98	0	189	3	0.97	1.00
Maity et al.	SPAN Diagnostics, Indi, other information is not available	300	132	0	168	0	1.00	1.00
Kant et al.	Toyo anti-HCV test (Turklab, Izmir, Turkey)	185	82	12	90	1	0.99	0.88
Kosack et al.	The ImmunoFlow HCV test (Core Diagnostics,United Kingdom)	82	55	0	26	0	1.00	1.00
Scalioni Lde et al.	WAMA Imuno-Rápido HCV Kit (WAMA Diagnóstica, Brazil)	194	119	3	45	27	0.82	0.94
Scalioni Lde et al.	WAMA Imuno-Rápido HCV Kit (WAMA Diagnóstica, Brazil)	194	134	3	45	12	0.92	0.94
Hui et al.	Not reported	197	91	0	88	18	0.83	1.00

Pooled test accuracy for RDT versus EIA alone

Overall, five studies evaluated RDT compared to the EIA alone, with a total sample of 15 943. Of the five studies, sample sizes ranged from 197 to 2754, sensitivities ranged from 0.83 to 1.00, and specificities ranged from 0.99 to 1.00. The pooled sensitivity and specificity were 0.98 (95% CI 0.98–1.00) and 1.00 (95% CI 1.00–1.00), respectively, while heterogeneity was observed between the included studies (*P*<0.001) (Fig. 3, Table 4).

For the three studies that were conducted within past 10 years,[8, 31, 42] the total sample size was 12 992, with pooled sensitivity and specificity of 0.99 (95% CI 0.99–1.00) and 1.00 (95% CI 1.00–1.00), respectively.

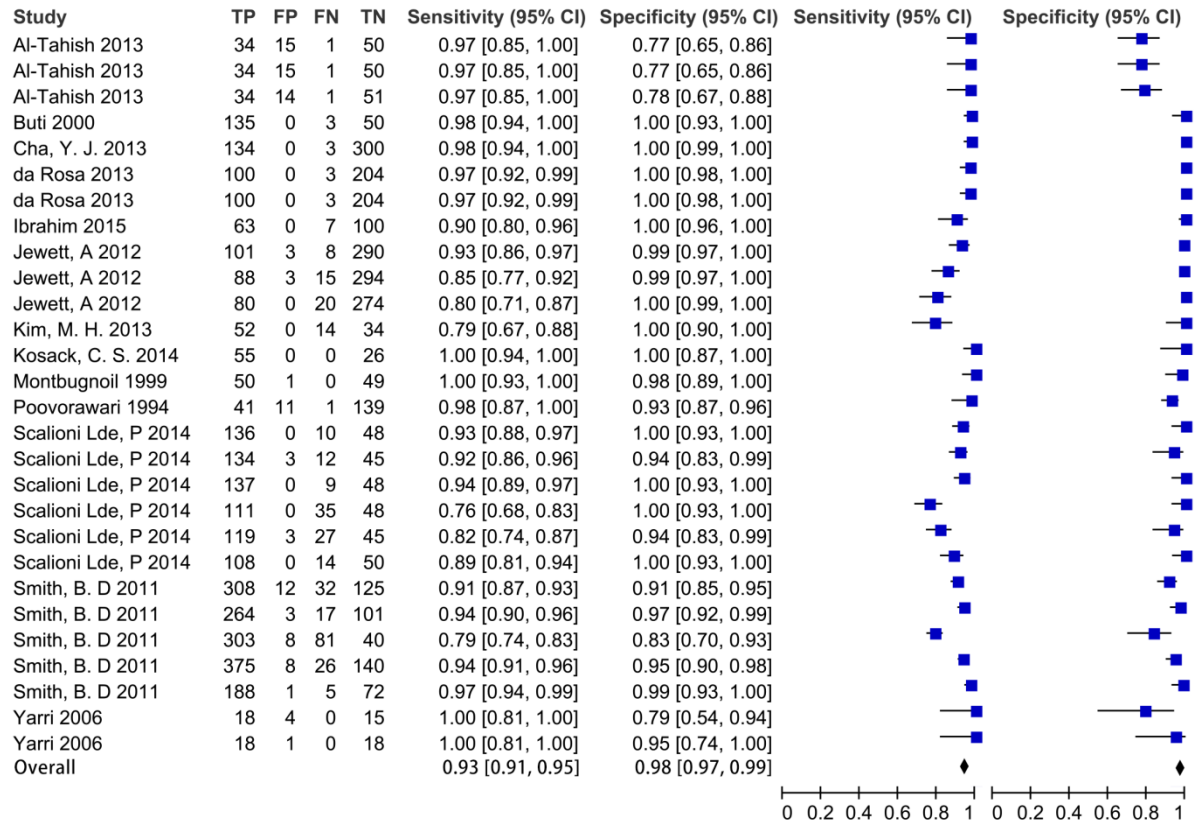
Fig. 3. Pooled test accuracy of HCV Ab RDTs compared to an EIA reference (5 studies)



RDT accuracy compared to NAT or immunoblot

Overall, 13 studies evaluated RDTs compared to NAT or immunoblot, with a total sample of 6 683. Among these studies, sample sizes ranged from 36 to 549, sensitivities ranged from 0.76 to 1.00, and specificities ranged from 0.77 to 1.00. The pooled sensitivity and specificity were 0.93 (95% CI 0.91–0.95) and 0.98 (95% CI 0.97–0.99), respectively, while heterogeneity was observed between the included studies (*P*<0.001) (Fig. 5, Table 4).

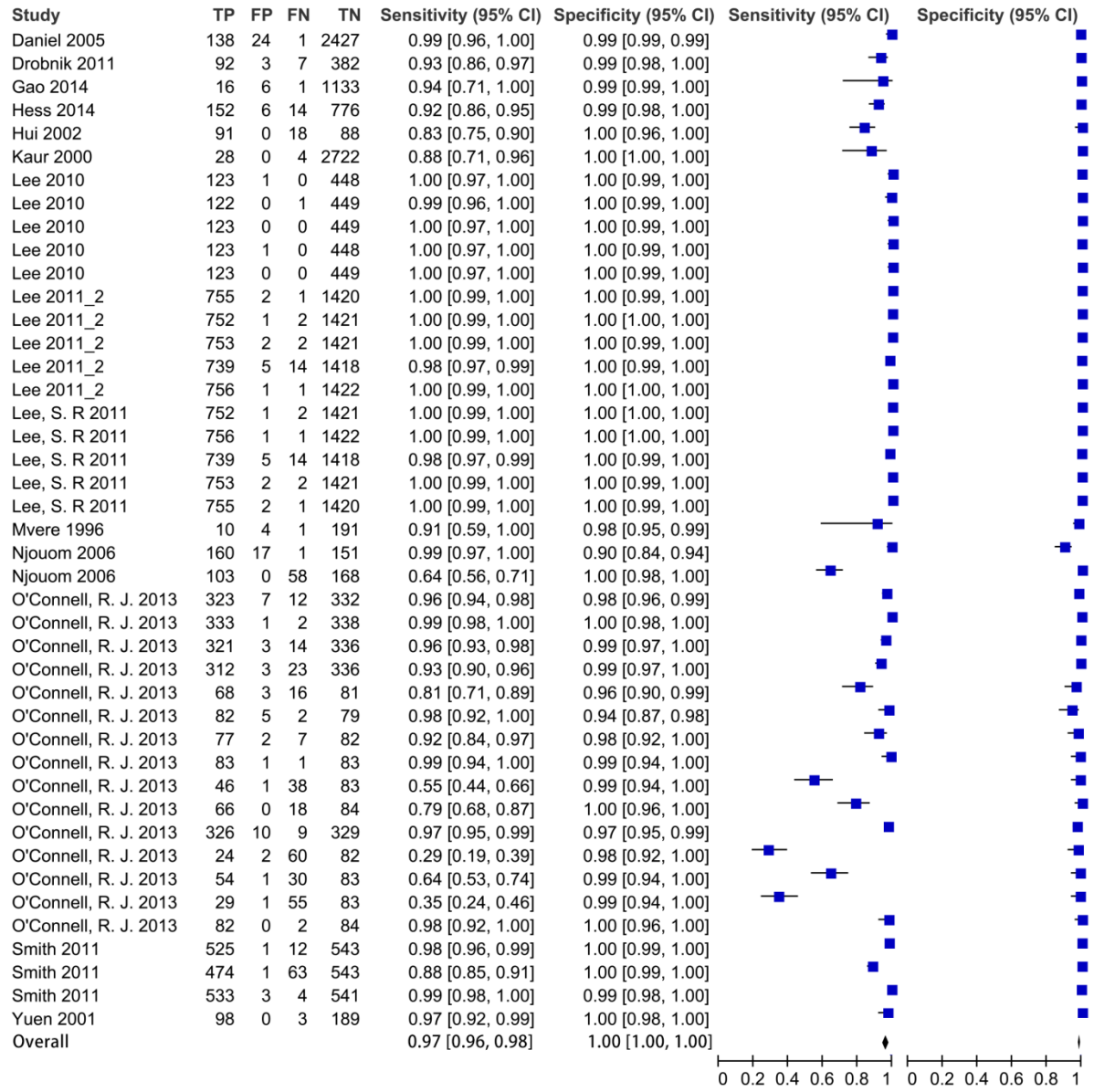
Fig. 5. Pooled test accuracy of HCV Ab RDTs compared to a NAT or immunoblot reference (n=13 studies)



RDT test accuracy compared to EIA, NAT or Immunoblot

Overall, 14 studies evaluated RDT by referencing to EIA with NAT and/or immunoblot, with a total sample of 42 212. Of the 14 studies, sample sizes ranged from 168 to 2754, sensitivities ranged from 0.29 to 1.00, and specificities ranged from 0.90 to 1.00. The pooled sensitivity and specificity were 0.97 (95% CI 0.96–0.98) and 1.00 (95% CI 1.00–1.00), respectively, while heterogeneity was observed between studies (P<0.001) (Fig. 4, Table 4).

Fig. 4. Pooled test accuracy of HCV Ab RDTs compared to EIA, NAT or immunoblot reference standards (n=14 studies)

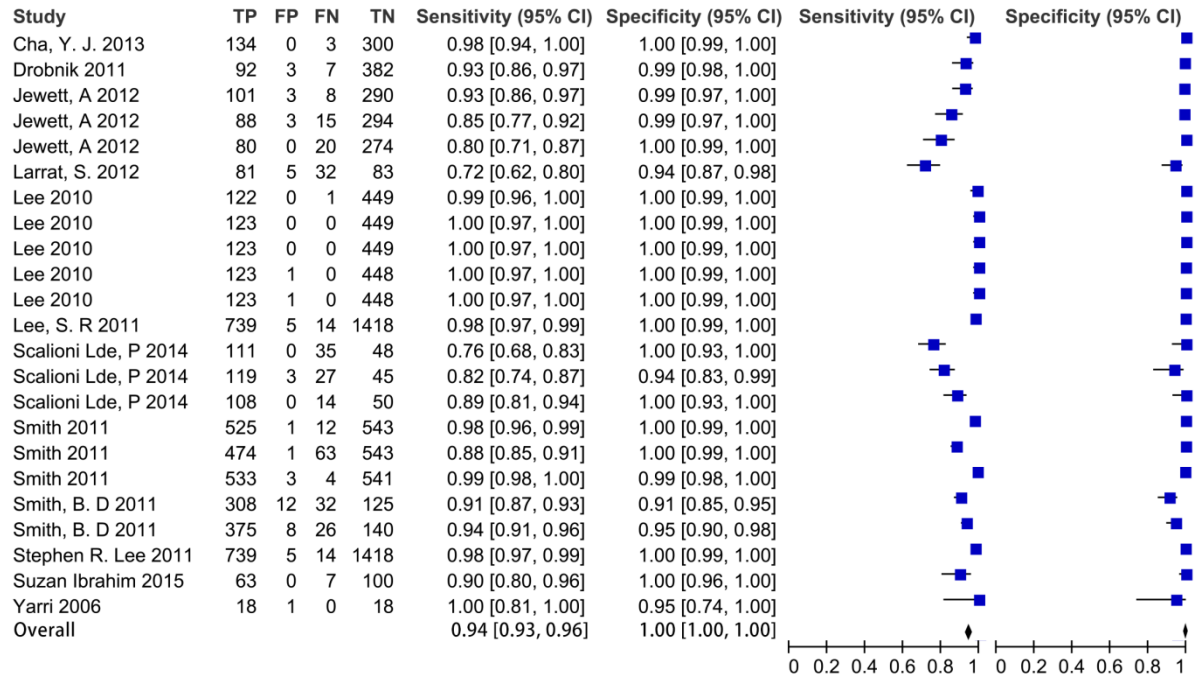


Pooled test accuracy for oral versus blood samples

EIAs using oral fluid samples

Overall, 12 studies compared the accuracy of EIAs using oral fluid samples to a blood sample as a reference, with a total sample size of 14 546. Of the 12 studies, sample sizes ranged from 37 to 2176, sensitivities ranged from 0.72 to 1.00, and specificities ranged from 0.91 to 1.00. The pooled sensitivity and specificity were 0.94 (95% CI 0.93–0.96) and 1.00 (95% CI 1.00–1.00), respectively. Heterogeneity was observed between the included studies (P<0.001) (Fig. 6, Table 4).

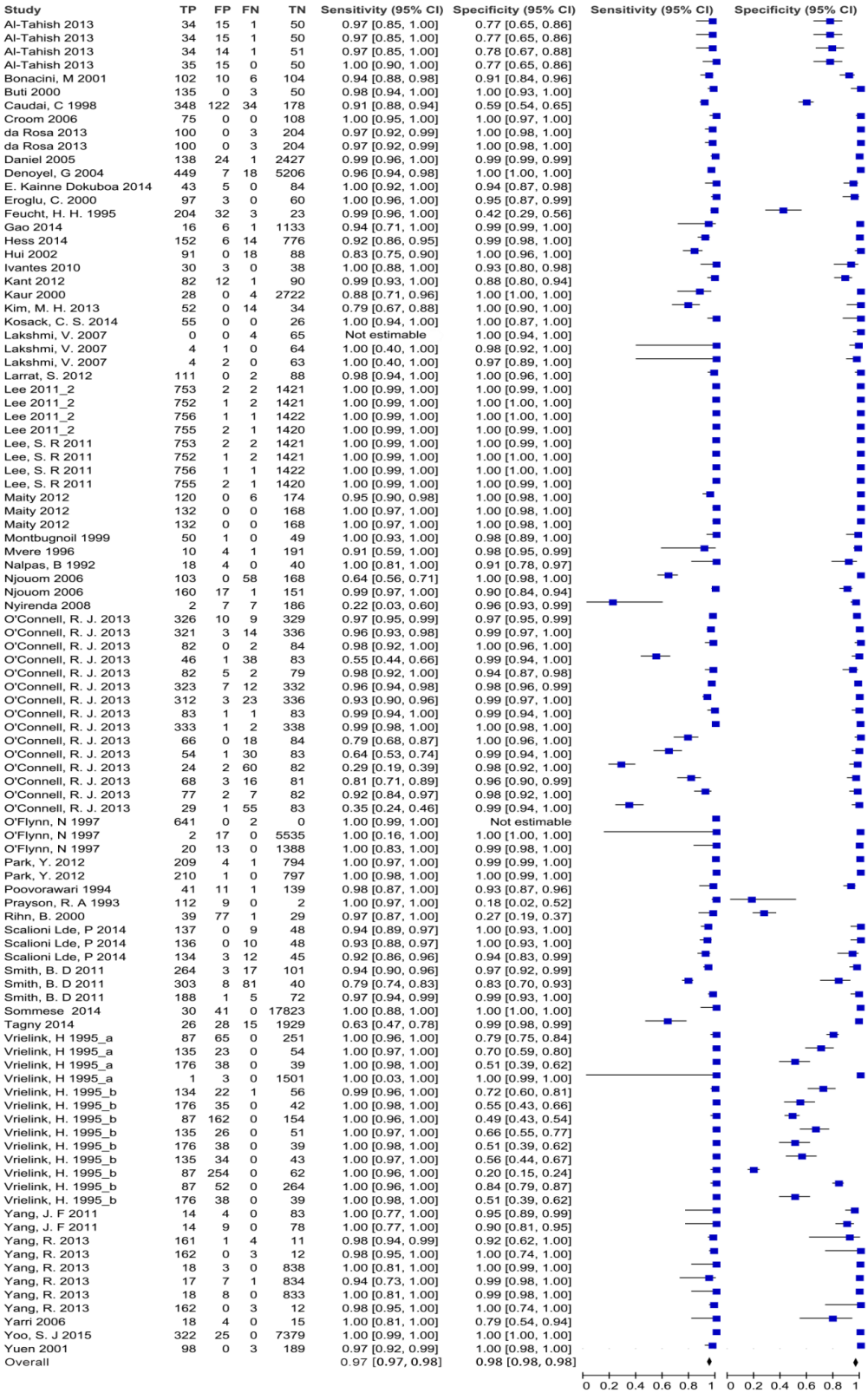
Fig. 6. Pooled test accuracy for oral HCV Ab RDTs compared to blood as a reference (n=12 studies)



Blood samples

Overall, 45 studies used blood samples for evaluations, with a total sample of 89 608. Sample sizes ranged from 37 to 17 894, sensitivities ranged from 0.29 to 1.00, and specificities ranged from 0.18 to 1.00. The pooled sensitivity and specificity were 0.98 (95% CI 0.97–0.98) and 0.98 (95% CI 0.98–0.98), respectively. Heterogeneity was observed between the included studies (P<0.001) (Fig. 7, Table 4).

Fig. 7. Pooled HCV Ab test accuracy for blood samples (n = 45 studies)

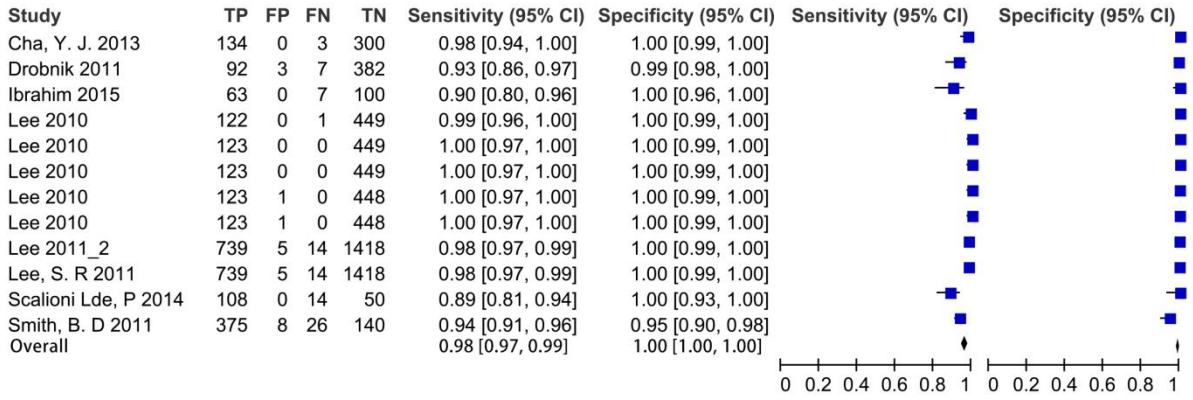


Pooled test accuracy for OraQuick versus other bands on Oral kits

OraQuick

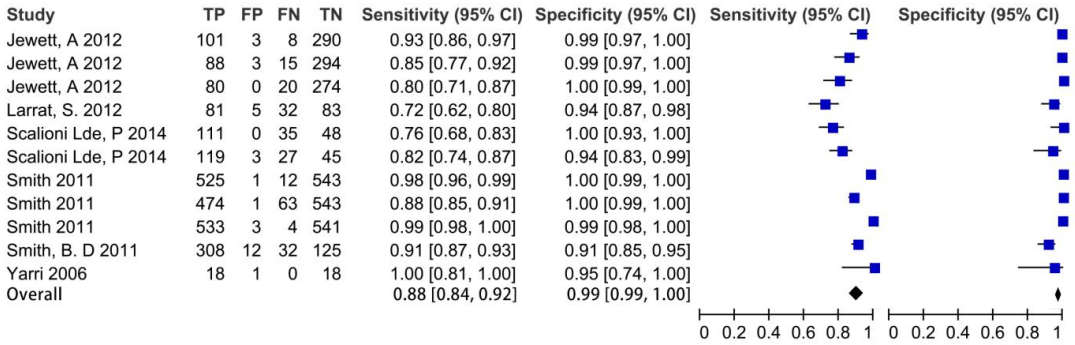
Overall, eight studies reported sensitivity and specificity of OraQuick (OraSure Technologies, PA, USA), with a total sample of 9024. The sample size of these studies ranged from 172 to 2183, sensitivities ranged from 0.90 to 1.00, and specificities ranged from 0.95 to 1.00. The pooled sensitivity and specificity were 0.98 (95% CI 0.97–0.99) and 1.00 (95% CI 0.90–1.00), respectively. Heterogeneity was observed between the included studies (*P*<0.001) (Fig. 7, Table 4).

Fig. 8. Pooled test accuracy for HCV Ab OraQuick kits (n = 8 studies)



Overall, six studies reported sensitivity and specificity for other three brands of oral kits, with a total sample of 6652. The sample size of these studies ranged from 37 to 1081, sensitivities ranged from 0.72 to 1.00, and specificities ranged from 0.91 to 1.00. The pooled sensitivity and specificity were 0.88 (95% CI 0.84–0.92) and 0.99 (95% CI 0.99–1.00), respectively, while heterogeneity was observed between the included studies (*P*<0.001) (Fig. 8, Table 4).

Figure 9. Pooled test accuracy for other brands of oral HCV Ab test kits (n = 6 studies)



Other findings

Our study further found that the overall sensitivity and specificity of studies conducted among general populations were 0.95 (95% CI 0.94–0.96) and 0.99 (95% CI 0.98–0.99), among key populations were 0.97 (95% CI 0.96–0.98) and 0.94 (95% CI 0.94–0.95), and among hospital patients were 0.97 (95% CI 0.96–0.98) and 1.00 (95% CI 1.00–1.00), respectively.

Table 4. Pooled test accuracy for different testing strategies (*n* = 52 studies)

Comparison	Pooled SE	95%CI		Tau-square *P*-value for heterogeneity	Pooled SP	95% CI		Tau-square *P*-value for heterogeneity
RDT versus EIA only (*n* = 5)	0.99	0.98	1.00	<0.001	1.00	1.00	1.00	<0.001
RDT versus NAT or Immunoblot (*n* = 13)	0.93	0.91	0.95	<0.001	0.98	0.97	0.99	<0.001
RDT versus EIA, NAT or Immunoblot (*n* = 14)	0.97	0.96	0.98	<0.001	1.00	1.00	1.00	<0.001
Oral RDT versus blood reference (*n* = 12)	0.94	0.93	0.96	<0.001	1.00	1.00	1.00	<0.001
Sample type								
Blood samples (*n* = 45)	0.98	0.97	0.98	<0.001	0.98	0.98	0.99	
Oral samples (*n* = 12)	0.94	0.93	0.96	<0.001	1.00	1.00	1.00	<0.001
Source population								
General screening (*n* = 17)	0.95	0.94	0.96	<0.001	0.99	0.98	0.99	<0.001
Key population (*n* = 19)	0.97	0.96	0.98	<0.001	0.94	0.94	0.95	<0.001
Hospital patients (*n* = 16)	0.97	0.96	0.98	<0.001	1.00	1.00	1.00	<0.001
Antibody and antigen combo testing (*n* = 6)	0.86	0.79	0.94	<0.001	0.99	0.98	1.00	<0.001
Oral kits brand								
OraQuick (*n* = 8)	0.98	0.97	0.99	<0.001	1.00	1.00	1.00	<0.001
Other brands (*n* = 6)	0.88	0.84	0.92	<0.001	0.99	0.99	1.00	<0.001

* Studies conducted in both LMICs and high-income countries were not included here

Studies conducted across these regions were not included here.

SE: sensitivity; SP: specificity

GRADE

GRADE for RDT versus EIA

HCV Ab RDTs showed comparable sensitivity and specificity compared to that of EIAs. For the 5 studies evaluated RDT versus EIA, 15 943 of samples were evaluated, and moderate risk of bias was observed, and precision was present, while inconsistency was present for sensitivity, as the sensitivities of the included tests varied. But the consistency of specificity is observed. Since the unit of the analysis varied among studies (Table 4), indirectness was observed. In addition, the overall

strength of the pooled evaluation was moderate, with pooled sensitivity and specificity of 0.99 (95% CI 0.98–1.00) and 1.00 (95% CI 1.00–1.00), respectively. Under the pre-test probability of 5%, the post-test probability after a positive test result is 97%, and the post-test probability after a negative test result is 100%.

GRADE for oral RDT versus blood reference

The use of oral RDT HCV Ab had comparable sensitivity and specificity compared to blood reference standards (Table 4). For the 12 studies evaluated oral RDT versus blood reference, 14 547 samples were evaluated. A moderate risk of bias was observed. Inconsistency present for sensitivity, as the sensitivities of the included studies varied. But the consistency of specificity was observed. Since the unit of the analysis varied with each other among the included studies (Table 4), indirectness was observed for included studies. In addition, the overall strength of the pooled evaluation was moderate, with pooled sensitivity and specificity of 0.94 (95% CI 0.93–0.96) and 1.00 (95% CI 1.00–1.00), respectively. Assuming a pre-test probability of 5%, the post-test probability after a positive test result was 94%, and the post-test probability after a negative test result was 100%.

Table 5. GRADE table

	RDT versus EIA	**Oral RDT versus blood**
Unit of analysis	Hospital patients, blood donors, injection drug users and other high-risk populations	General population, hospital patients, blood donors, injection drug users and other high-risk population
Sample type	Oral fluid serum or plasma	
Studies, *n*	5	12
Risk of bias	Moderate	
Consistency	Se: Inconsistent Sp: Inconsistent	
Directness/Precision	Indirect/ Precise	
# of samples	15 943	14 547
Strength of evidence	Se: Moderate Sp: Moderate	
Sensitivity (95% CI)	0.99(0.98–1.00)	0.94 (0.93–0.96)
Specificity (95%CI)	1.00 (1.00–1.00)	
Pretest probability (%)	0.05	
Positive LR (95% CI)	618.5 (350.6–2493.2)	314.5 (202.0–684.1)
PPV	0.97	0.94
Negative LR (95% CI)	0.01 (0.002–0.02)	0.06 (0.04–0.07)

NPV	1

6. Discussion

Acute HCV infection is usually asymptomatic, therefore there is an urgent need to increase screening for individuals who may be at increased risk. In this meta-analysis, we found HCV Ab RDTs, including those using oral fluid, showed a high overall sensitivity and specificity compared to laboratory-based EIAs. This extends the literature by including several new studies that were not included in prior reviews, including a subanalysis focused on RDTs that used oral fluid.

Our data suggest that RDTs can be used for HCV Ab detection in a wide range of clinical settings. High HCV Ab RDT sensitivity and specificity were observed across several different populations (general population, key populations, hospital patients). The use of an EIA to detect HCV Ab followed by NAT to confirm active infection is standard practice for diagnosis of HCV infection and recommended by the US Centers for Disease Control and Prevention (CDC) and the WHO.[70, 71] However, despite these recommendations, HCV Ab assays have not been widely used because of the complexity of laboratory-based assays, long turnaround time, high cost and requirements for specialized apparatus and trained technicians. To overcome this barrier, companies have developed RDTs for HCV Ab screening.[72] They obviate the need for multiple follow-up appointments, shorten wait times, and allow for the simplification and decentralization of testing. However, it is essential for policy-makers, government officials, and health care practitioners engaged in HCV screening, care and treatment to know that the performance of individual RDTs for detection of HCV Ab vary widely. Individual diagnostic accuracy for specific brands should be examined to ensure acceptable performance.

In recent years, RDTs that used oral fluid has been developed. Tests that can be used with non-invasive samples allow testing to be decentralised further and can be used in outreach settings.[73] Our data suggest that oral tests have slightly lower pooled sensitivity (0.94, 95% CI: 0.93–0.96) compared to blood-based tests (0.98, 95% CI: 0.97–0.98) but comparable specificity. Oral HCV Ab RDTs tests may be particularly useful in contexts where venepuncture may be difficult, such as subsets of people who inject drugs who have difficult veins to access.

With the increasing availability of DAAs, countries are seeking information on diagnostic accuracy of different tests, their operational characteristics and cost, to allow them to scale up HCV Ab screening, especially of at risk populations. Deploying which tests at which level of the health care system and for what settings require policy-makers to consider the different attributes of laboratory-based EIA versus blood-based or oral RDTs. Advantages and disadvantages of EIAs and RDTs are listed in Table 6. Performance, cost and accessibility need to be considered. Each country needs to decide on what cannot be compromised and what trade-offs are acceptable, based not only on disease prevalence and the health-care infrastructure, but also on technical, socioeconomic, cultural, behavioural considerations. For example, is it acceptable to buy Test X, which is 10% less accurate than Test Y but is considerably cheaper so that many more people can be screened? Although oral RDTs are less accurate than blood-based RDTs, would their use be more acceptable for outreach to at-risk

populations and allow the control programme to identify more HCV cases? In a low-prevalence setting, even a test with 98% specificity can yield more false-positive than true-positive results. All these trade-offs can be modelled to give an estimate of the cost–effectiveness and potential impact of different strategies for HCV Ab screening.

Table 6. Advantages and disadvantages of laboratory-based EIAs vs RDTs

	Laboratory-based EIA	RDTs
Advantages	• Accurate • High throughput • Objective, automated reading of results • Within-assay quality control	• Accessible to lowest level of the health care system (including outreach) • Can be used with non-invasive specimens, and facilitate self-testing • Rapid result to enable treatment initiation at the same clinic visit • Can be stored at ambient temperature
Disadvantages	• Requires laboratory facility, equipment and highly trained staff • Reagents need refrigeration • Need venepuncture to obtain sera • Time to result = 3–4 hours so patients need to return for results	• Lower accuracy than EIAs • Subjective reading and interpretation of results • No built-in quality control • Higher cost/test

Our review also underlines some of the common methodological problems encountered in evaluating diagnostic accuracy. Cross-sectional method or case–control methods were used by all 52 included studies, introducing a potential risk of bias. These studies used a broad range of reference standards, which makes the pooled performance data less meaningful. Within the evaluation of diagnostic accuracy, even cross-sectional studies in patients with diagnostic uncertainty and direct comparison of test results with an appropriate reference standard can be considered high quality.[74] The majority of the included studies used convenience sampling. In this review, we excluded panel studies because there are not based on clinical settings and our purpose was to produce data that would be relevant in clinical settings as part of detection of HCV Ab.

Most studies that reported HIV or HBV coinfection only reported the test performance of the kits among all samples, instead of disaggregated diagnostic accuracy. It may be useful for policy-makers and hospital administrators to know that the diagnostic accuracy of different kits among individuals with coinfections, particularly HIV coinfection.[69] Further research is needed to understand HCV Ab test characteristics among coinfected individuals.

Our study is subject to a number of limitations. First, we included studies conducted among the general population, hospital patients and key populations. HCV prevalence is variable among these different populations.[75] Diagnostic performance can be influenced by disease prevalence.[76] Second, we detected substantial heterogeneity that could influence our confidence in the review findings.[77] To deal with this problem, we undertook a number of subanalyses. Third, about 20 brands of RDT kits were used in the included studies, and the performance of these RDT kits vary. This limited our ability to summarize the accuracy of

different brands, with the exception of comparing OraQuick to other brands. Another concern is publication bias, as studies with poor test performance may be less likely to be published, leading to distorted estimates of accuracy.[78]

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Annex 5.5

PICO 3 - Testing strategies (HBV)

Diagnostic strategies for hepatitis B surface antigen detection: a meta-analysis and review of the literature

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1. Executive study

Background: Most individuals with chronic HBV infection are not aware of their serostatus, contributing to delayed diagnosis and complications from advanced disease. Chronic HBV infection, defined as persistence of hepatitis B surface antigen (HBsAg) for at least six months, is a major cause of preventable morbidity and mortality worldwide. Advances in hepatitis B virus (HBV) detection technology create new opportunities for enhancing screening, referral, and treatment. This review will look into what is the best testing strategy (diagnostic accuracy, cost, cost–effectiveness, and other resource utilization) for detection of HBsAg.

Methods: A comprehensive literature search algorithm, including Internet searches, using the components hepatitis B, screening, and testing strategies were applied**.** We reviewed observational and RCT studies that provided original data from patient specimens. Our goal was to compare two broad strategies for HBsAg detection – one-test strategies and two-test strategies.

Results: Our search resulted in 3655 literature review references and 7 additional Internet references for PICO 3. Screening of titles/abstracts resulted in 7 selected articles for possible data extraction. None of these 7 articles met all of the data extraction inclusion criteria so no articles were identified as final selection for PICO 3; comparing the diagnostic accuracy, cost, or effectiveness of two different testing algorithms, where possible. These 7 articles are discussed in more detail – 4 of the articles provided 3 national HBV algorithms (Australia, UK, US); 1 discussed testing strategies for select populations; 2 provided a look at cost–effectiveness of given testing strategies.

Conclusions: No study compared the diagnostic accuracy, cost, or cost–effectiveness of one- versus two-step HBsAg testing strategies. Studies that may provide contextual information about testing strategies were briefly summarized.

2. Background

Hepatitis B virus

An estimated 240 million individuals worldwide[1] are chronically infected with hepatitis B virus (HBV) and there are an estimated 4 million acute HBV infections each year. Twenty per cent to 30% of those with chronic hepatitis B infection will develop cirrhosis[2] or hepatocellular carcinoma,[3] leading to approximately 650 000 deaths each year.[4] However, most individuals with chronic HBV infection are not aware of their serostatus, contributing to delayed diagnosis and complications from advanced disease.[5] HBV testing is critically important in order to refer infected individuals to HBV treatment and care, to refer uninfected individuals to vaccination, and to mobilize prevention and control efforts.

In March 2015, the World Health Organization published the first guidelines for the prevention, care, and treatment of individuals with chronic HBV infection.[5] These guidelines focused on assessment for treatment eligibility, initiation of first-line therapies, switching, and

monitoring. These initial guidelines did not include recommendations on testing strategies that included what test to use and how to test. Given the large burden of HBV in low- and middle-income settings where there are limited or no existing HBV testing guidelines, there is a substantial need for HBV testing guidelines.

Description of HBV Ag detection

Chronic HBV infection is defined as persistence of hepatitis B surface antigen (HBSAg) for at least six months. However, interpretation of HBV serologies is complex (Table 1). The serological markers most frequently used for HBV testing include HBsAg, total anti-HBc, and anti-HBs (Table 1).

Table 1. Hepatitis B serological marker interpretation

	Serological marker				Interpretation
	HBsAg (hepatitis B surface antigen)	**Total anti-HBc (antibody to hepatitis B core antigen)**	**IgM anti-HBc (immunoglobulin M to anti-HBc)**	**Anti-HBs (antibody to HBsAg)**	
Test results	–	–	–	–	Never infected and susceptible to infection
	+	+	–	–	Chronic infection
	–	+	–	+	Recovered from past infection and immune
	+	+	+	–	Acute infection
	–	+	–	+	Immune by natural infection
	–	–	–	+	Immune by hepatitis B vaccination
	–	+	–	–	Immune by natural infection or possible false positive

Source: US Centers for Disease Control and Prevention. (Available at: http://www.cdc.gov/immigrantrefugeehealth/ guidelines/ domestic/hepatitis-screening-guidelines.html)

One test vs two test serological testing strategy

The most important marker for the diagnosis of chronic hepatitis B infection requiring further assessment or treatment remains HBsAg. The case definition of chronic hepatitis B is the detection of HBsAg twice six months apart.

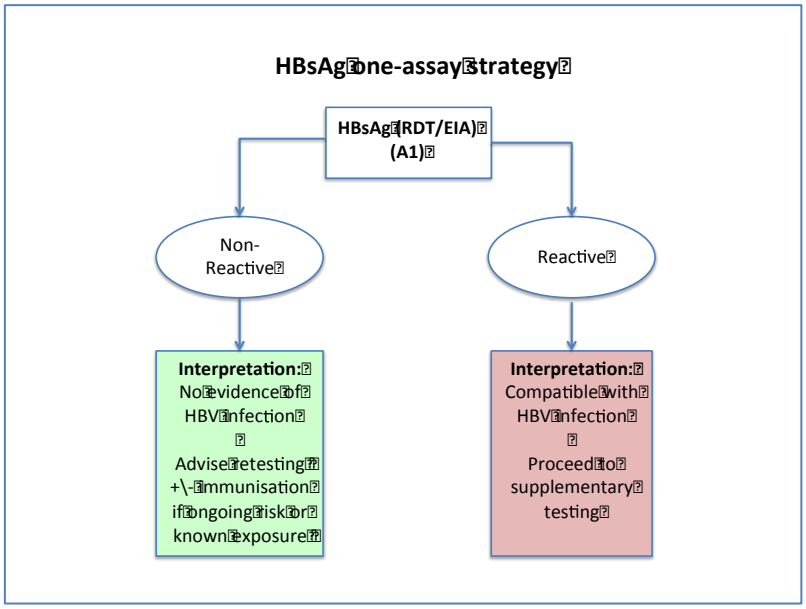
After an initial positive result for HBsAg, supplementary testing can be undertaken in order to facilitate entry into a care pathway. The detection of HBsAg in blood can include rapid diagnostic tests, or enzyme immunoassays. Confirmation of the specificity of a reactive HBsAg first-line test result is usually carried out by either:

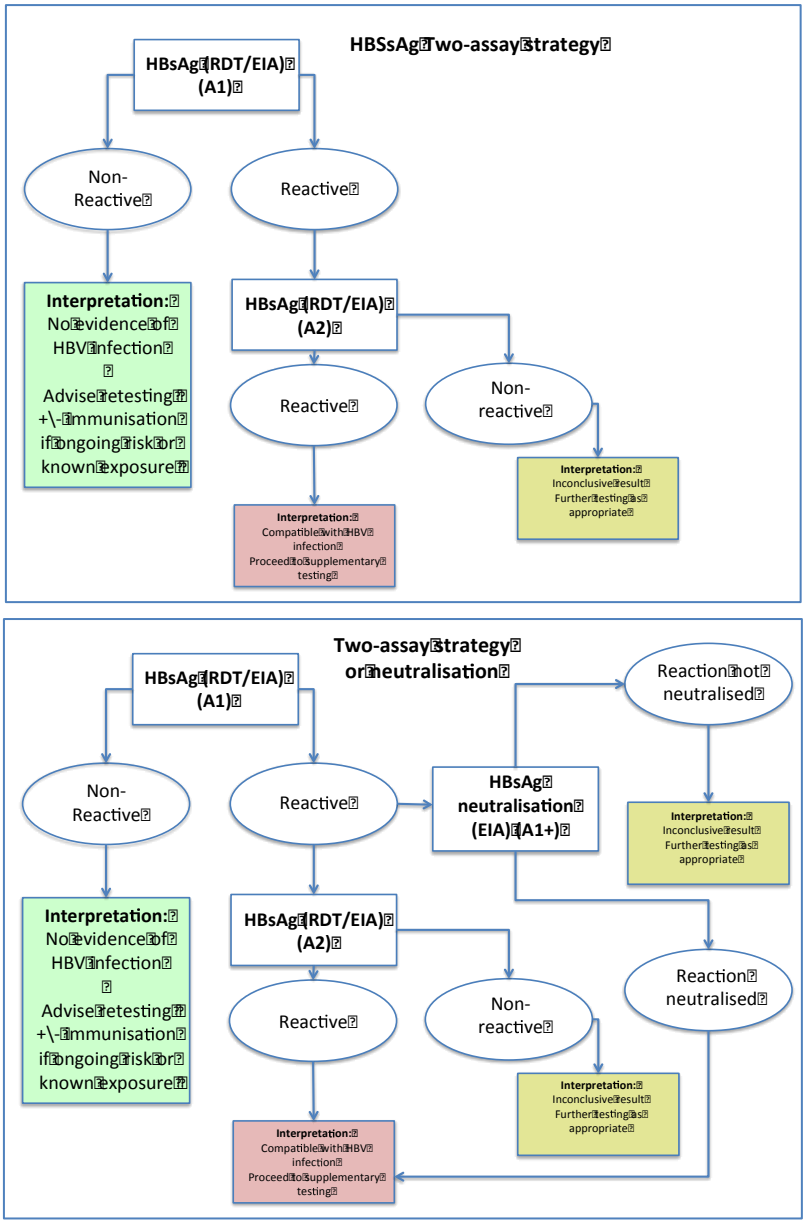
i) repeating the HBsAg testing in a different assay of similar sensitivity, or

ii) performing a neutralization test using a specific anti-HBs-containing reagent in the same first-line assay after appropriate dilution of the specimen under test. Specificity is confirmed when this reagent abolishes reactivity in the assay.

WHO recommends standardized testing strategies to maximize the accuracy of hepatitis B and C testing while minimizing cost and increasing simplicity. This PICO question addresses the issue of whether a positive result from a single HBsAg assay has sufficient specificity in order to proceed to supplementary testing and/or entry into a care pathway, or whether confirmatory testing on the same specimen with a different HBsAg assay (or neutralization), performed sequentially after the first assay is required. This is particularly relevant in low prevalence settings where more than one assay may be required to confirm specificity. Two previous reviews[6,7] on hepatitis testing focused on the test performance but did not compare testing strategies.

Fig. 1. Options for HBV screening, which may include HBsAg in a one-test strategy (e.g. a single HBsAg using a rapid diagnostic test [RDT] or enzyme immunoassay [EIA]) and two-test strategies (second RDT or EIA or neutralization with EIA)





PICO 3	HBsAg testing strategy: Among persons identified for hepatitis B testing, what is the best testing strategy (diagnostic accuracy and other outcomes) for detection of HBsAg? (One-test versus two-test strategy) (Figs 1A, 1B)
P	Persons identified for HBV testing
I	One-test strategy; one HBsAg test (Fig. 1A)
C	Two-test strategy; two different HBsAg tests (Fig. 1B)
O	Diagnostic accuracy True negatives (TN), who are screen negative, and do not have HBV infection

	False negatives (FN), who are screen negative but have HBV infection. These are incorrectly misclassified, and this may result in missed opportunities to recognize and present progression of liver disease. True positives (TP), who are screen positive and have HBV infection. False positives (FP), who are screen positive, but do not truly have HBV infection. These will have additional unnecessary tests and evaluation. Costs (cost of testing strategy, including lab reagents and running costs, cost of further evaluation of a false positive) Cost–effectiveness Acceptability to health-care worker and patients Other outcomes (missed cases of liver disease because of false negative results, unnecessary referral, investigations and/or treatment in false positives)

3. Objectives

- To identify quantitative evidence on the sensitivity and specificity of one-test compared to two-test algorithms for detection of HBsAg
- To evaluate the cost–effectiveness, acceptability, and other outcomes (missed liver disease because of false-negative results, unnecessary referral, investigations) associated with these two types of testing strategies
- To inform models to optimize hepatitis B screening algorithms.

4. Methods

We reviewed observational studies and randomized controlled trials (RCTs) that provided original data from patient specimens. Our goal was to compare two broad strategies for HBsAg detection – one-test strategies and two-test strategies.

Search algorithm

Literature search strategies were developed by a medical librarian with expertise in systematic review searching. Our search algorithm consisted of the following components: hepatitis B, screening, and testing strategies (**Annex 1**).

We searched MEDLINE (OVID interface, 1946 onwards), EMBASE (OVID interface, 1947 onwards), the Cochrane Central Register of Controlled Trials (Wiley interface, current issue), Science Citation Index Expanded (Web of Science interface, 1970 onwards), Conference Proceedings Citation Index-Science (Web of Science interface, 1990 onwards), SCOPUS (1960 onwards), Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS) (BIREME interface) and WHO Global Index Medicus. The search was supplemented by searching for ongoing studies in WHO's International Clinical Trials Registry. The literature search was limited to the English language and human subjects.

We formulated a comprehensive and exhaustive search strategy in an attempt to identify all relevant studies. After the MEDLINE strategy was finalized, it was adapted to the syntax and subject headings of the other databases.

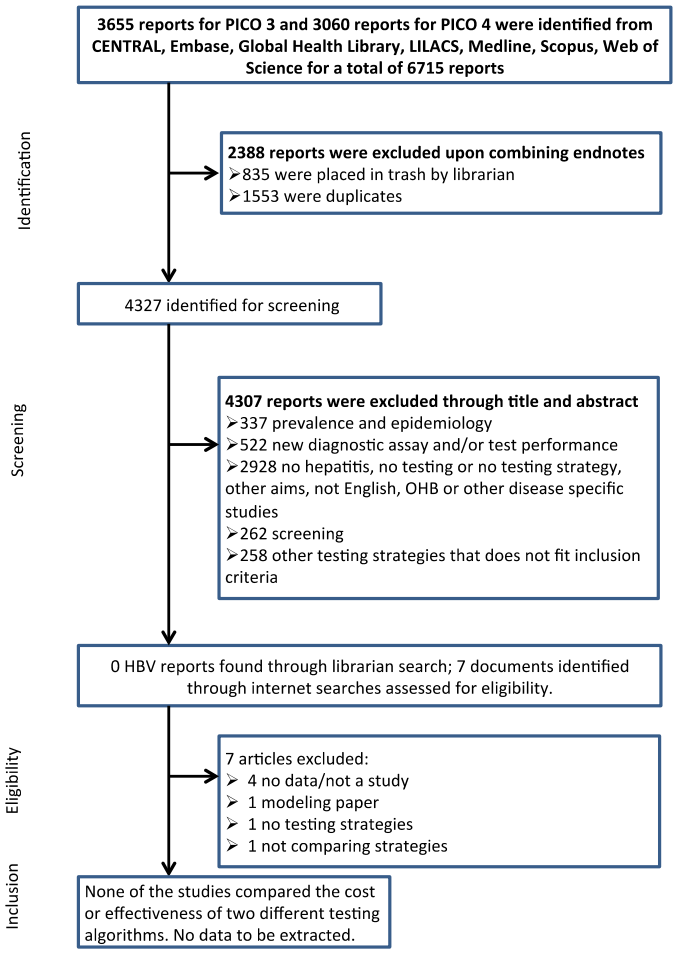
In addition to searching databases, we also searched the Internet for any peer-reviewed articles and conference abstracts that might have been missed through our librarian search and also expanded our search to national guidance documents.

5. Results

Study selection

The librarian search resulted in 3655 references for PICO 3. Because of overlap with objectives and search strategies between PICOs 3 and 4, and to expedite the initial screening, PICO 3 references were combined with the 3060 references identified through the librarian search for PICO 4 (HCV); 2388 searches were immediately excluded. The librarian excluded 835 for not being relevant and there were 1553 duplicates. Thus, 4327 remained for screening. Titles/abstracts were screened according to protocol inclusion and exclusion criteria, for both PICOs 3 and 4. Reasons for excluding 4307 reports were noted (Fig. 2).

Fig. 2. PRISMA for PIC0 3 HBV (Diagnostic strategies for hepatitis B surface antigen and hepatitis C antibody detection)



From the librarian search, no reports were identified for possible data extraction. The Internet searches resulted in 7 additional reports for possible data extraction. Full documents (manuscripts, abstracts, guidelines, etc.) were obtained and assessed against inclusion criteria. Papers were either accepted or rejected and reasons for rejection were explained.

The following inclusion criteria were used to evaluate the final selection: evaluations of HBV testing strategies; evaluations based on human clinical materials. The following exclusion criteria were used: studies focused only on evaluation of single-test assays without a two-test comparator group; studies focused on two-test strategies that include other types of test (e.g. anti-HBsAg) studies with primary aims other than evaluation of testing strategies; studies related to disease prevalence, drug resistance, genotyping, sequencing, or non-diagnostic purposes; articles in languages other than English, conference abstracts.

Data abstraction and data synthesis

Of the 7 selected for possible data extraction, the following variables were collected, when available: first author, title, year, objective, and exclusion criteria (**Table 2**).

Table 2. Seven reports assessed for eligibility

	Author or source year	Title	Objective	Exclusion criteria	Conclusions
1.	Fan et al. 2014	Cost-effectiveness of testing hepatitis B-positive pregnant women for hepatitis B e antigen or viral load	To estimate cost-effectiveness of testing with hepatitis B (hepatitis B surface antigen [HBsAg]-positive) for hepatitis B e antigen (HBeAg) or hepatitis B virus (HBV) DNA	Decision tree model to estimate the costs and effects of two sequential testing strategies	Either sequential HBeAg testing or sequential HBV load testing was cost-effective. Sequential HBeAg testing dominated sequential HBV load testing with 1000 QALYs and $6.6 million saved
2.	US Preventive Services Task Force, 2014	Hepatitis B, non-pregnant adolescents and adults: screening, May 2014	To recommend screening for hepatitis B virus (HBV) infection in persons at high risk for infection	No data/not a study. USPSTF makes recommendations about the effectiveness of specific clinical preventive services for patients without related signs or symptoms.	Document makes screening recommendation; not relevant to data synthesis for this report
3.	Prepared for the US Preventive Services Task Force by Peter W. Pendergrass and Carolyn DiGuiseppi (Texas Dept State Health and Univ. Colorado), 2014	Screening for hepatitis B virus infection	To develop recommendations for USPSTF	No data/not a study	Document makes screening recommendation; not relevant to data synthesis for this report
4.	Chen et al. 2015	Cost-effectiveness of augmenting universal hepatitis B vaccination with immunoglobin treatment	To compare the cost-effectiveness of hepatitis B virus (HBV) control strategies combining universal vaccination	Not testing strategies – vaccination strategies	Universal vaccination plus screening for hepatitis B surface antigen (HBsAg) and HBIG treatment for HBsAg-positive mothers' neonates averted the most infections

			with hepatitis B immunoglobulin (HBIG) treatment for neonates of carrier mothers		
5.	Public Health England (PHE), National Health Service (NHS), 2014	UK Standards for Microbiology Investigations	To develop a set of standards for hepatitis B diagnostic serology in the immunocompetent (including hepatitis B in pregnancy)	No data/not a study	No conclusions
6.	Australian Government, 2012	National HBV Testing Strategy	To provide diagnostic strategies for HBV	No data/not a study	No conclusions
7.	Peng et al. 2011	Development of an economic and efficient strategy to detect HBsAg: Application of “grey-zones” in ELISA and combined use of several detection assays	To thoroughly assess the performance of the HBsAg assays and testing algorithm currently used in clinical settings	Does not compare strategies	Combined use of “grey-zones” in ELISA and several different detection assays can significantly increase the efficiency of HBsAg detection

References found in **Annex 2.**

None of the reports compared the cost or effectiveness of two different testing algorithms. Of the 13 documents selected, 4 referenced algorithms and are therefore shown in Table 3. Types of tests performed and exclusion criteria are also included.

Table 3. Four reports that examined testing strategies

	References	Test 1	Test 2	Test 3	Test 4	Exclusion criteria
1	Fan et al. 2014	Sequential HBeAg				Decision tree model to estimate the costs and effects of two sequential testing strategies
		Sequential HBV load				
2*	Public Health England (PHE), National Health Service (NHS), 2014	HBsAg	Repeat HBsAg	Confirm by neutralization		No data/not a study
		HBsAg	Repeat HBsAg	Anti-HBc	HBV DNA or IgM	
3*	Australian Government, 2012	HBsAg	Anti-HBs	Anti-HBc		No data/not a study
4*	Peng et al. 2011	ELISA	ELISA	CMIA	Confirm by HBsAg	Does not compare strategies

***** Algorithm schematics attached (**Annex 3**)

Extensive review of the literature found no articles, reports, etc. that met all of the eligibility criteria for data extraction to be used to address this question. Most of the literature focused on screening blood donations.

Seven reports were identified that might be useful for modelling exercises to address this PICO question. This short narrative will provide an overview of these 7 articles, also drawing on other informative reviews and personal communications.

Cost

Fan et al. (2014) examined the cost–effectiveness of testing hepatitis B-positive pregnant women for hepatitis B e antigen or viral load. In this select population of mothers of a neonate birth cohort, either sequential HBeAg testing or sequential HBV load testing was found cost–effective. Sequential HBeAg testing dominated sequential HBV load testing with 1000 QALYs

and $6.6 million saved. It is important to note that this study used a decision tree model to estimate the costs and effects of two sequential testing strategies.

Chen et al. (2015) also examined cost–effectiveness of three strategies using a cohort of hospital patients in China. In this case, costing was not related to testing strategies but vaccination strategies, specifically universal HBV vaccination with immunoglobulin treatment. Their study found that while screening tests may be cost–effective, they require more infrastructure than is needed for vaccination, including laboratory services and adequate numbers of medical professionals to interpret test results and administer HBIG. As previously mentioned, it appears that vaccination should strongly be taken into account with considering HBV testing strategies.

Quality assessment

Study quality was not evaluated using the QUADAS-2 tool[8] and the STARD checklist, as it was not applicable since none of the studies met inclusion criteria.[9]

6. Discussion

Testing strategies

Although four of the seven reports identified provided national testing algorithms for Australia and the UK, none published data on supporting evidence. This has been confirmed by personal communications.

In general, HBV screening typically includes HBsAg in both the one-test (e.g. HBsAg) and two-test strategies. Beyond this it is unclear how to select other tests. This may depend on findings from PICO 1 to better understand the performance characteristics of HBV tests, and from this to model various testing strategies for feasibility and utility.

The “simplest” testing strategies seemed to include all 3 tests below (as seen in the US and Australia algorithms).

- hepatitis B surface antigen (HBsAg)
- hepatitis B core antigen (anti-HBc)
- hepatitis B surface antibody (anti-HBs)

Public Health England (PHE) provides testing strategies to confirm HBsAg by an alternative assay or neutralization. Without much other evidence to support this, it seems as though confirming with an alternative assay might be a simple, cost–effective approach. It is also important to note that almost all reports reviewed also discussed comparing cost of screening and a testing strategy to cost of vaccination.

Testing recommended for select populations

During domestic medical examination of refugees, CDC screens using the above tests for chronic HBV (HBsAg) for all persons from countries with intermediate (≥2%–7%) or high (≥8%)

prevalence of chronic HBV infection. The only exception would be if a negative HBsAg test result is documented on their medical form.

Peng et al. (2011) examined novel strategies to detect HBsAg using ELISA "grey-zones", in combination with other detection assays. As noted, clinical HBV detection methods differ between countries with high and low levels of endemic HBV infection. This study focused on a select population to test the algorithm currently used in clinical settings in China, specifically assessing the performance of the KHB (Kehua Bio-engineering Co. Ltd., Shanghai, China) and CMIA (Chemiluminescent Micropartical Immunoassay) HBsAg tests. While KHB is one of the most commonly used kits in China, this was a major limitation for the purposes of this systematic review. Yet, they presented a novel approach of combining strategies using ELISA "grey-zones", which was found to significantly increase the efficiency of HBsAg detection. Although this approach "broadened the range" to allow for increased sensitivity, it may prove to be rather complicated requiring the establishment of numerous population-specific "grey-zones" and complex interpretations.

Again, almost all reports discussed testing based on select populations but this study did not focus on identifying algorithms to be used on select populations.

The choice between a one-test versus two-test strategy depends on the diagnostic accuracy of HBsAg tests. Results from a systematic review of the diagnostic accuracy of HBsAg tests (PICO 1) across 21 studies that evaluated 25 brands of RDTs using 15 EIA reference assays, with 36 919 total samples, including serum, plasma, venous and capillary whole blood, showed that the overall pooled clinical sensitivity and specificity of rapid HBsAg tests were 90.0% (95% CI: 89.1, 90.8) and 99.5% (95% CI: 99.4, 99.5), respectively, compared to laboratory-based immunoassay reference standards. Sensitivities ranged from 50% to 100% with overall pooled sensitivity of 90.0% (95% CI: 89.1, 90.8). Specificities ranged from 69% to 100%, with overall pooled specificity of 99.5% (95% CI: 99.4, 99.5). Pooled positive likelihood ration (PLR) and negative likelihood ratio (NLR) were 117.5 (95% CI: 67.7, 204.1) and 0.095 (95% CI 0.067, 0.136), respectively, with tau-square 3.89, 1.72, respectively, suggestive of significant heterogeneity between studies.

Pooled sensitivity in studies of HIV-positive persons was lower than in known HIV-negative patients; 72.3% (95% CI: 67.9, 76.4) compared to 92.6% (95% CI: 89.8, 94.8), respectively. Pooled sensitivity and specificity in blood donors were 91.6% (95% CI: 90.1, 92.9) and 99.5% (95% CI: 99.5, 99.9), respectively. Samples using whole blood specimens (venous or capillary) were 91.7% (95% CI: 89.1, 93.9) and 99.9% (95% CI: 99.8, 99.9) sensitive and specific compared to serum. The overall pooled clinical sensitivity and specificity of laboratory-based HBsAg tests were 88.9% (95% CI: 87.0, 90.6) and 98.4% (95% CI: 97.8, 98.8) sensitivity and specificity, respectively, compared to state-of-the-art chemiluminescent microparticle enzyme immunoassays.

Although these tests appeared to have excellent specificity and would have required a 2-test strategy, in a low-prevalence setting, even tests that have excellent specificities may produce false-positive results. This would then require the use of a second test to reduce the number of false-positive results. This can be illustrated as follows:

In a hypothetical population of 1000 people where the HBV prevalence is 2%, a test with a sensitivity of 100% and a specificity of 99% may lead to 10 false-positive and 20 true-positive results. This means that 1 in 3 positive results may be a false-positive result.

Table 4. Diagnostic accuracy in a low-prevalence setting example

	Reference test		
	+	–	
Index test +	20	10	30
Index test –	0	970	970
Total	20	980	1000

Sensitivity = 20/20 = 100%; Specificity = 970/980 = 99%; PPV = 20/30 = 67%; NPV = 970/970 = 100%.

The systematic review also showed that HBsAg tests have a lower sensitivity in HIV-positive individuals. A second test may be useful for increasing the performance of testing overall.

Worked example to illustrate the effect of prevalence on predictive values for the two different testing strategies

Assuming the following assay performance characteristics:

If Assay 1 has sensitivity of 99% and specificity of 98%

If Assay 2 has sensitivity of 99.4 and specificity of 99.5%

Table 5. Effect of prevalence on predictive values for the two different testing strategies

	Prevalence of analyte		
	0.1%	**1%**	**10%**
Positive predictive values			
Assay 1	4.7%	33.3%	84.6%
Assay 1 + Assay 2 (serial)	90.7%	99%	99.9%
Negative predictive values			
Assay 1	99.9%	99.99%	99.99%

Using the following equation for PPV and NPV that incorporates prevalence more correctly,

$$PPV = \frac{sensitivity \times prevalence}{sensitivity \times prevalence + (1 - specificity) \times (1 - prevalence)}$$

$$NPV = \frac{specificity \times (1 - prevalence)}{(1 - sensitivity) \times prevalence + specificity \times (1 - prevalence)}$$

Reference: Altman DG, Bland JM. Diagnostic tests 2: predictive values. BMJ. 1994 Jul 9; 309(6947):102. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/ PMC2540558 /pdf/bmj00448-0038 za.pdf

7. Conclusions

No study compared diagnostic accuracy, cost, cost–effectiveness of one- vs two-step testing strategies for the detection of HBsAg. Diagnosis of HBV is very complex and there may not be simple algorithms that will cover all settings. All PICOs related to HBV will need to be looked at together to address PICO 3.

The decision tree model described by Fan et al. may prove to be useful for modelling costs of testing strategies. Vaccination strategies should also be taken into account.

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3. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology. 2004;127(5 Suppl 1): S35–S50.
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8. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011;155(8):529–36.
9. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. Ann Intern Med. 2003;138(1):W1–W12.

Appendices

Appendix 1. Librarian search

1. Hepatitis, Viral, Human/
2. Hepatitis Viruses/
3. Hepatitis Antibodies/
4. exp Hepadnaviridae Infections/
5. Hepatitis B Antibodies/
6. Hepatitis B virus/
7. Hepadnaviridae/
8. Hepatitis B Surface Antigens/
9. (heptatitis-b or hep-b or (hepatitis adj5 b) or (hep adj5 b) or hbv).ti,ab.
10. hbsag.ti,ab.
11. exp Hepatitis C/
12. Hepacivirus/
13. Hepatitis C Antibodies/
14. (heptatitis-c or hep-c or (hepatitis adj5 c) or (hep adj5 c) or hcv or aghcv or hepacivirus*).ti,ab.
15. hcvab.ti,ab.
16. or/1-15 [HEP B or HEP C]
17. exp Mass Screening/
18. screen*.ti,ab.
19. 17 or 18 [MASS SCREENING]
20. (one-test* or two-test*).ti,ab.
21. ("1-test*" or "2-test*").ti,ab.
22. ((one or two or "1" or "2" or strateg* or algorithm* or approach or procedure* or system*) adj5 (test or tests or testing or detect* or diagnos* or kit or kits or assay* or device*)).ti,ab.
23. or/20-22 [TESTING STRATEGIES]
24. 16 and 19 and 23
25. Humans/
26. Animals/
27. 25 and 26
28. 26 not 27
29. 24 not 28
30. Limit 29 to English language

Appendix 2. Seven full text articles assessed for eligibility (comparing algorithms, including costing)

1. Final recommendation statement: screening for hepatitis B virus infection in non-pregnant adolescents and adults. In: US Preventive Services Task Force [website]. May 2014. (http://www.uspreventiveservicestaskforce.org/Announcements/News/ Item/final-recommendation-statement-screening-for-hepatitis-b-virus-infection-in-nonpregnant-adolescents-and-adults, accessed 08 June 2016).
2. UK Standards for Microbiology investigations: hepatitis B diagnosis in the immunocompetent (including hepatitis B in pregnancy). Standards Unit, Microbiology Services, PHE Virology. V 4, Issue no: 5.3, Issue date: 31.03.14. (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/344145/V_4i5.3.pdf, accessed 8 June 2016).
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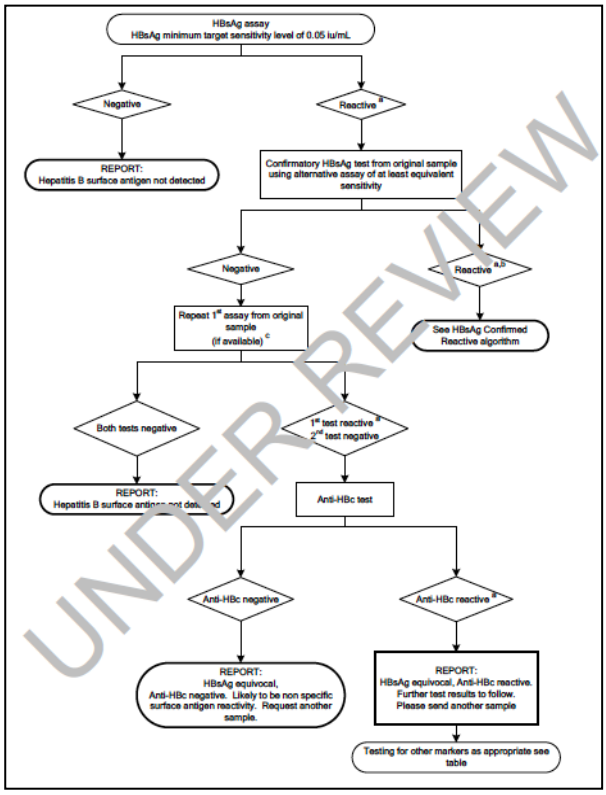
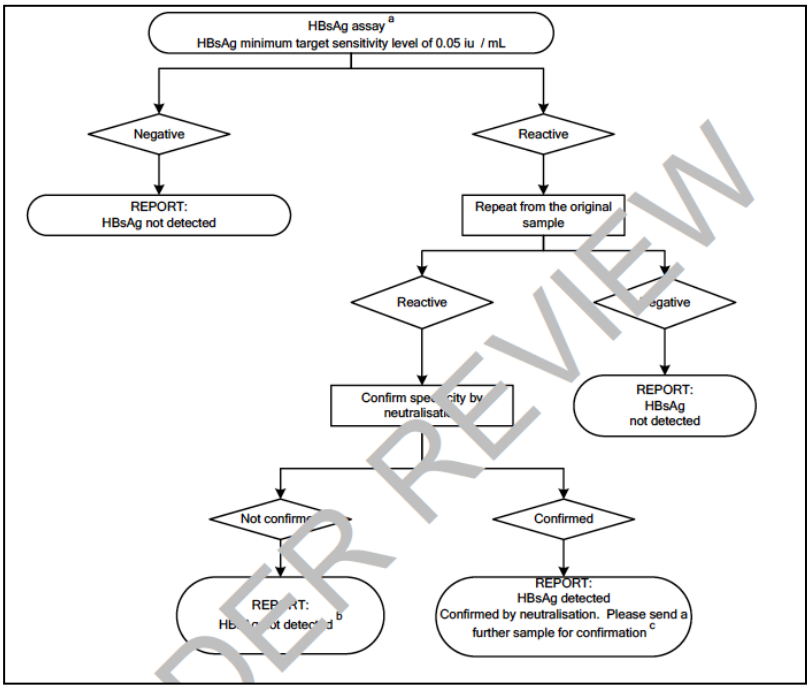
5. Fan L, Owusu-Edusei K, Jr., Schillie SF, Murphy TV. Cost-effectiveness of testing hepatitis B-positive pregnant women for hepatitis B e antigen or viral load. Obstet Gynecol. 2014;123(5):929–37.
6. Peng J, Cheng L, Yin B, Guan Q, Liu Y, Wu S et al. Development of an economic and efficient strategy to detect HBsAg: application of "gray-zones" in ELISA and combined use of several detection assays. Clin Chim Acta. 2011 412(23–24):2046–51.
7. Summaries for patients. Screening for hepatitis B virus infection: US Preventive Services Task Force recommendation statement. Ann Intern Med. 2014;161(1):I–28.

Appendix 3. Testing schematics

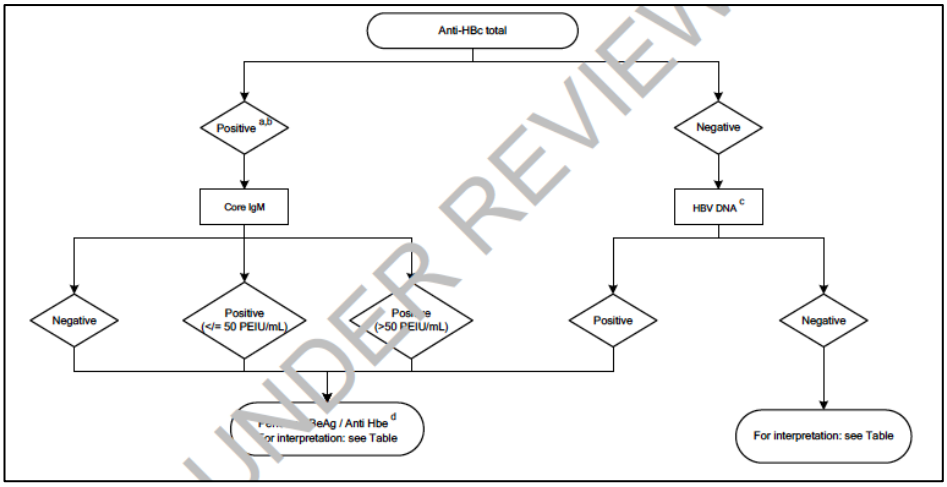


Public Health England, 2014: hepatitis B virus serology – HBsAg confirmation by alternative assay

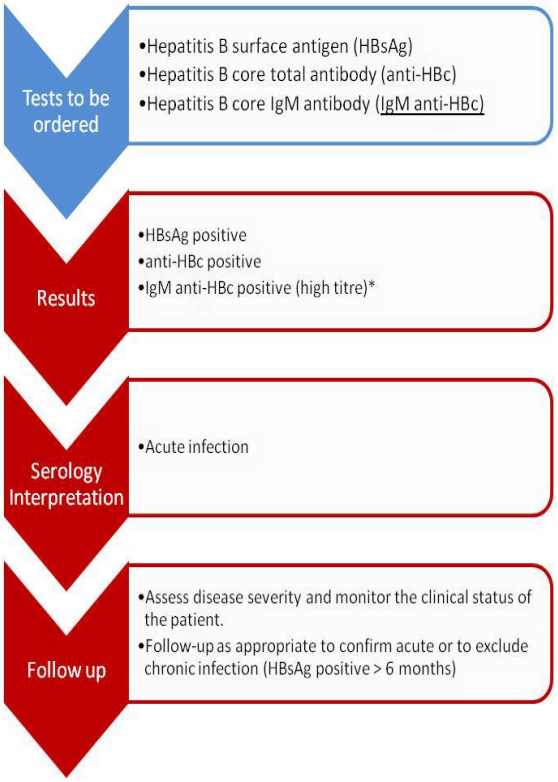


Public Health England 2014: hepatitis B surface antigen (HBsAg) confirmation by neutralization

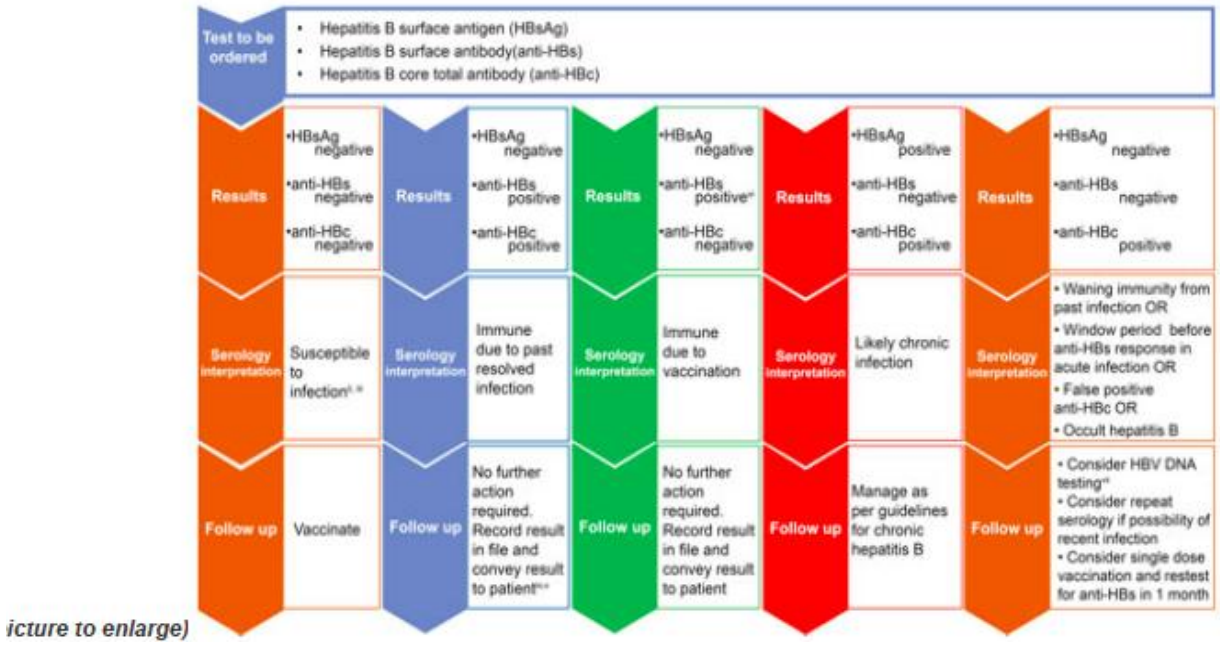
UNDER REVIEW



Public Health England, 2014: hepatitis B surface antigen confirmed reactives



Australia Government, 2012: suspected acute HBV



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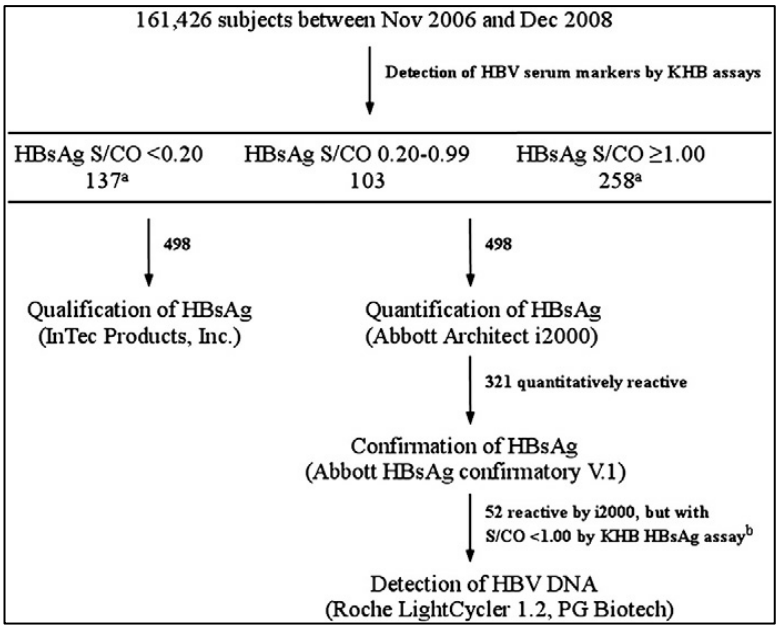
Australia Government 2012: suspected chronic HBV



Peng et al. 2011

Annex 5.6

PICO 4 - How to test (HCV)

Diagnostic strategies for hepatitis C antibody detection: a meta-analysis and review of the literature

London School of Hygiene and Tropical Medicine team
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London School of Hygiene and Tropical Medicine team
*Co-leaders of this review

September 2015

1. Executive summary

Background: An estimated 130–150 million people have chronic hepatitis C infection worldwide, leading to 350 000–500 000 deaths per year. Although HCV treatment is successful in a majority of people, most HCV-infected individuals remain undiagnosed and untreated. Advances in HCV detection technology create new opportunities for enhancing screening, referral, and treatment.

Methods: A comprehensive literature search algorithm, including Internet searches, using the components hepatitis C, screening, and testing strategies were applied**.** We reviewed observational studies and randomized controlled trials (RCTs) that provided original data from patient specimens. Our goal was to compare the effects of two broad strategies for hepatitis C antibody detection – one-test strategies and two-test strategies on diagnostic accuracy, costs, and resource utilization.

Results: Our search resulted in 3060 literature review references and 3 additional Internet references for PICO 4. Screening of titles/abstracts resulted in the selection of 8 articles for possible data extraction. Two of these 8 articles met all of the data extraction inclusion criteria so no articles were identified as final selection for PICO 4; comparing the diagnostic accuracy, cost or effectiveness of two different testing algorithms. These 8 articles are discussed in more detail – 1 of the articles provided a comprehensive overview of antibody/antigen testing; 2 articles delved into core antigen testing; 3 articles exemplified other testing such as recombinant immunoblot (IB) tests, signal-to-cut-off ratios, point-of-care tests (POCT), and antibody-based rapid diagnostic tests (RDT); 1 discussed testing strategies; 1 provided a look at comparison and cost–effectiveness of given testing strategies.

Conclusions: Two studies compared the diagnostic accuracy, cost, cost–effectiveness of a 1-test versus 2-test strategy for detection of HCV antibody. One study found that in individuals who are HCV antibody positive, the use of an IB assay with defined signal-to-cut-off ratios can be used to distinguish between those who are viraemic and those who are not. This reduces the number of nucleic acid tests (NATs) required to confirm active infection is a cost–effective strategy. Another study found that screening with a highly sensitive EIA followed by another EIA as confirmation assay in a routine clinical laboratory can be effective in nonimmunocompromised populations. In immunocompromised patients, IB may be more effective as these patients tend to have low antibody levels.

2. Background

Hepatitis C virus

Hepatitis C virus (HCV) causes acute infection which can progress to chronic infection and liver disease.[1,2] An estimated 130–150 million people have chronic hepatitis C infection worldwide, leading to 350 000–500 000 deaths per year. [1–3] Approximately 15–45% of individuals who have acute HCV infection will spontaneously clear it without any treatment. Most individuals will go on to develop chronic active HCV infection which is defined by the presence of HCV

RNA.[1–3] Although HCV treatment is successful in a majority of people, most HCV-infected individuals remain undiagnosed and untreated.[4] As a result, approximately 15–30% of individuals with chronic HCV infection progress to cirrhosis, leading to end-stage liver disease and hepatocellular carcinoma.[1,2]

The recent introduction of direct-acting antivirals (DAAs) have led to sustained virological response (SVR) in greater than 90% of all individuals[5,6] and are recommended by the WHO.[7] DAAs will not only improve SVR rates, but also may simplify HCV management algorithms and allow smaller health facilities to manage HCV-infected individuals.[8]

In April 2014, the World Health Organization published guidelines for the screening, care, and treatment of individuals with HCV infection.[9] These guidelines included recommendations on who to screen for HCV and how to confirm HCV infection, but not which tests are optimal for initial screening. A test for HCV antibody (Ab) is an important first step in the diagnosis of hepatitis C infection as the presence of Ab is a marker of exposure to HCV.

After an initial positive result for HCV Ab, supplementary testing can be undertaken in order to confirm active infection and facilitate entry into a care pathway. The detection of HCV Ab in blood can include rapid diagnostic tests, or enzyme immunoassays (EIA). Confirmation of the specificity of a reactive HCV Ab first-line test result can be carried out by repeating the HCV Ab testing in a different assay of similar sensitivity. Specificity is confirmed when this reagent abolishes reactivity in the assay.

WHO recommends standardized testing strategies to maximize the accuracy of hepatitis B and C testing while minimizing cost and increasing simplicity. This PICO question addresses the issue of whether a positive result from a single HCV Ab assay has sufficient specificity in order to proceed to supplementary testing and/or entry into a care pathway, or whether confirmatory testing on the same specimen with a different HCV Ab assay performed sequentially after the first assay is required. This is particularly relevant in low-prevalence settings where more than one assay may be required to confirm specificity.

Description of HCV antibody testing

Antibodies to HCV infection begin during early infection and persist throughout life in most individuals. Hence, an HCV Ab test is the best marker of exposure to HCV but cannot be used to distinguish between active and treated or resolved past infection.

Screening for exposure to HCV is dependent on assays that detect antibodies to HCV (anti-HCV). Once antibody status is confirmed, the patient can undergo supplementary testing to determine the presence of HCV RNA or core antigen (HCV cAg) as markers of active infection. It is important to note that the latest generation of assays designed to detect anti-HCV are combined with cAg to increase the sensitivity of the assay in detecting active infection.

The question this PICO aims to address is whether one or two serological assays (anti-HCV or HCV Ag/Ab combo assays) performed sequentially are required, in terms of specificity and positive predictive value, in order to proceed to supplementary testing.

PICO 4	**Among persons identified for hepatitis C testing, what is the best testing strategy (diagnostic accuracy and other outcomes) for detection of HCV antibodies? (One-test versus two-test strategy) (Fig. 1A,1B)**
P	Persons identified for HCV testing
I	**One-test strategy; One HCV Ab test (Fig. 1A)**
C	**Two-test strategy; Two different HCV Ab tests (Fig. 1B)**
O	Diagnostic accuracy True negatives (TN), who are screen negative, and do not have HCV infection. False negatives (FN), who are screen negative but have HCV infection. These are incorrectly misclassified, and this may results in missed opportunity to recognize and prevent progression of liver disease. True positives (TP), who are screen positive and have HCV infection. False positives (FP), who are screen positive, but do not truly have HBV infection. These will have additional unnecessary tests and evaluation. **Costs (cost of testing strategy including lab reagents and running costs, cost of further evaluation of a false positive)** **Cost–effectiveness** Acceptability to health-care worker and patients Other outcomes (missed cases of liver disease because of false-negative results, Unnecessary referral, investigations and/or treatment in false positives)

Two systematic reviews on diagnostic performance of different hepatitis C serological assays focused on evaluating point-of-care tests compared to EIAs and other reference tests.[10,11] None of the existing reviews compared one-test and two-test strategies for detection of hepatitis C Ab.

Fig. 1: What is the best testing strategy for detection of HCVAb? (A. One test, B. Two-test strategy)

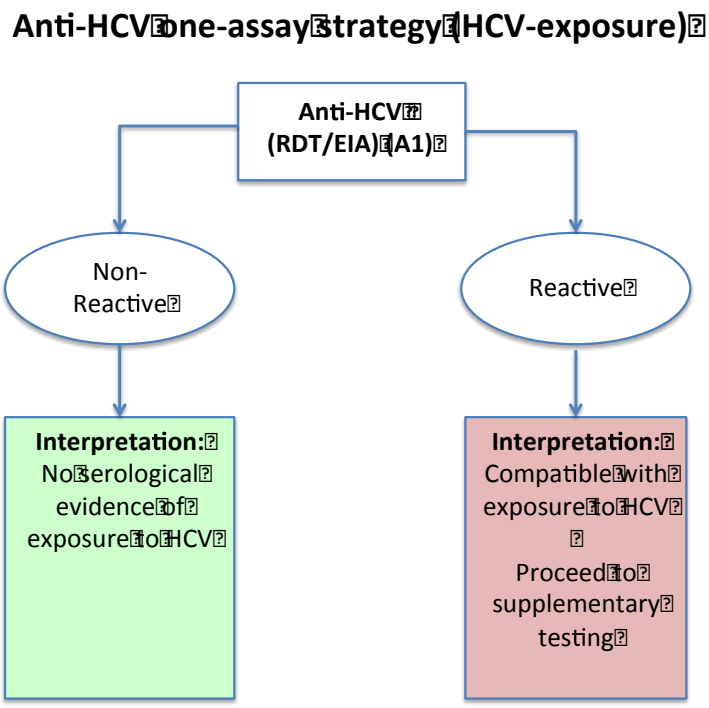


Fig. 1a. One-assay testing strategy for exposure to HCV (detection of anti-HCV)

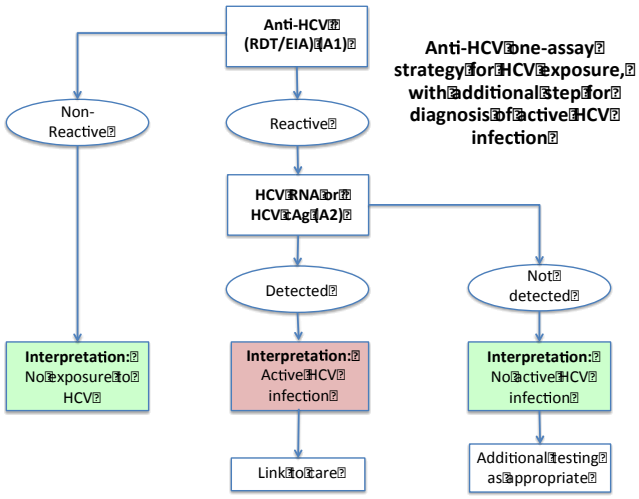


Fig. 1b. Two-assay testing strategy for diagnosis of HCV (detection of anti-HCV, followed by HCV RNA/core Ag)

3. Objectives

- To identify quantitative evidence on the sensitivity and specificity of one-test compared to two-test algorithms for detection of hepatitis C antibody

- To evaluate the cost–effectiveness, acceptability, and other outcomes (missed liver disease because of false-negative results, unnecessary referral, investigations) associated with these two types of testing strategies
- To inform models to optimize hepatitis C screening algorithms.

4. Methods

We reviewed observational studies and RCTs that provided original data from patient specimens. Our goal was to compare two broad strategies for hepatitis C antibody detection – one-test strategies and two-test strategies.

Search algorithm

Literature search strategies were developed by a medical librarian with expertise in systematic review searching. Our search algorithm consisted of the following components: hepatitis C, screening, and testing strategies (**Annex 1**).

We searched MEDLINE (OVID interface, 1946 onwards), EMBASE (OVID interface, 1947 onwards), the Cochrane Central Register of Controlled Trials (Wiley interface, current issue), Science Citation Index Expanded (Web of Science interface, 1970 onwards), Conference Proceedings Citation Index-Science (Web of Science interface, 1990 onwards), SCOPUS (1960 onwards), Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS) (BIREME interface) and WHO Global Index Medicus. The search was supplemented by searching for ongoing studies in WHO's International Clinical Trials Registry. The literature search was limited to the English language and human subjects.

We formulated a comprehensive and exhaustive search strategy in an attempt to identify all relevant studies. After the MEDLINE strategy was finalized, it was adapted to the syntax and subject headings of the other databases.

In addition to searching databases, we also searched the Internet for any peer-reviewed articles and conference abstracts that might have been missed through our librarian search and also expanded our search to national guidance documents.

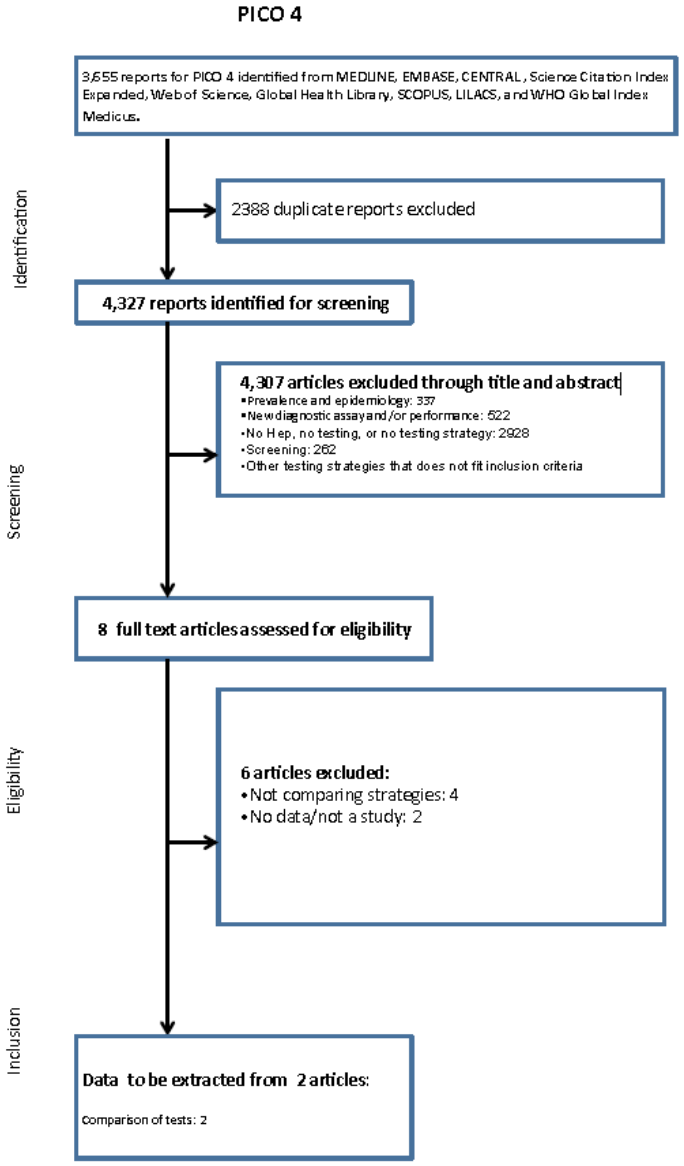
5. Results

Study selection

The librarian search resulted in 3060 references for PICO 4. Because of overlap with objectives and search strategies between PICOs 3 and 4, and to expedite the initial screening, PICO 4 references were combined with the 3655 references identified through the librarian search for PICO 3 (HBV) for a total of 6715 references. 2388 searches were immediately excluded: the librarian excluded 835 as not relevant and there were 1553 duplicates; 4327 remained for screening. Titles/abstracts were screened according to protocol inclusion and exclusion criteria, for both PICOs 3 and 4; 4307 reports were excluded. Reasons for excluding them were noted (Fig. 2).

From the librarian search, 5 reports were identified for possible data extraction. The Internet searches resulted in 3 additional reports for possible data extraction. Full documents (manuscripts, abstracts, guidelines, etc.) were obtained and assessed against inclusion criteria. Papers were either accepted or rejected and reasons for rejection were explained.

Fig. 2. PRISMA for PIC0 4 HCV (diagnostic strategies for hepatitis C antibody detection)



The following inclusion criteria were used to evaluate the final selection: evaluations of HCV testing strategies; evaluations based on human clinical materials. The following exclusion criteria were used: studies only focused on evaluation of single-test assays without a two-test comparator group; studies focused on two-test strategies that include other types of test (e.g. HCV RNA) studies with primary aims other than evaluation of testing strategies; studies related to disease prevalence, drug resistance, genotyping, sequencing, or non-diagnostic purposes; articles in languages other than English, conference abstracts.

Study characteristics

Of the 8 selected for possible data extraction, the following variables were collected, when available: first author, title, year, objective, and exclusion criteria (Table 2).

Table 2. Eight reports assessed for eligibility

	Author or source, year	**Title**	**Objective**	**Exclusion criteria**	**Conclusions**
1.	Cresswell. et al. 2014	Hepatitis C core antigen testing: a reliable, quick and potentially cost-effective alternative to hepatitis C polymerase chain reaction in diagnosing acute hepatitis C virus infection	To compare the utility of HCV core-antigen compared to qRT-PCR in the diagnosis on acute HCV in an HIV-positive cohort	No comparison of testing strategies	HCV core-antigen detection compared to HCV PCR is a quick, simple, cost–effective test in screening for acute HCV
2.	Krajden 2000	Hepatitis C virus diagnosis and testing	To identify how anti-HCV serology and NAT can be combined to provide a definitive answer as to whether or not an individual has been or is actively infected	No data/not a study	Report describes how anti-HCV serology and NAT can be combined to provide a definitive answer as to whether or not an individual has been or is actively infected
3.	Njouom 2006	A cost-effective algorithm for the diagnosis of hepatitis C virus infection and prediction of HCV viraemia in Cameroon	To describe the accuracy of an algorithm that combines two HCV rapid tests to diagnose and predict viraemia of HCV in Cameroon	No comparison of testing strategies	A comparison of 2 HCV rapid tests suggests an algorithm using the more sensitive test first to screen followed by the 2nd test to discriminate between viraemic and non-viraemic HCV seropositive subjects. Not relevant for this review as the second test is for HCV RNA

4.	Shivkumar 2012	Accuracy of rapid and point-of-care screening tests for hepatitis C: a systematic review and meta-analysis	To review evidence on the diagnostic performance of globally available RDTs and POCTs to screen for hepatitis C	No comparison of testing strategies	POCTs (blood) have highest accuracy, followed by RDTs (serum, plasma) and POCTs (oral fluids). RDTs and POCTs may be useful in expanding first-line screening for hepatitis C
5.	Tillmann 2014	Hepatitis C virus core antigen testing: role in diagnosis, disease monitoring and treatment	To review the current knowledge on 4 newer assays with decreased sensitivity, in different scenarios and reflect on their utility	No comparison of testing strategies	HCV core antigen has relative strong role in a diagnostic algorithm for HCV infection, while it is too insensitive in its present form to substitute for HCV RNA testing in the blood bank setting
6.	Barreto 2008	Cost–effective analysis of different algorithms for the diagnosis of hepatitis C virus infection	To compare diagnostic performance and cost–benefit of two new algorithms with the conventional one in Brazilian blood donors who showed positive or inconclusive anti-HCV results in screening tests	Study was performed using blood donors	Study evaluated and costed 3 algorithms (2 CDC algorithms and Brazilian). The more practical and economical algorithm requires the establishment of a specific level of signal-to-noise ratio to determine the need for reflex supplemental testing (i.e. immunoblot anti-HCV)
7.	Vermeersch 2008	Validation of a strategy for HCV antibody testing with two enzyme immunoassays in a routine clinical laboratory	To compare the performance of a strategy using AxSYM HCV 3.0 as screening test and Monolisa Plus anti-HCV version 2 as confirmation to AxSYM-pos sera with PCR and immunoblot	Comparison of testing strategies	Monolisa Plus can be used as an alternative to immunoblot for the confirmation of AxSYM-positive sera
8.	CDC MMWR 2013	Testing for HCV infection: an update of guidance for clinicians and laboratorians	To provide guidance to for clinicians and laboratorians on testing for HCV infection	No data/not a study	Update to CDC guidance for diagnosis of acute hepatitis C: rapid or a laboratory-conducted assay for HCV antibody, reactive followed by NAT for HCV RNA

References listed in **Annex 2.**

Of the 8 included reports, 6 described algorithms with the types of tests used (**Table 3**). Reports 1–4 were excluded from the systematic review as they did not compare testing

strategies. Two studies, Boretto et al. (2008) and Vermeersch et al. (2008), determined costs and effectiveness.

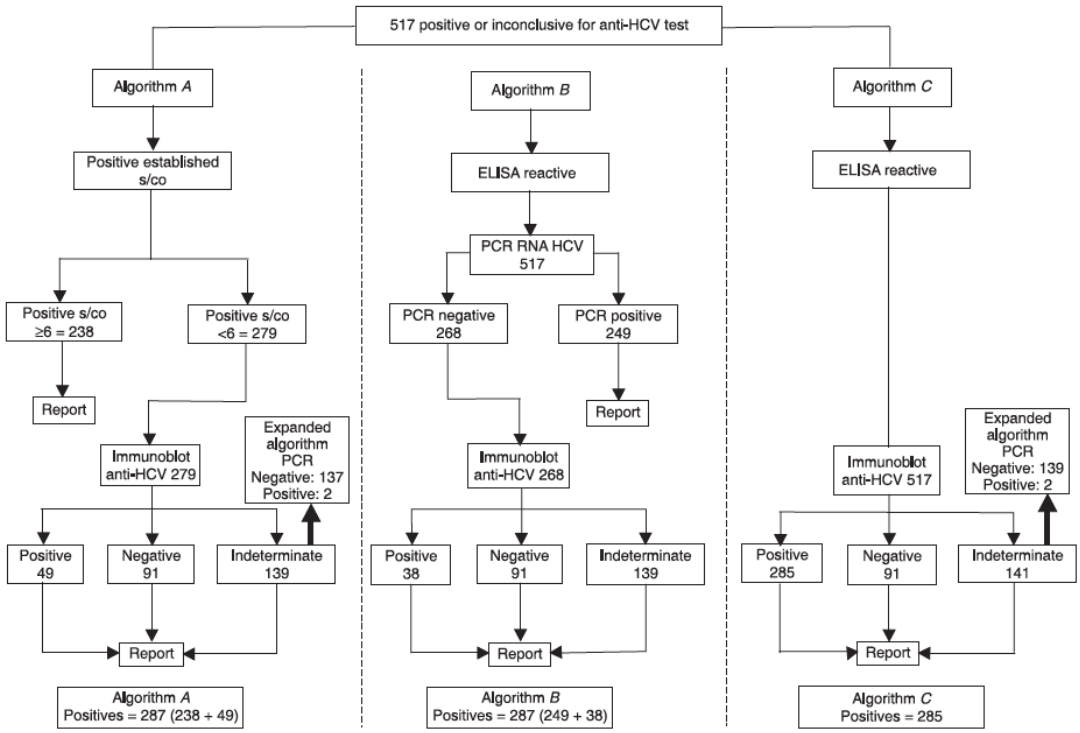
Table 3. Six reports of HCV testing algorithms

	Report	Test 1	Test 2	Test 3	Test 4	Exclusion Criteria
1	Cresswell 2014	HCV core-antigen	HCV RNA	HCV Ab		No comparison of testing strategies
2*	Njouom 2006	Anti-HCV EIA	HCV RNA PCR			No comparison of testing strategies
		Anti-HCV EIA	HCV RNA PCR	RT	RT	
3*	Tillmann 2014	Anti-HCV testing	RIBA	RNA PCR		No comparison of testing strategies
4*	CDC 2013	HCV antibody	HCV RNA			No data/not a study
5*	Barreto 2008	See schematic below (algorithm depends on a specific level of signal-to-cut-off ratio)				Study was performed using blood donors
6	Vermeersch	MEIA	Confirm by EIA			
		MEIA	Confirm by PCR	Confirm by immunoblot		

***** Algorithm schematics shown in **Annex 3.**

Although the study of **Barreto** et al. was conducted in a blood donor setting, the study did compare 3 testing strategies and determined cost–effectiveness. In this study the authors recognized that new anti-HCV tests have increased sensitivity but it means that there may be more false-positive results. These tests would be falsely negative in individuals who are newly infected as antibodies are absent or at low levels during this immunological window period. The use of a confirmatory diagnostic assay that targets different antigens can lower the risk of detecting false reactive results. Supplemental testing can be used to ensure a reliable diagnosis but this also means increased costs.

The authors compared 2 CDC algorithms to the national Brazilian algorithm to determine effectiveness and cost–benefit. The figure below depicts the testing of 517 individuals identified as ELISA-positive or inconclusive by anti-HCV test using 3 different algorithms. Algorithms A and B are the CDC recommended algorithms while Algorithm C is the national Brazilian algorithm.



The authors found that all three algorithms had similar diagnostic performance, revealing a remarkable agreement in the results obtained by the algorithms. As shown above, PCR was performed to resolve indeterminate results from immunoblots (139 samples from algorithm A and 141 samples from conventional algorithm C).

Algorithm A (CDC) was recommended for populations with a high prevalence of HCV infection. The algorithm showed high concordance with true-positive results. IB testing was required only for weakly reactive samples.

Algorithm B (CDC) used PCR to speed up clinical decision and was found more suitable for the immunosuppressed patient population for whom the IB test could represent a problem because of its low antibody level, leading to occasional false-negative results.

Algorithm C (Brazil) was found to be useful for determining the immune status of the patients against HCV infection and also for confirming the specificity of positive enzyme-linked immunoassay (ELISA) results. It is recommended for low prevalence populations for which false-positive antibody results are usually high. However, in the present study, this algorithm yielded a high frequency of IB-indeterminate results, producing no conclusive diagnosis. This algorithm also did not differentiate between active and past infections.

While algorithms A and B were found to be highly sensitive, the choice of an algorithm must take into account its purpose, the population and the prevalence of HCV infection, as well as the financial and infrastructure conditions of the laboratory. In the end they concluded that algorithm A is the best in terms of cost and feasibility, and particularly suitable for laboratories in resource-limited settings as it minimizes the number of samples requiring supplemental testing. Supplemental PCR tests were still required to detect active infection.

The **Vermeersch** study also investigated the CDC guidelines, specifically the required confirmation of HCV screening-test-positive sera with a low signal/cut-off (S/CO) ratio by

recombinant immunoblot or PCR. The UK Health Protection Agency suggested that a second EIA could be used as an alternative for confirmation in non-immunocompromised patients. A total of 17 936 consecutive in-house sera were evaluated in this study; AxSYM-positive sera were tested by Monolisa Plus and confirmed with IB (per CDC guidelines) or PCR.

This study specifically determined the performance of a strategy using AxSYM as screening test and Monolisa Plus as confirmation assay in a routine clinical laboratory and found that Monolisa Plus can be used as an alternative to immunoblot for the confirmation of AxSYM-positive sera in nonimmunocompromised. Although the study of Barreto et al. was conducted in a blood donor setting, the study did compare 3 testing strategies and determined cost–effectiveness.

Cost

Barreto et al. performed a cost–effective analysis of the two CDC-recommended algorithms compared to the current Brazilian national algorithm for the diagnosis of HCV infection. The cost of each algorithm depended on the number of supplemental tests required.

Algorithm A (CDC)	**Algorithm B (CDC)**	**Algorithm C (Brazil)**
Based on signal-to-cut-off (s/co) ratio of ELISA anti-HCV samples that show s/co ratio ≥95% concordance with immunoblot (IB) positivity. **US$ 21 299.39** This was determined to be the more practical and economical one since it requires supplemental tests for only 54% of the samples	Reflex nucleic acid amplification testing by PCR was required for ELISA-positive or -inconclusive samples and IB for PCR-negative samples **US$ 32 397.40** This one provided early information about the presence of viraemia	All positive or inconclusive ELISA samples were submitted to immunoblot **US$ 37 673.79**

Quality assessment

Study quality was not evaluated using the QUADAS-2 tool[12] and the STARD checklist, as these do not apply to the two studies.[13]

6. Discussion

Although none of the studies met inclusion criteria, eight references were identified that might be useful for modelling exercises to address this PICO question. This short narrative will provide an overview of these 8 articles, also drawing on other informative reviews and personal communications.

Antibody and antigen tests

In 2000, Krajden described strengths and weaknesses of serological and molecular tests for diagnosing hepatitis C. In general, serological tests detect antibodies to hepatitis C while molecular tests detect or quantify HCV RNA. This combination of an antibody test followed by a confirmatory NAT (RNA) has generally been accepted.

HCV antibody detection by enzyme immunotests (EIA) are simple, inexpensive, and often less time consuming, although they cannot distinguish between acute, active or chronic, non-viraemic HCV infection. In chronically infected persons, EIA sensitivity approaches 97–99% while in acutely infected individuals, EIA sensitivity is as low as 50–70%. The rapid antibody tests are typically more expensive and not designed for testing large batches of specimens. However, in non-clinical (field) settings and laboratories that conduct low-volume testing, adoption of rapid testing can be cost-effective.

Nucleic acid testing (NAT) remains the gold standard for identifying active infection (HCV RNA is detectable in serum or plasma as early as 1 week after exposure) but is costly, requires skilled technicians, extensive equipment and reagents, and a robust transport system to ensure sample integrity. The various forms of NAT testing include polymerase chain reaction (PCR), branched DNA signal amplification, and transcription-mediated amplification. NATs exhibit high specificities of up to 99% across all 6 genotypes of HCV.

Recently, HCV core antigen testing has become widely commercially available. Two of the eight papers selected for this narrative discussed HCV core antigen testing (Cresswell 2014; Tillmann 2014). Tillmann describes the use of core testing as a serological test capable of identifying active infection, and as a possible replacement for NAT as a confirmatory test. Overall, the core test is less sensitive than HCV RNA tests, but as Tillmann reports, more than 50% of anti-HCV positive persons will be HCV core antigen positive making core antigen testing a cost–effective reflex test to confirm infection, and can easily be applied on the same platform.

(Current HCV RNA assays have a lower level of detection between about 5–15 IU/mL. The sensitivity for the currently available HCV core antigen assay by Abbott was improved to about 3.00 fmol/L [0.0 6 pg/mL].)

Cresswell examined the efficacy and cost of HCV core antigen in diagnosing acute HCV in a high-risk, high-prevalence population (HIV-positive cohort of MSM). Compared to HCV NAT PCR, core antigen proved sensitive (100%), specific (97.9%), and cost–effective. In their cohort, they calculated cost per individual tests to be $108 for PCR versus $23.4 for HCV cAg. Their conclusion was that in high-risk, high-prevalence populations, the core test can be used as a quick, simple and cost–effective test in screening for acute HCV.

Other possible tests

Three other possibilities for testing were discussed in the literature and briefly mentioned here; recombinant immunoblot tests, signal-to-cut-off ratios and point-of-care tests (POCT) and antibody-based rapid diagnostic tests (RDT).

IBs are highly specific serological tests. They can be performed on the same sample used in the screening test; however, they are not amenable to routine use, as they do not have high sensitivity, are costly, with a testing procedure that is technically complex, and lengthy. Confirmation of active infection still requires testing for HCV RNA.

The CDC guidelines now include an option to use signal-to-cut-off ratios to limit the number of samples needing supplemental testing. Signal-to-cut-off ratios are test specific and slightly complicated to put in use and interpret. This approach might be better suited in a clinical laboratory setting (reference laboratory) that would use only one test, employ skilled technicians, and have a high volume throughput.

Shivkumar (2012) published a meta-analysis specifically on diagnostic accuracy of POCTs and RDTs to screen for hepatitis C. This analysis showed POCTs of blood (serum, plasma, or whole blood) have the highest accuracy, followed by RDTs of serum or plasma and then by POCTs of oral fluids. *More evidence is needed to consider using these newer tests in a diagnostic algorithm.*

Testing recommended for select populations

Many of the articles identified through the librarian search did not meet the inclusion criteria because they recommended HCV testing in select populations based on demography, prior exposures, high-risk behaviours, and medical conditions.

For example, one-time HCV testing is recommended for persons born between 1945 and 1965, without prior ascertainment of risk. Smith et al. (2012) note that the cost–effectiveness of one-time birth cohort testing is comparable to that of current risk-based screening strategies. Other major groups discussed under “risk behaviours” or “risk exposures” include injection drug use, children born to HCV-infected women, HIV infection. Because these studies were so specific to populations, they were not included as applicable to PICO 4.

Testing strategies

CDC MMWR (2013) describes CDC guidelines for HCV diagnostic testing: an anti-HCV test, and if the result is positive, active infection should be confirmed by a sensitive HCV RNA test. CDC recommends using US Food and Drug Administration (FDA)-approved tests (laboratory-based tests and POCT) such as OraQuick HCV rapid antibody test which has sensitivity and specificity similar to those of FDA-approved laboratory-based HCV antibody tests). An FDA-approved quantitative or qualitative NAT with a detection level of 25 IU/mL or lower should be used to detect HCV RNA.

Persons positive for an anti-HCV test and negative for HCV PCR RNA are informed that they do not have current (active) HCV infection, with no further testing necessary, unless there are ongoing risk factors for and suspicion of recent infection. In this case, repeat HCV RNA test is recommended.

To determine if the HCV antibody test represents a remote HCV infection that has resolved (true positivity) or a false-positive result (biological false positivity), CDC recommends

a second FDA-approved HCV antibody test that is different from the test used for initial antibody testing. A biological false result is not likely to occur with 2 different tests (Vermeersch 2008).

For patients with no apparent risk for HCV infection, the likelihood of a false-positive HCV antibody test is directly related to the HCV prevalence in the tested population; false-positive test results for anti-HCV are most common for populations with a low prevalence of HCV infection.

Njoum et al. (2006) performed a study in Cameroon comparing HCV rapid tests. In this study, using the more sensitive test first to screen followed by the second test to discriminate between viraemic and non-viraemic HCV seropositive proved to be a cost–effective algorithm for the diagnosis of HCV infection and prediction of HCV viraemia in Cameroon.

The two rapid tests evaluated were the ImmunoComb® II HCV assay and Hexagon® HCV assay. The ImmunoComb® II HCV test had a higher sensitivity than the Hexagon® HCV assay for detecting anti-HCV.

	ImmunoComb II	**Hexagon**	**Reference assay**
Sensitivity	99.4	64.0	HCV antibody detection
Specificity	89.9	100.0	
Sensitivity	100.0	87.7	HCV RNA detection
Specificity	2.1	93.6	

Their study did not actually report on cost but mentioned that EIAs are less expensive than PCR technology and in this case a second EIA can be substituted in the algorithm for the confirmatory PCR test.

7. Conclusions and recommendations for research

- Two studies compared the diagnostic accuracy, cost, cost–effectiveness of a one-test versus two-test strategies for detection of HCV antibody. One study found that in individuals who are HCV antibody positive, the use of an immunoblot assay with a defined signal-to-cut-off ratio can be used to distinguish between those who are viraemic and those who are not. This reduces the number of NATs required to confirm active infection is a cost–effective strategy.
- The challenge of using immunoblot assays is that they are lengthy and technically complex laboratory procedures, often leading to indeterminate results.
- Another study found that screening with a highly sensitive EIA followed by another EIA as confirmation assay in a routine clinical laboratory can be effective in non-immunocompromised populations. In immunocompromised patients, immunoblot is more effective as these patients tend to have low antibody levels.

References

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9. Duskova D, Darebnicek L. Nucleic acid testing of hepatitis B virus, hepatitis C virus, and human immunodeficiency virus 1, 2 in blood donors in the General University Hospital, Prague. Acta Virol. 2014;58(2):146–51.
10. Khuroo MS, Khuroo NS, Khuroo MS. Diagnostic accuracy of point-of-care tests for hepatitis C virus infection: a systematic review and meta-analysis. PloS One. 2015;10(3):e0121450.
11. Shivkumar S, Peeling R, Jafari Y, Joseph L, Pant Pai N. Accuracy of rapid and point-of-care screening tests for hepatitis C: a systematic review and meta-analysis. Ann Intern Med. 2012;157(8):558–66.
12. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011;155(8):529–36.
13. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. Ann Intern Med. 2003;138(1):W1–W12.

Appendices

Appendix 1. Librarian search

31. Hepatitis, Viral, Human/
32. Hepatitis Viruses/
33. Hepatitis Antibodies/
34. exp Hepadnaviridae Infections/
35. Hepatitis C Antibodies/
36. Hepatitis B virus/
37. Hepadnaviridae/
38. Hepatitis B Surface Antigens/
39. (heptatitis-b or hep-b or (hepatitis adj5 b) or (hep adj5 b) or hbv).ti,ab.
40. hbsag.ti,ab.
41. exp Hepatitis C/
42. Hepacivirus/
43. Hepatitis C Antibodies/
44. (heptatitis-c or hep-c or (hepatitis adj5 c) or (hep adj5 c) or hcv or aghcv or hepacivirus*).ti,ab.
45. hcvab.ti,ab.
46. or/1-15 [HEP B or HEP C]
47. exp Mass Screening/
48. screen*.ti,ab.
49. 17 or 18 [MASS SCREENING]
50. (one-test* or two-test*).ti,ab.
51. ("1-test*" or "2-test*").ti,ab.
52. ((one or two or "1" or "2" or strateg* or algorithm* or approach or procedure* or system*) adj5 (test or tests or testing or detect* or diagnos* or kit or kits or assay* or device*)).ti,ab.
53. or/20-22 [TESTING STRATEGIES]
54. 16 and 19 and 23
55. Humans/
56. Animals/
57. 25 and 26
58. 26 not 27
59. 24 not 28
60. limit 29 to english language

Appendix 2. Eight full-text articles assessed for eligibility (comparing algorithms, including costing)**.**

1. Barreto AM, Takei K, E CS, Bellesa MS, Salles NA, Barreto CC et al. Cost-effective analysis of different algorithms for the diagnosis of hepatitis C virus infection. Braz J Med Biol Res. 2008;41(2):126–34.
2. Centers for Disease C, Prevention. Testing for HCV infection: an update of guidance for clinicians and laboratorians. MMWR Morb Mortal Wkly Rep. 2013;62(18):362–5.
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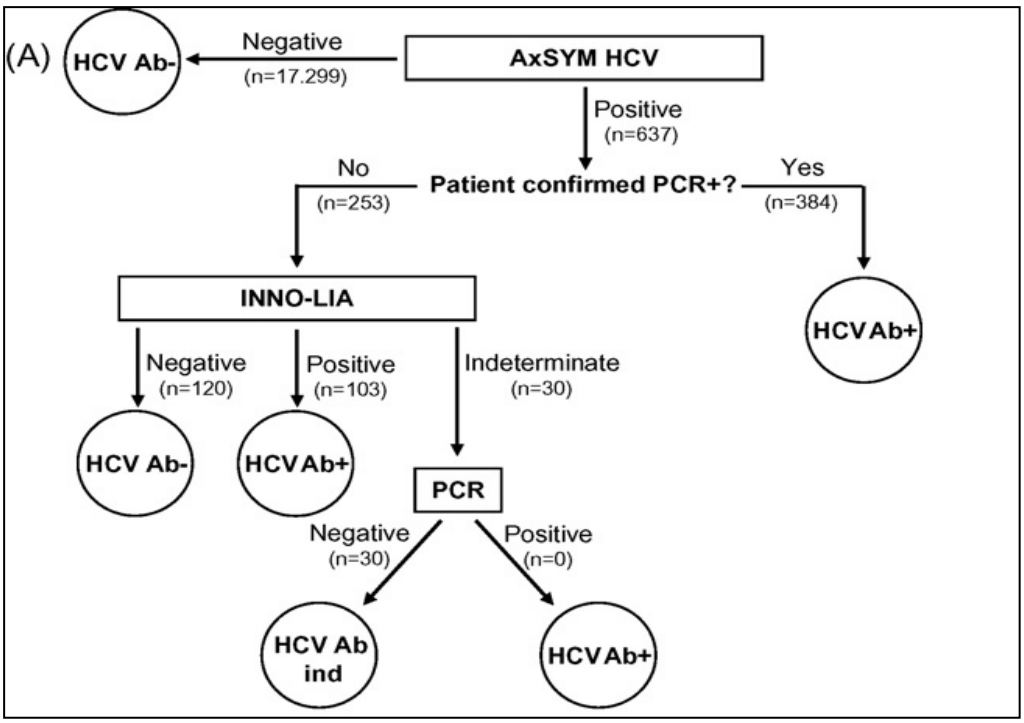
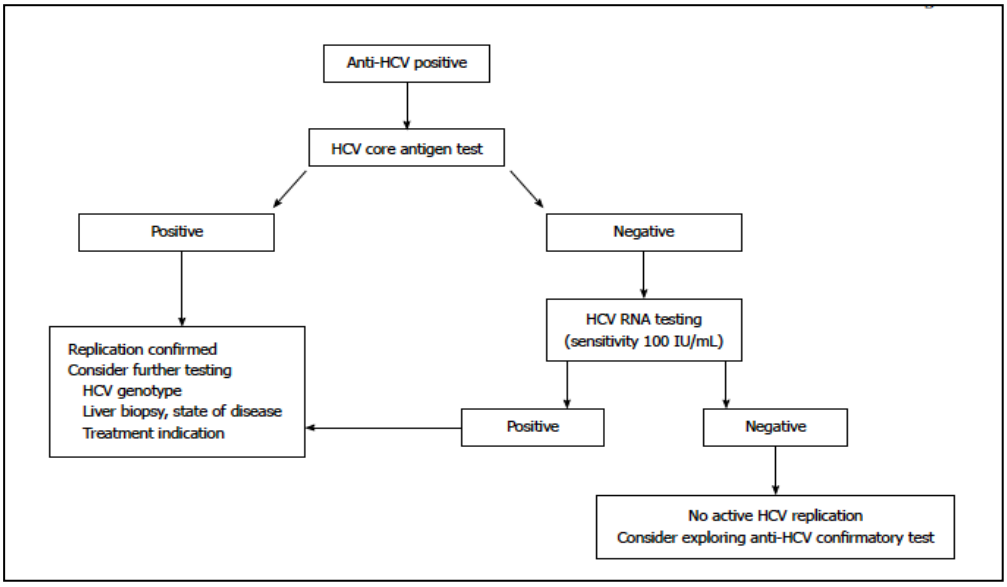
7. Tillmann HL. Hepatitis C virus core antigen testing: role in diagnosis, disease monitoring and treatment. World J Gastroenterol. 2014;20(22):6701–6.
8. Vermeersch P, Van Ranst M, Lagrou K. Validation of a strategy for HCV antibody testing with two enzyme immunoassays in a routine clinical laboratory. J Clin Virol. 2008;42(4):394–8.

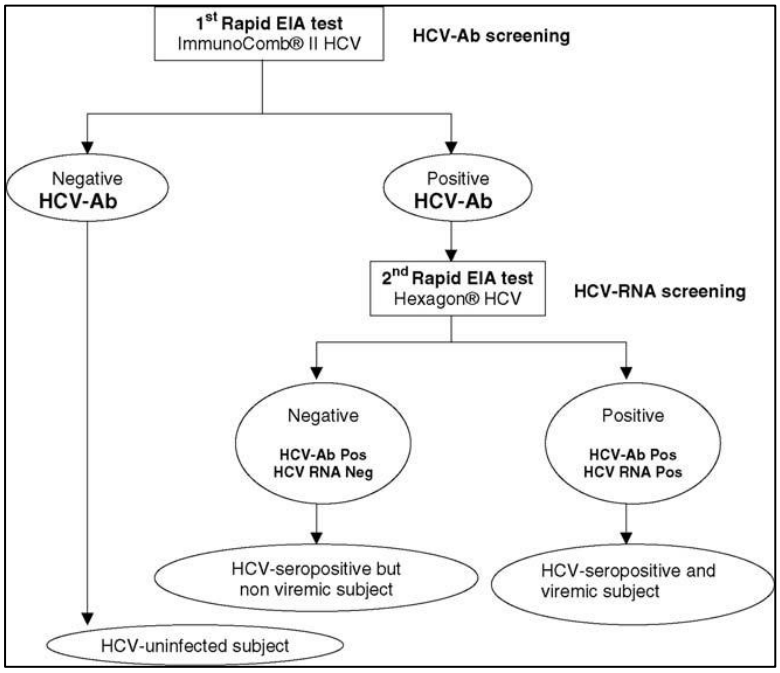
Appendix 3. Testing schematics



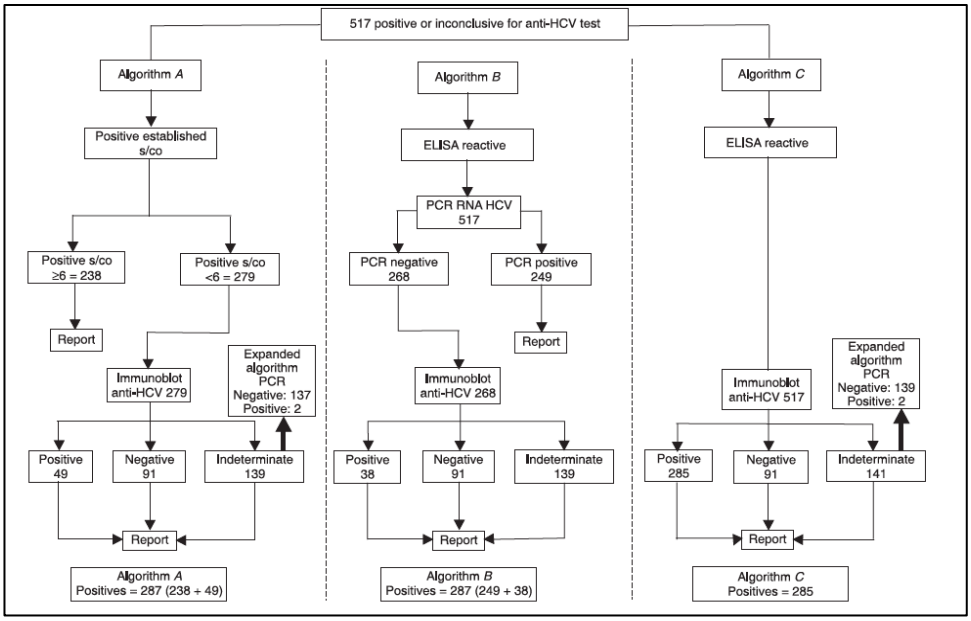
Vermeersch et al. 2008



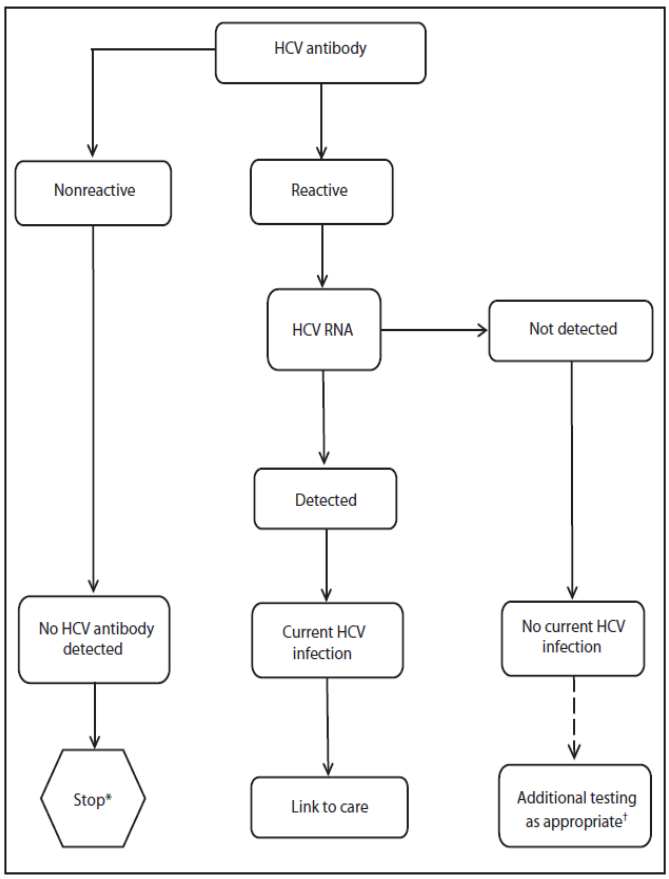
Tillman et al 2014



Njoum et al. 2006



Baretto et al. 2008



CDC MMWR 2013

Annex 5.7

PICO 6 - How to test (confirmation of HCV viraemia)

Diagnostic accuracy of HCV RNA tests to detect active HCV infection: a meta-analysis and review of the literature

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*Co-leaders of this review

September 2015

1. Executive summary

Background: Advances in hepatitis C virus detection technology create new opportunities for enhancing screening, referral and treatment. The purpose of this review was to determine the accuracy of qualitative NAT methods versus quantitative NAT methods for HCV RNA for detection and/or quantification to confirm active HCV infection.

Method: A literature search was conducted focused on hepatitis C, diagnostic tests and diagnostic accuracy. Studies were included if they evaluated an assay to determine the sensitivity and specificity of a single qualitative hepatitis C RNA test compared to a quantitative HCV RNA reference among humans. Two reviewers performed a quality assessment of the studies and extracted data for estimating test accuracy.

Results: Traditionally, qualitative nucleic acid amplification (NAT) assays are at least 10 times more sensitive than quantitative assays. This systematic review shows that for HCV, the lower limit of detection of most commercial qualitative assays was in the 10–15 IU/mL range measured against a WHO standard, whereas the lower limit of detection for quantitative assays is at 600–1100 IU/mL. This systematic review shows that the sensitivity of HCV viral quantitative assays range from 87% to 100% compared to qualitative assays.

Conclusions: Although HCV qualitative assays have a lower limit of detection than quantitative assays, the range of sensitivity found in this systematic review demonstrate that HCV viral loads are rarely in the lower range of the limit of detection of these quantitative assays. New technology platforms are now available which have linear range of quantitation between 12 and 10^8 IU/mL, with the result that there is no longer any difference between the lower limit of detection of a qualitative assay compared to a quantitative assay. New point-of-care (POC) devices for quantitation of HCV viral load will soon be available. These devices are more affordable than the laboratory-based assays and can potentially be used to improve access to HCV detection and treatment monitoring.

2. Background

Hepatitis C is a liver disease caused by the hepatitis C virus (HCV) that causes acute and chronic infection.[1,2] An estimated 130–150 million people have chronic hepatitis C infection worldwide, leading to 350 000–500 000 deaths per year.[1–3] Although HCV treatment is successful in a majority of people, most HCV-infected individuals remain undiagnosed and untreated.[4] As a result, approximately 15–30% of individuals with chronic HCV infection progress to cirrhosis, leading to end-stage liver disease and hepatocellular carcinoma.[1, 2]

Rapid detection of HCV is essential for prevention of the progression of the disease into the chronic phase. Qualitative nucleic acid testing (NAT) allows for a rapid and sensitive detection of the virus as well as evidence of viral RNA load falling below a clinical threshold.[4] Quantitative testing is useful for measuring of viral burden and treatment response.[5] Both methods are essential in the detection of active HCV infection, though there is scare research comparing the two NAT methods for this purpose.

In April 2014, the World Health Organization (WHO) published guidelines for the

screening, care and treatment of individuals with HCV infection.[6] These guidelines included recommendations on who to screen for HCV and how to confirm HCV infection, but not which tests are optimal for initial screening. The World Health Assembly has passed several resolutions highlighting the importance of viral hepatitis for global health.

Advances in HCV detection technology create new opportunities for enhancing screening, referral and treatment. Previous systematic reviews on hepatitis C infection have focused on treatment response,[7,8] clinical complications,[9] and epidemiology.[10,11] Two systematic reviews on hepatitis C testing focused on evaluating point-of-care tests compared to EIAs and other reference tests.[12,13] This review instead focuses on individuals with detectable HCV antibodies to evaluate qualitative versus quantitative detection methods to confirm active HCV infection.

The purpose of this review was to identify evidence on the sensitivity and specificity of qualitative HCV RNA tests compared to quantitative HCV RNA tests for the detection of active HCV infection, to summarize the key test characteristics associated with detection of active HCV infection.

PICO 6	Among HCV Ab positive patients, what is diagnostic test accuracy of qualitative NAT methods versus quantitative NAT methods for HCV RNA for detection and/or quantification to confirm active HCV infection?
P	Persons with detectable HCV antibodies
I	Qualitative NAT methods
C	Quantitative NAT methods
O	Diagnostic accuracy: True negatives (TN), who are screen negative and do not have HCV infection. False negatives (FN), who are screen negative but have HCV infection, These are incorrectly misclassified and this may results in missed opportunity to recognise and present progression of liver disease. True positives (TP), who are screen positive and have HCV infection. False positives (FP), who are screen positive, but do not truly have HCV infection. These will have additional unnecessary tests and evaluation. Costs (Cost of testing strategy including lab reagents and running costs, cost of further evaluation of a false positive) Cost–effectiveness Acceptability to health-care worker and patients

3. Objectives

The purpose of this review was to identify evidence on the sensitivity and specificity of qualitative HCV RNA tests compared to quantitative HCV RNA tests for the detection of active HCV infection and to summarize the key test characteristics associated with detection of active HCV infection.

4. Methodology

We followed standard guidelines and methods for systematic review and meta-analyses of diagnostic tests.[14,15] We prepared a protocol for the literature search, article selection, data extraction and assessment of methodological quality.

Selection criteria

Types of studies

We included observational and RCT studies that provide original data from patient specimens, including cross-sectional and case–control studies and studied qualitative NAT tests used to detect HCV RNA compared to a reference standard of quantitative HCV RNA tests.

Participants

Little information on participants was provided in the selection of papers included in the systematic review; therefore, we set a wide inclusion criterion. We included patients of all age groups from all settings and countries as well as all types of specimens.

Index tests

Studies that utilized a commercially available HCV NAT test were eligible for inclusion. The following seven are the index tests included:

- AMPLICOR HCV test, version 2.0, Roche
- CAP/CTM, Roche
- COBAS AMPLICORTM HCV Test v1.0 assay, Roche
- COBAS AMPLICORTM HCV Test v2.0 assay, Roche
- COBAS HCM-2, Roche
- Real-Time Assay, Abbott
- Versant HCV genotype assay, Bayer.

Reference standard

The reference standards accepted for a definitive diagnosis included tests for detection of HCV RNA by the following quantitative NAT techniques: polymerase chain reaction (PCR), branched-chain DNA (bDNA), or transcription mediated amplification (TMA). The performance characteristics of NATs are very similar above 50 IU/mL; thus all NATs were considered as one reference standard.

Outcome measures

Sensitivity refers to the proportion of samples with true HCV infection diagnosed with positive qualitative NAT tests confirmed with a positive quantitative NAT tests.

Specificity refers to the proportion of samples with negative qualitative NAT tests confirmed with a negative quantitative NAT tests.

Search methods

A database search of LILACS, MEDLINE, EMBASE, PubMed, Scopus, Web of Science, Cochrane and WHO Global Index Medicus was performed through April 2015. No language restriction was applied. The references of published articles found in the above databases were searched for additional pertinent materials.

Study selection proceeded in three stages. First, titles/abstracts were screened by a single reviewer according to standard inclusion and exclusion criteria. Second, full manuscripts were obtained and assessed against inclusion criteria. Papers were accepted or rejected and reasons for rejection were specified. Third, two independent reviewers assessed each manuscript and differences were resolved by a third independent reviewer.

Data extraction

Information on the following variables were extracted by a reviewer if the study met the exclusion and inclusion criteria: first author, total sample size, country (and city) of sampling, sample type (oral fluid, finger-prick, venous blood, etc.), point-of-care (Y/N), eligibility criteria, reference standard, manufacturer, raw cell numbers (true positives, false negatives, false positives, true negatives), sources of funding and reported conflict of interest. We define point-of-care as being able to give a result within 60 min and having the results guide clinical management at the same encounter.

Assessment of methodological quality

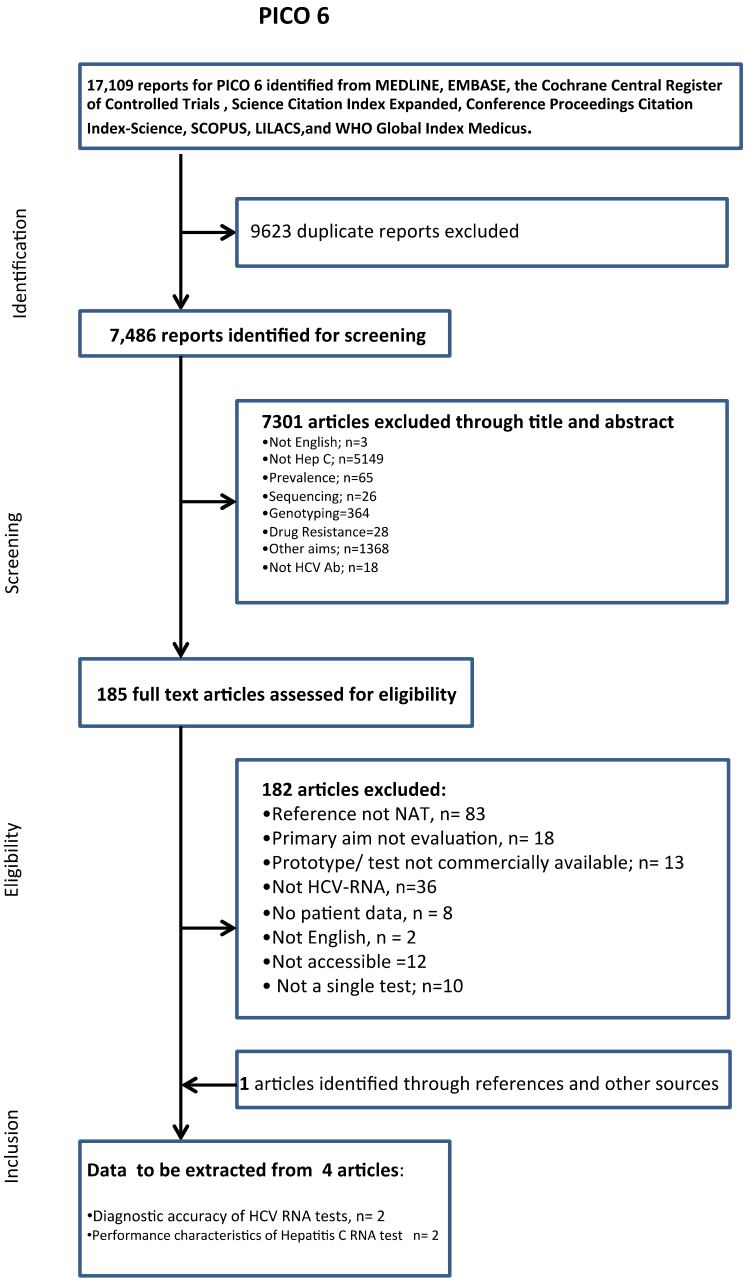
Study quality was evaluated using the QUADAS-2 tool,[14] the STARD checklist[15] and the GRADE method.[16] QUADAS includes domains to evaluate bias in the following categories: risk of bias (patient selection, index test, reference standard, flow and timing); applicability concerns (patient selection, index test, reference standard). The GRADE method evaluates the strength of evidence by assessing the risk and probability of bias, imprecision and inconsistency as well as dose-respondent gradient and residual confounding.[16]

5. Results

PRISMA flowchart

A total of 17 109 citations were identified and 9623 duplicates were removed. Each of the 7486 titles was examined according to pre-specified inclusion and exclusion criteria. A total of 4 research studies were included in the final analysis (Fig. 1 below).

Fig. 1. PRISMA flow diagram outlining study selection examining diagnostic accuracy HCV RNA tests to detect active HCV infection



Characteristics of included studies

A total of four studies met the PICO criteria and data was extracted from each of these studies. Two of the four studies took place in the United States of America, with the remaining two in Taiwan and Germany. Of these studies only one included a population of patients at risk of HCV infection, while the others were either patients who have an acute or chronic HCV infection. The assays evaluated in these analyses were Abbott Real-Time Assay, AMPLICOR

HCV test, v2.0 assay, COBAS AMPLICORTM HCV Test v2.0 assay and Versant HCV genotype assay.

This systematic review shows that for HCV, the lower limit of detection of most commercial qualitative assays was in the 10–15 IU/mL range measured against a WHO standard, whereas the lower limit of detection for quantitative assays is at 600–1100 IU/mL. The sensitivities of qualitative NAT methods reported in the selected articles showed a relatively wide range (87–100%), contrary to the narrow range reported for specificity (97–100%).

A large limitation in the quality of the studies was a lack of information on the populations studied, randomization and sample collection as well as poor standardization in the evaluation of diagnostic test accuracy.

Table 1. Description of study design, study population and setting of all studies (*n*=4)

No	First author, country	Sample type and number	Study population	Diagnostic test (quantitative)	Reference test (qualitative)	Sensitivity	Specificity
1	Lee, 2000, United States of America	Serum *N* = Not stated	Patients at risk of HCV infection	AMPLICOR HCV test, version 2.0 *Roche*	COBAS AMPLICORTM HCV Test v2.0 assay. *Roche*	94%	97%
2	Yu 2000, Taiwan	Serum *N* = 215	Patients with chronic hepatitis C	COBAS HCM-2 Roche	COBAS AMPLICORTM HCV Test v2.0 assay. *Roche*	95%	–
3	Ferreira-Gonzalez, 2007, United States of America	Plasma *N* = 76	Patients with HCV infection	Versant HCV genotype assay, *Bayer*	COBAS AMPLICORTM HCV Test v1.0 assay *Roche*	100%	100%
4	Sarrazin, 2008, Germany	Serum *N* = 65	Patients with HCV infection	Versant HCV genotype assay. *Bayer* CAP/CTM* *Roche*	Abbott Real-time Assay	87%	–

*CAP/CTM= Roche Cobas Ampliprep/Cobas Taqman HCV assay

Lee et al. (2000) investigated the performance characteristics of AMPLICOR HCV test, version 2.0 Roche (quantitative assay) and COBAS AMPLICORTM HCV Test v2.0 assay Roche (qualitative assay). This was done by measuring the limit of detection, sensitivity, specificity, linear range, agreement between test formats and genotypic reactivity for both tests. The genotypic reactivity for both tests showed that samples with 10 copies per reaction yielded positive results at least 95% of the time for all genotypes with the exception of genotype 5. When testing for the agreement between test formats, it was shown that the COBAS AMPLICOR v.2 assay format produced values with a range of 0.02–0.13 $\log_{10}$ higher than those obtained for the AMPLICOR assay with samples containing titres within the linear range of the assays.

Yu et al. (2000) investigated the performance characteristics of COBAS HCM-2 (quantitative assay) compared to the COBAS AMPLICORTM HCV Test v2.0 assay (qualitative) and evaluated the clinical utility of COBAS HCM-2. This study looked at quantitative range, reproducibility of COBAS HCM-2 and linearity of HCV RNA quantifications. The quantitative range for the COBAS HCM-2 assay from 1.0 X 10^3 to 3.88 X 10^6 copies/mL and the within-run reproducibility showed serum HCV RNA levels with standard deviations of 0.03, 0.09 and 0.12. The linearity of HCV RNA quantifications ranged from 6.11, 6.44, 6.46 to 6.49 logs for genotypes 1b, 2a, 2b and 1b.

Ferreira-Gonzalez et al. (2007) evaluated the qualitative (COBAS Taqman HCV) against the quantitative (Versant HCV genotype assay, Bayer) using the same clinical specimens. Analytical sensitivity was measured by the ability of a system to detect replicates, both tests were capable of detecting all six replicates with 10 HCV RNA IU/mL and 100% of all replicates with 1.0 Log_{10} HCV RNA IU/mL.

Sarrazin et al. (2008) focuses on the evaluation and comparison of performance characteristics of HCV qualitative (CAP/CTM Roche Cobas Ampliprep/Cobas Taqman HCV assay, Roche) and quantification (Versant HCV genotype assay, Bayer) methods. The study compared intra-assay variability, analytic sensitivity, limit of detection, HCV WHO standard RNA unitage, genotypic specific assay linearity. The intra-assay variability of the two assays varied from 0.72% to 1.3% for CAP/CTM assay and 1.4–3.02% for Versant HCV genotype assay. For sensitivity, the positive hit rates for WHO HCV RNA standards for the Abbott RealTime HCV assay were 87% at 15 IU/mL, where as for the CAP/CTM they were 100% at 15 IU/mL. The limit of detection was higher for RealTime HCV assay at 16.8 (95% CI; 13.1 to 27.9) than for CAP/CTM 10.3 (95% CI; 8.4 to 15.1). HCV WHO standard RNA unitage deviated between –0.2 log_{10} IU/mL at 3.2 Log_{10} for RealTime HCV assay and –0.3 log_{10} IU/mL at 3.2 Log_{10}. For RealTime HCV assay and CAP/CTM assay, the quantification of HCV RNA of five different HCV genotypes was mostly linear between concentrations of 4.0 X 10^3 and 1.0 X 10^6 IU/mL though the sample harbouring genotype 4 showed lower results than expected at concentrations above 1.0 X 10^4 UI/mL.

Narrative summary of each systematic review's findings

Traditionally, qualitative nucleic acid amplification (NAT) assays are at least 10 times more sensitive than quantitative assays. This systematic review shows that for HCV, the lower limit of detection of most commercial qualitative assays was in the 10–15 IU/mL range measured against a WHO standard, whereas the lower limit of detection for quantitative assays is at 600–1100 IU/mL.

Though very sensitive for determining the presence of virus, qualitative assays do not allow for determination of viral load. The sensitivity of qualitative assays makes them essential during screening blood donors and monitoring treatment progression as they are used to show the presence of virus as a marker of an on-going HCV infection. [17–20] This systematic review showed sensitivities as low as 5 IU/mL for HCV NAATs. Verification of the presence of RNA is complicated due to the lack of a standardized commercial HCV assay with sufficient sensitivity that is capable of testing discrepant specimens at such low concentrations.[21] This is

particularly important when qualitative assays are used prove the absence of HCV-RNA at the end of treatment (ETR) or at the end of follow up (EFU).[22] Another use of qualitative assays is to discriminates sustained responders (SRs) from relapsers (RELs).[23–25]

Despite the lower sensitivity, quantitative assays have been found to be a reproducible method to detect and quantify HCV RNA in plasma or serum.[25] This systematic review also highlighted potential issues with accuracy as the level of imprecision in a number of quantitative tests (COBAS) AMPLICOR HCV Monitor assay) was seen to be between a factor of 3–5 (0.5–0.7 $\log_{10}$) difference from the actual titre.[26] These assays have been proven to be crucial in the measurement of the viral load at the start of therapy and after 12 weeks of treatment to decide about the usefulness of further treatment (stopping rule). These assays have also been proven to have a broad dynamic range of 615–7 700 000 IU/mL ([COBAS] AMPLICOR HCV Monitor assay, Roche). The ability of a quantitative tests to detect viral loads as low as 650 IU/mL allow them to measure early response to treatment as often viral loads drop rapidly at the start of interferon treatment. Viral loads seldom exceed the upper limit of this assay so that retesting of diluted samples is often unnecessary.[27–28] This explains the reasoning behind laboratories in high-income countries employ HCV RNA quantitative assays in serum or plasma, notwithstanding its higher cost, as it can be used to monitor treatment efficacy and chronic HCV disease progression.[29–32]

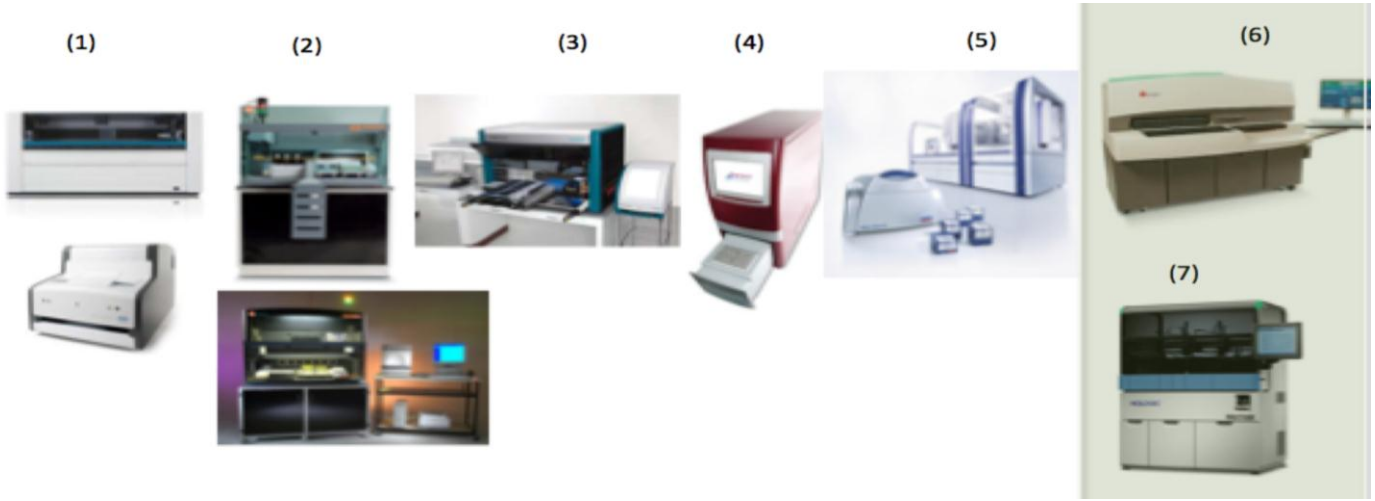
Since these studies were published, companies have been steadily improving the sensitivity of quantitative assays. Table 1 shows that currently 5 HCV quantitative assays are commercially available with another two in the pipeline (UNITAID Hepatitis C diagnostic technology landscape report, 2015).[39] These assays have linear range of quantitation of 12–10^8 IU/mL using plasma or serum and the time to result ranges from 70 min to 5–6 h. With these new technology platforms, there is no longer any difference between the lower limit of detection of a qualitative assay compared to a quantitative assay. From this systematic review, the finding of sensitivities of 87–100% for a quantitative assay compared to qualitative assays demonstrates that viral loads are rarely in the lower range of the limit of detection of these older quantitative assays.

These new quantitative assays and the equipment are costly and can be prohibitive to control programmes. However, since other assays such as HIV viral load can be performed on these platforms, HCV control programmes can leverage investments made by HIV programmes for the procurement of these technologies. In resource-limited settings where these assays are not affordable, HCV antigen detection can be considered as a surrogate marker of ongoing virus replication.

Investments in the development of point-of-care (POC) devices that can be used to measure HIV viral load have yielded several technological platforms that can be used to quantitate HCV viral load. Table 2 shows that four companies have developed HCV quantitative assays that would soon be available with another three in the pipeline. These devices cost much less than the laboratory based instruments and can be used outside of laboratory settings as some of them can run on batteries. They are sample in-answer out type of technologies that will require minimal training and all have connectivity capacity so that surveillance for hepatitis C can be automated. The evaluation of these POC platforms will be

important to inform countries of whether these devices can be used to improve access to HCV detection and treatment monitoring.

Table 2. Qualitative/quantitative HCV RNA platforms currently available (1–5) and soon to be available (6–7)



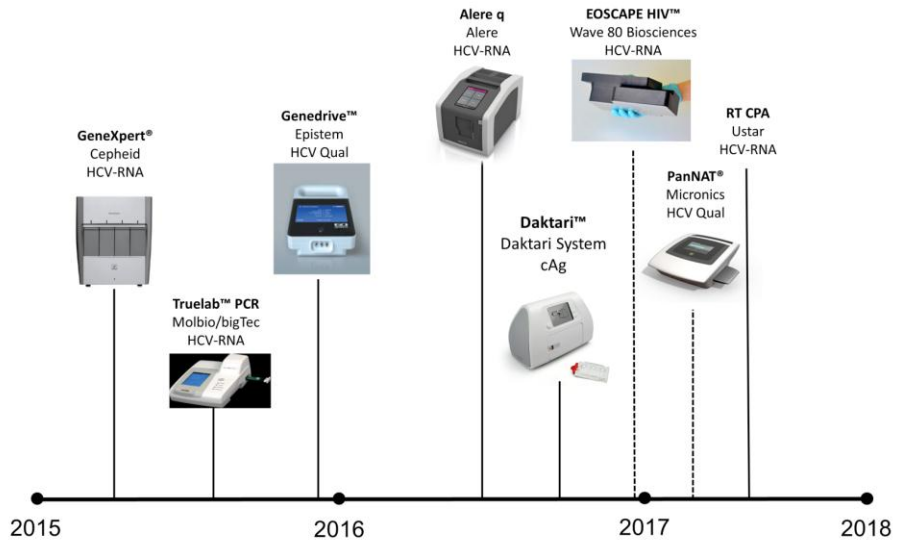
	Roche Molecular Systems (1)	Abbott Diagnostics (2)	Siemens Healthcare Diagnostics (3)	Sacace Biotechnologies (4)	QIAGEN (5)	Beckman Coulter (6)	Hologic Inc (7)
Qualitative assays	COBAS AmpliPrep /COBAS TaqMan HCV Qualitative Test v.2 (LLOD: 15 IU/mL)	–	–	–	–	–	–
Quantitative assays	COBAS AmpliPrep /COBAS TaqMan HCV Quantitative Test v.2	Abbott RealTime HCV Assay	VERSANT kPCR HCV RNA Assay	HCV Real-TM Quant Dx Assay	artus HCV QS-RCQ Kit	VERIS MDx	RT-TMA Technology for the Panther® System
Linear range of quantitation, IU/mL	**15–10^8**	**12–10^8**	**15–10^8**	**13–10^8**	**35–1.77x10^6**	–	–
Sample type (mL)	**0.650 mL plasma/ serum**	**0.5 mL plasma/serum**	**0.5 mL plasma or serum**	**1 mL plasma**	**1 mL plasma**	**plasma, serum**	**0.24 mL plasma**
Cost/test (US$)	36–38/43–51	13–35	72–100	>20	16–45	–	10–15
Price of instrument (US$)	COBAS AmpliPrep: 80 000–100 000 COBAS Taqman 48:	248 000 (45 000 + 162 000 + 80 000)	Pricing for the assay and instrument is available from Siemens	113000 (95 000+18 645)	Available from company	–	–

	40–50 000						
# Specimen/run and Time to result	24 specimens in 2 h, can process up to 72 samples at one time	96 samples at a time in about 3 h	89 samples per run with a total time to result of <6 h	24 samples/run in 5–6 h	Continuous loading in batches of up to 24 samples plus internal controls	48 samples can be lined up on 12 racks; DNA tests takes ~70 min and RNA tests ~110 min	First results available **3** h after loading samples and five results after every 5 min thereafter. Samples can be continuously loaded, with up to 120 samples on the Panther® System

	Alere	**Molbio Diagnostics Pvt Ltd**	**Cepheid**	**Ustar Biotechnologies**
Quantitative Assays	Alere q HCV VL	Truelab Real Time micro PCR System	GeneXpert HCV quantitative assay	RT-CPA HCV Viral Load Test
Linear range of quantitation, IU/mL	–	–	10^1–10^8	10^4–10^6
Sample type (mL)	0.5–1 plasma	0.1 plasma	1 plasma/serum	0.1 blood
Cost/test (US$)	15–25 –	14 per chip; 2 per extraction	<US$ 20	–
Price of instrument (US$)	–	8000	17 000	–
Time to result (min)	<60	60	105	20–45 (<500 IU/mL)
Other tests on platform	HIV, Ebola	MTB, HBV, dengue, chickungunya, HINI, malaria, HIV VL, HCV VL	HIV	–

Table 3. Point-of-care HCV RNA platforms in the pipeline

Hepatitis C virus point-of-care diagnosis and treatment monitoring platforms: pipeline*



*Estimated as of September 2014 - timeline and sequence may change. ---- No market launch date set by company.

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Annex 5.8

PICO 5a and PICO 9 - How to test (confirmation of HCV viraemia)

HCV core antigen testing for presence of active HCV infection and monitoring for treatment response and cure: a systematic review

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2015

Abstract

Background: Chronic hepatitis C virus (HCV) infection with viraemia is prevalent in approximately 1.1% of the world population.[1] Current diagnosis of active infection requires a positive HCV antibody (Ab) as well as nucleic acid testing (NAT) to detect HCV ribonucleic acid (RNA) indicative of active replication. HCV core antigen (HCVcAg) testing was developed as an alternative to NAT. This systematic review aims to summarize (1) the diagnostic accuracy of HCVcAg testing in those with and without positive HCV Ab (PICO 5a), (2) inform the best testing strategy for identification of active HCV infection (PICO 5b), and (3) examine the utility of HCVcAg monitoring for those on HCV treatment (PICO 9).

Methods: We performed a literature search in multiple databases for all published and peer reviewed literature without language restriction through March 2015. Studies were included if a commercially available HCV Core Ag test result was compared with NAT in at least 10 independent clinically collected samples. We contacted authors for missing data to complete extraction. We assessed the quality of studies using an adapted QUADAS-2 tool. Data were classified by HCV Core Ag test manufacturer. For PICO 5a, bivariate meta-analyses were performed for the Abbott ARCHITECT, Hunan Jynda, and Ortho ELISA to obtain pooled sensitivity (Se) and specificities (Sp) with 95% confidence intervals (CI). Due to limited number of studies and specificity data descriptive statistics were derived for the Murex EIA, Bio-RAD Monolisa, EIKEN Lumispot and Fujirebio Lumipulse. We assessed non-parametric regression of quantitative data and identified outliers. Due to the absence of published studies to inform PICO 5b, a decision analysis was performed and is reported separately. Only a descriptive analysis was possible on the use of HCV core Ag in treatment monitoring and assessment of SVR (PICO 9).

Results: We identified 50 published studies for inclusion in the analysis of PICO 5a, 1 study relevant to PICO 5b, and 5 studies relevant to PICO 9. For PICO 5a, 7 index tests were included with 30 studies utilizing Abbott ARCHITECT, 5 studies for Bio-RAD Monolisa, 4 for Murex Ag/Ab EIA, 6 for Ortho ELISA-Ag, 2 for EIKEN Lumispot HCV Ag, 1 for Ortho Lumipulse-Ag, and 4 for Hunan Jynda Bioengineering Group HCV Core Ag ELISA. Among these, 1 directly compared the ARCHITECT with the Lumipulse and Lumispot, and 1 compared the Monolisa with the Murex. From bivariate analyses, the pooled sensitivity and specificity with 95% CI were: ARCHITECT 93.4% (88.7, 96.2) and 98.7% (96.9, 99.4), Ortho ELISA 93.2% (81.6, 97.7) and 99.2% (87.9, 100), and Hunan Jynda 59.5% (46% 71.7) and 82.9% (58.6, 94.3). The sensitivity for the Lumipulse was 95% (90.2, 99.8) in one study; specificities could not be calculated. Three studies using the ARCHITECT provided quantitative data. The few points with negative HCVcAg were shown to occur at RNA levels below 3000 IU/mL where loss of linearity was also noted in pooled non-parametric regression. Accuracy of HCVcAg for treatment monitoring and as a test of cure was assessed by descriptive analysis in 5 studies (PICO 9). The sensitivity of ARCHITECT in EVR ranged from 74–100% with specificity from 70% to 100%. SVR was only assessed in 2 studies with 100% sensitivity and specificity ranging from 94% to 100%. Data on accuracy in prediction of SVR were limited and assessed in only 3 small studies.

Conclusions: HCV core antigen assays can have high sensitivity (up to 93.4% for Abbott ARCHITECT HCVcAg test), high specificity, and good correlation with HCV RNA to a detection limit of roughly 3000 IU/mL. The data on core antigen for treatment monitoring and as a test of cure is too limited to reach reliable conclusions.

GRADE summary tables

I. PICO 5a: What is the best strategy (diagnostic accuracy and other outcomes); comparing HCV core Ag test versus NAT for HCV RNA for detection (and/or) quantification to confirm active HCV infection?

SR Outcome: Diagnostic accuracy, sensitivity and specificity

Patients/population: Persons with detectable HCV RNA with or without positive HCV antibody

Setting: Any

Index tests: HCV core antigen assay

Importance: Inform best strategy for HCV diagnosis in a variety of clinical settings and economies

Reference standard: HCV RNA testing

Studies: Cohort, cross-sectional, or randomized controlled trials that use HCV NAT as gold standard reference test compared with a commercially available HCV core Ag index test

A) Strength of evidence

SR outcome: diagnostic accuracy				**Quality**				**Strength of Evidence**
Index test	**Outcome Measure**	**# Studies (# samples)**	**Design**	**Risk of Bias**	**Inconsistency**	**Indirectness**	**Imprecision**	
Abbott ARCHITECT HCV Ag Assay	Sensitivity	30 (12,788)	Cohort and cross-sectional	Low[1]	Low[2]	Moderate[3] (–1)	Low[4]	Moderate ⊕⊕⊕◯
	Specificity	20 (11,820)	Cohort and cross-sectional	Low[1]	Low[2]	Moderate[3] (–1)	Low[4]	Moderate ⊕⊕⊕◯
Ortho ELISA-Ag	Sensitivity	6 (1,423)	Cohort and cross-sectional	High[1] (–2)	Moderate[2] (–1)	Moderate[3] (–1)	Moderate[4] (–1)	Very low ⊕◯◯◯
	Specificity	5 (1,177)	Cohort and cross-sectional	High[1] (–2)	Moderate[2] (–1)	Moderate[3] (–1)	Moderate[4] (–1)	Very low ⊕◯◯◯
Bio-RAD Monolisa HCV Ag-Ab ULTRA	Sensitivity	5 (525)	Cohort and cross-sectional	Low[1]	High[2] (–2)	Moderate (–1)	Low[4]	Very low ⊕◯◯◯
	Specificity	1 (337)	Cross-sectional	Moderate[1] (–1)	NA[2] (–1)	Moderate[3] (–1)	NA[4]	Very low ⊕◯◯◯
EIKEN Lumispot HCV Ag	Sensitivity	2 (235)	Cross-sectional	Moderate[1] (–1)	Low[2]	Moderate[3] (–1)	Moderate[4] (–1)	Very low ⊕◯◯◯

	Specificity	0	NA	NA	NA	NA	NA	NA
Fujirebio Lumipulse Ortho HCV Ag	Sensitivity	1 (80)	Cross-sectional	Moderate[1] (–1)	NA[2] (–1)	Moderate[3] (–1)	NA[4]	Very low ⊕◯◯◯
	Specificity	0	NA	NA	NA	NA	NA	NA
Hunan Jynda HCV Core Ag ELISA	Sensitivity	4 (524)	Cohort and cross-sectional	Moderate[1] (–1)	High[2] (–2)	Moderate[3] (–1)	Low[4]	Very low ⊕◯◯◯
	Specificity	4 (524)	Cohort and cross-sectional	Moderate[1] (–1)	High[2] (–2)	Moderate[3] (–)	Low[4]	Very low ⊕◯◯◯
DiaSorin S.A. Murex Ag/Ab EIA	Sensitivity	4 (770)	Cohort and cross-sectional	Low[1]	High[2] (–2)	Moderate[3] (–1)	Low[4]	Very low ⊕◯◯◯
	Specificity	3 (658)	Cohort	Low[1]	Moderate[2] (–1)	Moderate[3] (–1)	Low[4]	Low ⊕⊕◯◯

NA= not applicable

Footnotes:

For each index test, quality of evidence started high when there were several high-quality observational studies (prospective cohort studies, cross-sectional studies with direct comparison of index test results with a reference standard). We then downgraded one point when there was moderate concern identified and two points when a there was a high concern identified in any of the four factors that may decrease the quality of evidence: risk of bias, inconsistency, indirectness, and imprecision.

[1] We used QUADAS-2 to assess risk of bias.

- For ARCHITECT, in half of the studies it was unclear how participants were selected and one study used only healthy blood donors; however, the data from all studies is consistent and unclear selection does not appear to cause bias thus we did not downgrade.
- For the Ortho ELISA, two studies of five used convenience enrolment for participant selection, and one enrolled only healthy blood donors thus we downgraded 2 points.
- For the Monolisa, four of five studies had unclear patient selection. For one it was unclear if the index and reference test were performed within 30 days. Given that there were no high-risk concerns for bias we did not downgrade. For specificity, there was only one study with data that had unclear participant selection, thus we downgraded one point, as there were no data from studies with random or consecutive selection to compare to and identify possible selection bias (as was possible with the ARCHITECT).
- For the Lumispot, both studies had unclear patient selection. As there were no data from studies with random or consecutive selection to compare, we downgraded one point.
- The Lumipulse only included one study with unclear participant selection and was downgraded 1 point.
- The Hunan Jynda had one of four studies with unclear participant selection, one in only healthy blood donors, and one for which it was unclear whether the index and reference were performed within 30 days. As the use of only healthy blood donors was considered a high-risk category, in combination with the other unclear factors, we downgraded one point.
- For the Murex test, three of four studies had unclear participant selection but no other high-risk concerns for bias and thus we did not downgrade.

[2] Unexplained heterogeneity in remaining studies may be related to covariates that could not be adjusted for in meta-regression due to limited data (HIV and HBV coinfections, HCV genotype). Additionally, not all studies identified HCV antibody status or stratified by acute and chronic infection thus variability of HCV replication could contribute to higher false negative HCVcAg.

- There was little heterogeneity noted in the ARCHITECT studies; thus we did not downgrade.
- For the Ortho ELISA, there was moderate heterogeneity with largely one outlier study, thus we downgraded 1 point.
- For the Monolisa sensitivity outcome, heterogeneity between studies precluded meta-analysis and thus we downgraded 2 points. For specificity, there is only 1 study and we cannot assess heterogeneity and downgrade 1 point.
- For the Murex sensitivity outcome there was too much heterogeneity to pool the data, and thus we downgraded 2 points. For specificity, there were not enough studies to perform meta-analysis and heterogeneity could not be formally assessed, however there is a broad range among results and thus we downgraded one point.
- The EIKEN Lumispot was only used in 2 studies. Sensitivity was similar in both studies suggesting little heterogeneity, thus we did not downgrade.
- For the Fujirebio Lumipulse, there is only 1 study and we cannot assess heterogeneity and downgrade 1 point.

[3] All studies were performed in reference laboratories, and the majorities were in high and middle-income countries. Thus the patient population, the viral population tested (e.g. genotype distribution), and the test users are not representative of the limited-resource settings for which these guidelines are envisioned. All were downgraded 1 point.

[4] We considered imprecision as present when the pooled confidence intervals were >10% and when there were fewer than 250 samples in the analysis. As such, we downgrade the Ortho ELISA, and Hunan Jynda one point for wide confidence intervals, and downgraded the Lumispot one point for small sample size. Additionally, imprecision could not be graded for the Monolisa specificity outcome, and the Lumipulse test as these only included one study.

B) Summary of findings, PICO 5a

SR outcome: diagnostic accuracy			**Effect accuracy (95% confidence interval)**		**Effect likelihood ratio (LR)**	
Index test	**# Studies (# samples)**	**Unit of analysis**	**Sensitivity**	**Specificity**	**Positive LR**	**Negative LR**
Abbott ARCHITECT HCV Ag Assay	20 (11,820)	Sample	93.4% (88.7, 96.2)	98.7% (96.9, 99.4)	71.8 (28.6, 160.3)	0.07 (0.04, 0.12)
Ortho ELISA-Ag	5 (1,177)	Sample	93.2% (81.6, 97.7)	99.2% (87.9, 99.9)	116.5 (6.7, 977)	0.06 (0.02, 0.07)
Bio-RAD Monolisa HCV Ag-Ab ULTRA	5 (525)	Sample	28.6–95%*	94.9% (89.9, 99.8)**	NA	NA
EIKEN Lumispot HCV Ag	2 (235)	Sample	97.5–98.1%*	ND	NA	NA
Fujirebio Lumipulse Ortho HCV Ag	1 (80)	Sample	95% (90.2, 99.8)**	ND	NA	NA
Hunan Jynda HCV Core Ag ELISA	4 (524)	Sample	59.5% (46, 71.7)	82.9% (58.6, 94.3)	3.5 (1.1, 12.6)	0.28 (0.2, 0.3)
DiaSorin S.A. Murex Ag/Ab EIA	4 (730)	Sample	50–100%*	83.8–100%*	NA	NA

ND: no data, NA = not applicable – if sensitivity and specificity results were not available from meta-analysis, likelihood ratios were not calculated.

* Meta-analysis not possible. Range of results seen across studies reported. **Result from one study only.

C) Impact of findings in different prevalence settings

Outcome	Effect per 1000 patients with presumed HCV for varying prevalence settings comparing HCV core Ag against HCV RNA		
	Prevalence 2%*	Prevalence 10%*	Prevalence 30%*
Abbott ARCHITECT HCV Ag Assay			
True positives (patients with HCV)	19 (18, 19)	93 (89, 96)	279 (267,288)
True negatives (patients without HCV)	967 (951, 974)	888 (873, 895)	691 (697, 696)
False positives (patients incorrectly classified as having HCV)	13 (6, 29)	12 (5, 27)	9 (4, 21)
False negatives (patients incorrectly classified as not having HCV)	1 (1, 2)	7 (4, 11)	21 (12, 33)
Ortho ELISA-Ag			
True positives (patients with HCV)	19 (16, 20)	93 (82, 98)	279 (246, 294)
True negatives (patients without HCV)	970 (862,980)	891 (792, 900)	693 (616, 700)
False positives (patients incorrectly classified as having HCV)	10 (0, 118)	9 (0, 108)	7 (0, 84)
False negatives (patients incorrectly classified as not having HCV)	1 (0,4)	7 (2, 18)	21 (6, 54)
Hunan Jynda HCV Core Ag ELISA			
True positives (patients with HCV)	12 (9, 14)	60 (46, 72)	179 (138, 216)
True negatives (patients without HCV)	813 (578, 921)	747 (531, 846)	581 (413, 658)
False positives (patients incorrectly classified as having HCV)	167 (59, 402)	153 (54, 369)	119 (42, 287)
False negatives (patients incorrectly classified as not having HCV)	8 (6, 11)	41 (28, 54)	122 (84, 162)

*Numbers in parentheses consider 95% confidence intervals of accuracy estimate

II. PICO 9: Among patients receiving treatment for HCV, what is the diagnostic accuracy of HCV core Ag test versus NAT for HCV RNA detection (and/or) quantification to confirm successful treatment response with viral clearance?

SR Outcome 1: Diagnostic accuracy, sensitivity and specificity of HCVcAg at SVR

SR Outcome 2: Timing and predictive accuracy of HCVcAg for SVR

Patients/population: Persons with detectable HCV RNA with or without positive HCV antibody

Setting: Any

Index tests: HCV core antigen assay

Importance: Inform best strategy for treatment monitoring and test of cure in a variety of clinical settings and economies

Reference standard: HCV RNA Testing

Studies: Longitudinal cohort or randomized controlled trials that use HCV NAT as gold standard reference test compared with a commercially available HCV core Ag index test

SR outcome 1: Diagnostic Accuracy at SVR				**Quality**				**Effect***	**Strength of evidence**
Index test	**Outcome measure**	**# Studies (# samples)**	**Design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**		
Abbott ARCHITECT HCV Ag Assay	Sensitivity	2 (67)	RCT, cohort	Low[1]	Low[2]	Moderate[3] (–1)	Low[4]	100%*	Moderate ⊕⊕⊕◯
	Specificity	2 (67)	RCT, cohort	Low[1]	Moderate[2] (–1)	Moderate[3] (–1)	Low[4]	94–100%*	Low ⊕⊕◯◯

SR outcome 2: Predictive accuracy of SVR				**Quality**				**Effect***	**Strength of evidence**
Index test	**Outcome measure**	**# Studies (# individuals)**	**Design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**		
Abbott ARCHITECT HCV Ag Assay	Sensitivity	1 (23)	Cohort	Low[1]	NA[2] (–1)	Moderate[3] (–1)	NA[4]	95.2%**	Low ⊕⊕◯◯
	Specificity	1 (23)	Cohort	Low[1]	NA[2] (–1)	Moderate[3] (–1)	NA[4]	70%**	Low ⊕⊕◯◯
Fujirebio Lumipulse Ortho HCV Ag	Sensitivity	2 (134)	Cohort	Moderate[1] (–1)	Moderate[2] (–1)	Moderate[3] (–1)	Moderate[4] (–1)	57.1–79.4%*	Very low ⊕◯◯◯
	Specificity	2 (134)	Cohort	Moderate[1] (–1)	Moderate[2] (–1)	Moderate[3] (–1)	Moderate[4] (–1)	88.5–99.3%*	Very low ⊕◯◯◯

* Results reported are range across studies or **individual result, NA= not applicable

Footnotes:

For each index test, quality of evidence started high when there were several high quality observational studies (prospective cohort studies, cross-sectional studies with direct comparison of index test results with a reference standard). We then downgraded one point when a serious issue was identified and two points when a very serious issue was identified in any of the four factors that may decrease the quality of evidence: risk of bias, inconsistency, indirectness, and imprecision.

SR outcome 1: diagnostic accuracy at SVR

1. We used QUADAS-2 to assess risk of bias. There were no concerns raised for the two studies that utilized the ARCHITECT assay.

2. The limited number of studies precluded a meta-analysis and formal assessment of heterogeneity. However, the data between studies for sensitivity is consistent thus we did not downgrade. There is some variability seen in the data for specificity, thus we downgrade 1 point.
3. Both studies were performed in reference laboratories in high and middle-income countries, which is not representative of broad use throughout the world thus we downgraded 1 point.
4. The range in specificity results was attributed to possible unexplained heterogeneity and is less likely from verification bias given the excellent reference standard. Given that we already downgraded for heterogeneity, we did not downgrade for imprecision.

SR outcome 2: predictive accuracy of early HCV Ag on SVR

1. We used QUADAS-2 to assess risk of bias. For the ARCHITECT, there were no concerns raised so we did not downgrade. In the 2 Fujirebio Lumipulse studies, participant selection was unclear in one, and one did not include all patients initially enrolled in the analysis so we downgraded 1 point.
2. For the ARCHITECT, there was only one study thus we could not assess heterogeneity and downgrade one point. For the Lumipulse assay, there were only 2 small studies and no formal heterogeneity could be assessed. However, neither study included covariate information aside from genotype and the results between studies are broad, thus we downgraded one point.
3. All studies were performed in reference laboratories in high- and middle-income countries, which is not representative of broad use throughout the world. All were downgraded 1 point.
4. For the ARCHITECT, imprecision could not be graded as there was only one study. There is a broad range of effect between the Fujino studies, which may in part be from unexplained heterogeneity already discussed, but may also be from imprecision as only absolute values of decline in HCVcAg were examined instead of log decline thus we downgraded 1 point.

2. Background

Chronic hepatitis C virus (HCV) infection with viraemia is prevalent in approximately 1.1% of the world population, or 64–103 million people, with an estimated 75% of all cases occurring in low- to middle-income countries (LMICs).[1] HCV is a small, enveloped, single stranded ribonucleic acid (RNA) virus belonging to the Flaviviridae family with seven genotypes and more than sixty-seven subtypes.[2] The genome is contained in an internal capsid formed by three domains of the HCV core protein, which is highly conserved and antigenic.[3,4] During viral assembly, nucleocapsid peptides 22 (p22) are released into plasma[5] and can be detected early in the course of infection.

Screening assays to assess for anti-HCV antibodies (HCV Ab) were among the first diagnostic tools developed to identify HCV infection, but can only inform about exposure to the virus and not active replication or ongoing infection. The serological window for conversion to a positive antibody is highly variable with an average of 60 days[6] and antibodies may remain persistently negative among patients on haemodialysis and those with poorly controlled HIV infection or other immunocompromised states. Thus, diagnosis of active HCV infection requires antibody testing followed by an assessment for viraemia both for confirmation of true infection in antibody-positive patients and for high-risk antibody negative patients. Confirmatory testing can be based on nucleic acid testing (NAT) to detect HCV-RNA or an antigen testing to detect core antigen.

HCV core antigen (HCVcAg) tests largely targeting p22 have been in development as an alternative to NAT since Tanaka et al. first demonstrated detection of circulating antigen in those

with chronic HCV infection in 1995[7] and the first commercial assay was released in 2000.[8] HCVcAg tests have the potential to be less costly and less centralized than NAT.

Detection of HCV viraemia is also important during treatment of chronic HCV infection. Current guidelines recommend virological confirmation pretreatment with the measurement of a baseline viral load with NAT. For interferon-based treatments, viral load is assessed at week 4 of therapy for the "rapid viral response" (RVR) to help predict efficacy of therapy, and repeated at week 6 if elevated at week 4 to see further viral response and guide whether treatment should be continued. NAT is performed again at week 12 (early viral response, EVR), at the end of treatment, and 12 and 24 weeks after therapy is completed to test for cure, "sustained viral response" (SVR). With the development of direct-acting antivirals (DAAs), NAT during therapy may no longer be necessary.[9] Additionally, DAA has made treatment for HCV possible in LMICs[10] making access to an affordable diagnostic and monitoring test even more important.

This systematic review of the published literature aims to assess the diagnostic accuracy of HCVcAg testing for HCV detection and inform the best testing strategy for identification of chronic HCV infection. Furthermore, the review looks at the utility of HCVcAg for monitoring on HCV treatment and to test for cure.

Tests included in this systematic review

Only commercially available tests were included in the systematic review. The most widely studied is the Abbott ARCHITECT HCV Ag assay, a two-step automated chemiluminescent microparticle immunoassay (CMIA) that allows quantitative determination of HCVcAg in serum or plasma. The assay uses the Abbott ARCHITECT *i* System (i2000/i2000SR/i1000SR modules), a reference laboratory instrument with ARCHITECT System Software version 5.0 or higher. The Fujirebio Lumipulse Ortho HCV Ag test and EIKEN Lumispot HCV Ag are similar automated chemiluminescent enzyme immunoassays (CLEIA) available in Japan and China.

There are two available Ab–Ag combination enzyme immunoassays (EIA), the DiaSorin S.A. Murex HCV Ag–Ab combination and Bio-RAD Monolisa™ HCV Ag-Ab ULTRA. The Monolisa uses a spectrophotometer to read absorbance values that detects presence or absence of Ab and/or HCVcAg with the colour intensity being proportional to quantity of Ab or Ag to HCV bound on the solid phase. Lastly, there are two enzyme-linked immunosorbent assay (ELISA)-based HCVcAg tests, Hunan Jynda Bioengineering Group HCV Core Ag ELISA and Ortho ELISA-Ag.

3. Objectives

This systematic review addresses predefined PICO questions 5a and 9. Question 5b will be addressed in a separate report.

PICO 5a	What is the best strategy (diagnostic accuracy and other outcomes); comparing HCV core Ag test versus NAT for HCV RNA for detection (and/or) quantification to confirm active HCV infection?
P	Persons with detectable HCV RNA with or without positive HCV antibody
I	HCV core antigen assay
C	HCV RNA testing
O	• **Diagnostic accuracy** 1. True negatives (TN), who are screen negative, and do not have HCV infection. 2. False negatives (FN), who are screen negative but have HCV infection. These are incorrectly misclassified, and this may results in missed opportunity to recognize and present progression of liver disease. 3. True positives (TP), who are screen positive and have HCV infection. 4. False positives (FP), who are screen positive, but do not truly have HCV infection. These will have additional unnecessary tests and evaluation.

PICO 5b	What is best testing strategy (diagnostic accuracy and other outcomes); between using sequential testing strategy (HCV core Ag followed by NAT if negative) versus NAT alone for diagnosis of active HCV infection?
P	Persons with detectable HCV RNA with or without positive HCV antibody
I	Sequential testing strategy (HCV core Ag followed by NAT if negative) (Fig. 4A)
C	Standalone NAT test (Fig. 4B)
O	• **Diagnostic accuracy** 1. *True negatives (TN)*, who are screen negative, and do not have HCV infection. 2. *False negatives (FN),* who are screen negative but have HCV infection. These are incorrectly misclassified, and this may results in missed opportunity to recognize and present progression of liver disease. 3. *True positives (TP),* who are screen positive and have HCV infection. 4. *False positives (FP),* who are screen positive, but do not truly have HCV infection. These will have additional unnecessary tests and evaluation.

PICO 9	Among patients receiving treatment for HCV, what is the diagnostic accuracy of HCV core Ag test versus NAT for HCV RNA detection (and/or) quantification to confirm successful treatment response with viral clearance? (Fig. 5A, 5B)
P	Patients receiving treatment for HCV
I	HCV core Ag test (Fig. 5A)
C	NAT for HCV RNA detection (and/or) quantification (Fig. 5B)
O	• **Diagnostic accuracy** 1. *True negatives (TN),* who are screen negative, and cleared the HCV infection. 2. *False negatives (FN),* who are screen negative but have HCV infection. (These will be misclassified, and treatment will be stopped resulting in disease progression leading to Liver related morbidity (fibrosis, cirrhosis, end-stage liver disease, hepatocellular carcinoma), progression of liver disease, and mortality. 3. *True positives (TP),* who are screen positive and truly have HCV infection, this will increase the number of treated cases and cured rate. 4. False positives (FP), who are screen positive, but do not have HCV infection. (These will continue treatment inappropriately, and will have unnecessary referral).

4.Methods

We followed standard guidelines and methods for systematic review and meta-analyses of diagnostic tests.11–13 We prepared a protocol for the literature search, article selection, data extraction, and assessment of methodological quality.

Selection criteria

Types of studies

We included case–control, cross-sectional, cohort studies and randomized trials that used HCV NAT as gold standard reference test compared with a commercially available HCV core Ag index test for the diagnosis of active HCV infection or in the monitoring of HCV infection while on treatment.

Participants

We included patients of all age groups from all settings and countries. Specimen types were limited to whole blood, plasma or serum, and we only included studies that examined at least 10 independent HCV NAT positive samples. Saliva specimens were also considered, but only one study was identified during the search and it did not use NAT as reference test and was thus excluded from further analysis. Commercially prepared reference panel specimens were excluded.

Index tests

Studies that utilized a commercially available HCV Core Ag test were eligible for inclusion. The following seven are the index tests included:

- Abbott ARCHITECT HCV Ag
- Bio-RAD Monolisa™ HCV Ag-Ab ULTRA
- EIKEN Lumispot HCV Ag
- Fujirebio Lumipulse Ortho HCV Ag
- Hunan Jynda Bioengineering Group HCV Core Ag ELISA
- DiaSorin S.A. Murex Ag/Ab EIA
- Ortho ELISA-Ag

Target conditions

PICO 5a/b

- Acute HCV infection: the 6-month time period following acquisition of hepatitis C virus. HCV Ab may be positive or negative; the time period between initial infection to seroconversion of antibody is the "window period". HCV RNA is detectable.
- Chronic HCV infection: duration of HCV infection more than 6 months from time of acquisition. HCV Ab and RNA are detectable.

PICO 9:

- Monitoring of viral clearance while on treatment with rapid viral response (RVR) at 4 weeks and early viral response (EVR) at 12 weeks and sustained viral response (SVR) at 24 weeks after completion of treatment

Reference standard

The reference standards accepted for a definitive diagnosis included tests for detection of HCV RNA by any of the following NAT techniques: polymerase chain reaction (PCR), branched-chain DNA (bDNA), or transcription mediated amplification (TMA). Tests were noted to be either qualitative or quantitative. The performance characteristics of NATs are very similar above 50 IU/mL, thus all NATs were considered as one reference standard.

Outcome measures

Sensitivity refers to the proportion of samples with true HCV infection diagnosed with positive HCV Core Ag test confirmed with a positive NAT result.

Specificity refers to the proportion of samples with negative HCV Core Ag test and no evidence of active HCV infection confirmed with a negative NAT result.

Search methods

A database search of EMBASE, PubMed, Scopus, Web of Science, and Cochrane was performed through March 2015. No language restriction was applied. The search terms used for each database are outlined in Appendix A.

Two review authors (JMF and TT) independently assessed titles and abstracts identified by the literature search to select potentially eligible studies (screen 1). Any citation identified by either review author during screen 1 was selected for full text-review. Full papers of each potentially eligible article were retrieved. Two review authors (JMF and TT) independently assessed the full text articles for inclusion using the predefined inclusion and exclusion criteria (screen 2). Three articles were excluded because of inability to find appropriate language interpretation. Discrepancies were resolved by discussion between the review authors, and for several studies by the decision of a third review author (CMD). The included studies were divided into those applicable for each PICO question. A list of excluded studies and reasons for exclusion can be found in Appendix D.

Data extraction

We created a data extraction form, pilot-tested the form with a subset of eligible studies, and then finalized the form (Appendix B). Two review authors (JMF and TT) independently extracted data from the included studies with the standardized form and crosschecked to ensure accuracy. Disagreement between review authors on data extraction was resolved by discussion or by a third reviewer (CMD). For studies without complete extraction information available, authors were contacted to request further data. Studies without extractable sensitivity and specificity data were excluded if no further information was acquired after three attempts to contact the study authors.

Assessment of methodological quality

We adapted the QUADAS-2 instrument, a validated tool for diagnostic studies,[14] to assess study quality. The information needed to answer QUADAS-2 questions was incorporated in the data extraction sheet. A description of the QUADAS-2 items and the interpretation in the study context can be found in Appendix C.

Statistical analysis and data synthesis

Statistical analyses were performed using STATA (version 14; STATA corporation, College Station, TX). The studies were grouped by type of index test used. QUADAS analysis was performed using Excel (version 14.5.3; Microsoft, Seattle, WA).

Approach to indeterminate index test results

We excluded indeterminate test results from the analyses for determination of sensitivity and specificity, as it was less than 1% for all index tests.

Assessment of publication bias

We did not perform formal assessment of publication bias (tests for funnel plot asymmetry), as these techniques are not recommended for diagnostic test accuracy studies. We reviewed the EASL and AASLD conference abstract books for abstracts of studies that have not been published subsequently and did not find anything between 2010 and 2013. We did not include unpublished data in this review.

Meta-analysis

Meta-analysis for each index test type was performed if at least four studies were available with the same index test with at least ten independent samples in each study. Bivariate random effects meta-analyses were performed for index tests with enough studies that included data to calculate sensitivity, specificity, and 95% confidence intervals for each. Several studies did not contribute to both sensitivity (no true positives and false negatives) and specificity (no false positives and true negatives) but only to one of the two. In such cases, we examined the correlation between sensitivity and specificity visually from a scatter plot of the sensitivity versus 1-specificity across studies. If the correlation was limited, we performed a univariate random effects meta-analysis of the sensitivity and/or specificity estimates separately, so as to make complete use of the available data. We then compared the results from the univariate analysis (including all studies) with the results from the bivariate analysis of the subset of studies that contributed to both sensitivity and specificity estimates. For index tests with data that contributed only to sensitivity but had at least 4 studies, we performed univariate random effects meta-analysis only for sensitivity. A descriptive analysis was performed for index tests with less than four studies available or when substantial heterogeneity was evident on forest plots that precluded a meta-analysis.

We visually assessed forest plots for heterogeneity among the studies within each index test and in the summary plots we examined the variability in estimates and the width of the prediction region, with a wider prediction region suggesting more heterogeneity. We also report an estimate of τ^2 (along with its standard error) corresponding to the variance of the logit-transformed specificity and sensitivity, which can be interpreted as a measure of between-study variability.

The initial protocol planned for sensitivity analysis excluding case-control studies, but none were identified amongst the studies included. We anticipated that studies included in the meta-analysis would be heterogeneous in many respects. Therefore, we pre-specified subgroups by antibody status. Furthermore, we planned to examine the effect of specimen condition (fresh vs. frozen), HBV and HIV status and genotype in a meta-regression. Where meta-regression to assess impact of covariates was not possible due to limited data, we showed descriptive statistics for HIV and HBV coinfection and genotype distribution. The impact of specimen condition could not be assessed as all studies either used frozen samples or did not specify condition.

Analysis of quantitative data

Where quantitative data were available from the studies, a locally weighted regression smoother was used to visually assess the correlation between quantitative HCV Ag measured in fmol/L to HCV RNA measured in IU/mL.[15] We identified outliers and performed descriptive statistics of these points. There was only enough quantitative data to assess the Abbott ARCHITECT assay.

5. Results

Results of the search

From the literature search, 8146 citations were identified and a total of 313 full-text articles were reviewed: 283 applied to PICO 5a, 11 applied to PICO 5b (reported separately), and 44 applied to PICO 9. For PICO 5a, 50 studies were included. For PICO 9, 4 studies were included. Figure 1a-d shows the PRISMA diagram with the flow of studies for each PICO and reasons for exclusion.

Description of studies

Core antigen for HCV detection (PICO 5a) – included studies

Fifty included studies utilized the 7 different HCV cAg assays described above, with two performing direct comparisons between two or more antigen tests.[16,17] Four studies were translated from Mandarin,[18] 1 from German,[19] 1 from French,[20] and 2 from Japanese.[21, 22] Characteristics for each study are presented in Table 1a.

The Abbott ARCHITECT HCV Ag assay was assessed in 30 studies.[5, 16, 18, 19, 23–49] All study designs were either cross-sectional or cohort, with a broad study population (included patients with HCV disease, and those susceptible to HCV disease) with the exception of one study that evaluated only healthy blood donors.[35] Only 20 had enough data to be included in the bivariate analysis.[34] Ten did not have enough data to calculate specificity[16, 17, 18, 28, 31, 36, 37, 41, 42, 45] and were only included in the univariate pooled sensitivity estimate. All but 3 studies specified positive HCV Ab status of specimens[19, 25, 26] and 4 included data for HCV Ab negative samples.[18,24,42,45, 48] Demographic data was available in 18 studies, the remainder utilized anonymous specimens and authors were unable to provide further information. HIV status was known in 15 of the studies with 2 including only HIV-coinfected subjects.[46, 48] HBV status was known in 13 studies and all but 4 excluded patients with HBV coinfection. The study with highest prevalence included 50.5% with HBV coinfection.[40] Only 1 study included children.[28]

The Bio-RAD Monolisa™ HCV Ag-Ab ULTRA was used in 5 studies;[17, 20, 50–52] all were cohort or cross-sectional in design with a broad study population. One study had an unknown amount of participants with at least 25 known subjects and an additional 94 samples from an unknown amount of donors.[48] Two included only HIV-coinfected adult subjects,[20, 51] the remaining 3 had unknown subject demographic information.

The EIKEN Lumispot HCV Ag was performed in one cross-sectional study with a broad study population.[53] Further demographic information was unavailable.

The EIKEN Lumispot HCV Ag, Fujirebio Lumipulse Ortho HCV Ag, and Abbott ARCHITECT HCV Ag were compared in 1 cross-sectional study,[16] with unknown demographic information.

Four studies assessed the Hunan Jynda Bioengineering Group HCV Core Ag ELISA.[54–57] Two studies had a cohort design, 2 cross-sectional, and 1 assessed a healthy blood donor population[56] while the others included broad study populations. HIV and HBV coinfection status was unknown in all studies. One included children,[57] and the remaining had unknown age groups included.

The DiaSorin S.A. Murex Ag/Ab EIA was used in 4 articles, 3 adult cohort studies [58–60] and 1 cross-sectional study that compared performance with the Bio-RAD Monolisa™ HCV Ag-Ab ULTRA;[17] this is the same study as above with an unknown total number of participants. One study included 25% HIV-coinfected patients,[59] and one included 6.1% HBV co-infected patients.[60]

Finally, 6 articles utilized the Ortho ELISA-Ag test.[61–64] All were either cross-sectional or cohort designs in broad study populations except for 1 study performed in healthy blood donors.[63] All had unknown demographic information.

Core antigen in best testing strategy for identification of active HCV infection (Pico 5b) – included studies

Only 1 study was found to meet inclusion criteria.[43] Given limited data, a decision analysis was performed to address this PICO question and was reported separately.

Core sntigen for treatment monitoring and test of cure (PICO 9) – included studies

Two studies evaluated the Abbott ARCHITECT compared to NAT at baseline, EVR and SVR, one used patients enrolled in a randomized controlled trial[65] and one used a cohort design.[66] One study used the ARCHITECT to assess correlation of HCVcAg and NAT at EVR only. Three cohort studies evaluated HCVcAg kinetics during EVR to assess predictive accuracy of SVR, but did not compare HCVcAg to NAT at SVR. One used the ARCHITECT,[67] and 2 employed the Fujirebio Lumipulse.[68,69] None required translation. Characteristics are presented in Table 1b. All studies included patients with active HCV infection who were initiated on interferon based treatment regimens.

Excluded studies

A list of excluded studies for each PICO and the reasons for exclusion is presented in appendix D.

Methodological quality (QUADAS-2)

The overall methodological quality of all included studies for each PICO question organized by QUADAS-2 domain is summarized in Fig. 2 and presented for each individual study in Fig. 3.

Patient selection

PICO 5a

In the "patient selection" domain, we judged 2 studies to have "high risk of bias" because they used convenience sampling of participants for enrolment. Twenty-three studies were judged to be "low risk of bias". In 25 studies risk of bias was "unclear" with 12 having both unspecified enrolment and prior exclusion strategies and the remaining 13 with a mix of the two. Applicability in this domain was judged to be "high risk" in 3 studies that included only healthy blood donors, and the remaining 47 were determined to be "low risk". Setting of testing was not considered for this review as currently available tests can only be operated in specialized laboratories.

PICO 9

In the "patient selection" domain, 4 studies were judged to be "low risk of bias" as sampling was consecutive or random. One was judged to be "unclear risk of bias" as patient selection was not specified. Applicability was judged to be "low risk" in all studies as all included patients with active HCV infection.

Index test

PICO 5a

All studies were determined to have "low risk of bias" as all index tests had predefined thresholds of positivity and interpretation does not require judgement thus all were considered blinded with respect to the results of the reference test. Applicability in this domain was assessed by whether or not the index test was performed per recommendations of the manufacturer. In 3 studies, this was unclear and information could not be obtained from the study authors, thus 3 were determined to be "unclear risk" while the remaining 47 were "low risk".

PICO 9

All studies were determined to have "low risk of bias" as all index tests had predefined thresholds of positivity and interpretation does not require judgement. For applicability, it was unclear in 1 study whether the index test was performed per recommendation of the manufacturer and was thus judged "unclear". The remaining 4 studies were "low risk".

Reference standard

PICO 5a and PICO 9

All studies were judged to be "low risk of bias" per our QUADAS-2 rules. Though studies used a variety of NAT techniques, all are considered highly sensitive and results are objective and do not require interpretation. As far as "applicability", this was also determined to be "low risk" for all studies as circulating virus detected by NAT is by definition associated with active infection and the specificity of the reference standard is high.

Flow and timing

PICO 5a

In the "flow and timing" domain, 42 studies were judged "low risk of bias". Eight studies were judged to be "unclear risk of bias". In 7 it could not be determined whether the index and reference tests were performed on the same specimen or within <1 month, and in 1 there were an unknown number of participants so we could not judge if all were included in the final analysis.

PICO 9

Four studies were judged to be "low risk" as index test and reference testing were performed on the same specimens at various time points throughout, and all patients were included in the final analyses. One study was judged to be "high risk" as not all patients enrolled were included in the analysis, only those who completed protocol.

HCV core Ag for diagnosis of active HCV infection

Abbott ARCHITECT

There were 20 studies included in the bivariate analysis with 11 820 total samples. Based on studies reporting paired (sensitivity and specificity) data, the pooled sensitivity regardless of HCV Ab status was 93.4% (95% CI 88.7, 96.2), sensitivity was 98.7% (95% CI 96.9, 99.4), positive likelihood ratio (LR) was 71.8 (95% CI 28.6, 160.3), and negative LR 0.07 (0.04, 0.12) (Table 2, Fig. 4a). The pooled sensitivity estimate from a univariate analysis was 94.1% (95% CI 92.4, 95.7) and included 10 additional studies that only contributed data for sensitivity with a total of 12,788 samples (Table 2, Figure 4b). Among 16 studies with known HCV Ab positive samples, the sensitivity was 92.5% (95% CI 86.9, 95.8) and specificity 97.8% (95% CI 94.7, 99.1) (Table 2, Figure 4c). From 4 studies that analysed HCV Ab negative samples, the pooled sensitivity was 74.4% (95% CI 6.2, 99.2) and specificity was 98.8% (97.2, 99.5) (Table 2, Fig. 4d). Figure 5 presents the pooled sensitivity and specificity estimates (a) regardless of HCV Ab status, (b) for HCV Ab-positive samples only, and (c) for HCV Ab-negative samples. In plots (a) and (b) the summary point approached the upper left corner suggesting good accuracy of the ARCHITECT test for diagnosis of HCV infection. Plot (c) demonstrated the broad 95% confidence interval among Ab-negative specimens.

Heterogeneity was visually assessed in Figs 4 and 5 and with τ^2 (Fig. 2). The studies were relatively homogeneous (Fig. 4a). A meta-regression was not possible given the limited amount of data on predefined covariates. There were three outlier studies in respect to sensitivity: Ergünay, Florea and Gu (72%, 74% and 44% sensitivity, respectively). Antibody status was known for Gu and performance was similar across antibody-positive and antibody-negative samples (44.0% and 41.7%, respectively). Other covariates were examined to assess reasons for low sensitivity. In the Ergünay study, HIV and HBV coinfection status were unknown, 60.2% of participants had HCV genotype 1b infection, 2.2% genotype 1a, 0.8% genotypes 3 and 4, and 35.8% were unknown (Table 3). In the Florea study, there were

no HIV or HBV infected patients, but genotype status was unknown. For specificity, the results are even more homogeneous with only 1 outlier, the Medici study. There are no demographic data for this study as it was performed on anonymous samples. Overall, genotype distribution was reported for 15 studies (Table 3a) with genotype 1b being the most prevalent and genotypes 5 and 6 minimally studied.

Ortho ELISA-Ag

Five studies were included in the bivariate analysis with 1177 total samples. The pooled sensitivity was 93.2% (95% CI 81.6, 97.7), specificity 99.2% (95% CI 87.9, 100), positive LR 116.5 (95% CI 6.7, 977), and negative LR 0.06 (95% CI 0.02, 0.07) (Fig. 6, Table 2). Univariate analysis with one additional study by Agha resulted in a pooled sensitivity of 90.8% (95% CI 83.5, 98.2) (Table 2). Figure 7 demonstrates the bivariate pooled sensitivity and specificity estimates, with the summary point approaching the upper left corner suggesting good accuracy of the Ortho ELISA-Ag test for diagnosis of HCV infection though the data exhibit some heterogeneity demonstrated by the wide 95% CI. Heterogeneity was also visually assessed in the forest plot (Fig. 6) and with τ^2 (Fig. 2) with two outlier studies, Nübling and El-Sayed. Both studies reported unknown HIV or HBV coinfection information, and genotype distribution was unknown for El-Sayed. The genotype distribution in the Nübling study was 11.5% genotype 1 not specified, 42.3% genotype 1a, 19.2% genotype 1b, 11.5% genotype 2, and 15.4% genotype 3. This study was performed in 494 total plasma samples from 52 subjects at various time points during HCV infection with varying levels of HCV RNA. The data were not stratified by antibody status, and the raw quantitative information was no longer available. The authors noted that panels later in the course of infection with higher and more consistent HCV RNA levels had improved correlation with HCVcAg detection but no sensitivity or specificity data were calculated.

Bio-RAD Monolisa HCV Ag-Ab ULTRA

Five studies with 525 total samples were included. Given heterogeneity observed in the forest plot, a pooled analysis was not performed and only descriptive statistics were examined (Fig. 8). The Nastouli and Schnuriger studies have substantially different results – sensitivities of 61.9 (95% CI 38.6, 81.9) and 95% (95% CI 75.1, 99.9), respectively. Each study was performed in participants with 100% HIV coinfection, though the genotype distribution differed with more genotype 1 patients in the Nastouli study, and more genotype 3 and 4 in the Schnuriger study (Table 3). The Tuke study demonstrated the lowest sensitivity of 28.6% (95% CI 20.4, 37.7). This study was performed in pre-seroconversion HCV Ab negative specimens only. Among the HCV genotypes, sensitivity was 33% for genotype 1a, 41% for genotype 1b, 29% for genotype 2, 0% for genotype 3, and 0% for unknown genotype (data not shown, obtained from original article). The authors also noted the sensitivity improved to 71% when limited to specimens with HCV RNA $>10^6$IU/mL, though remained negative in 7 genotype 3 samples with viral load >2 million IU/mL. The Laperche study was also performed in HCV Ab negative specimens with a broad distribution

of genotypes: 11.4% genotype 1a, 34.3% genotype 1b, 25.7% genotype 2, 14.3% genotype 3, 5.7% genotype 4, and 2.9% unknown. The sensitivity was 40.9% (95% CI 29.3, 53.2). The Vermeersch study was the largest with 337 samples and was the only with data to calculate specificity. The reported sensitivity was 93.83% (95% CI 90.2, 96.4) and specificity 94.9% (95% CI 89.9, 99.8). The study was done on anonymous samples without known HIV or HBV status or genotype. The reference standard was incomplete as RNA testing was done only on 61 random samples and all samples with discordant Ab and Ag result. Seventy-eight samples were antibody negative. Genotype distribution was unknown.

EIKEN Lumispot HCV Ag

Two studies only utilized the Lumispot assay. The first included 155 samples and the sensitivity reported was 98.1% (95% CI 95.9, 100) (Table 2).[53] The majority of samples were genotype 1 (65.2%) with the remaining genotype 2. The second study (Murayama et al.) compared the Lumispot to Fujirebio Lumipulse and Abbott ARCHITECT.16 There were 80 participants, and the reported sensitivity was 97.5% (95% CI 94.1, 100). The Abbott ARCHITECT sensitivity in that study was 100%, suggesting a bias towards better performance. Not enough data were reported to determine specificity in either study.

Fujirebio Lumipulse Ortho HCV Ag

Only one study was performed using the Lumipulse test with 80 participants comparing against Lumispot and Abbott ARCHITECT.[16] Sensitivity for the Lumipulse was reported as 95% (95% CI 90.2, 99.8) (Table 2). The Abbott ARCHITECT sensitivity in that study was 100%, suggesting a bias towards better performance. Not enough data was reported to determine specificity.

Hunan Jynda Bioengineering Group HCV Core Ag ELISA

There were 4 studies included in the bivariate analysis with 524 total samples. The pooled sensitivity was 59.5% (95% CI 46, 71.7), specificity 82.9% (95% CI 58.6, 94.3), positive LR 3.5 (95% CI 1.1, 12.6), and negative LR 0.28 (95% CI 0.2, 0.3; Table 2). Both the forest plot (Fig. 9) and bivariate analysis (Fig. 10; Table 2) demonstrated heterogeneity among the four studies, which limited confidence in the pooled estimate. No covariate assessment was performed as HIV status, HBV status and genotype distribution were unknown for all studies.

DiaSorin S.A. Murex Ag/Ab EIA

Four studies with a total sample size of 770 were available; however, given substantial heterogeneity in the forest plot (Fig. 11) a pooled estimate was not calculated. The sensitivity estimates varied between 50% and 100%. Heterogeneity was largely secondary to one outlier study by Tuke where a sensitivity of 50% (95% CI 40.4%, 59.6%) was reported. As reported above, this study was performed in HCV Ab-negative specimens in the "window period" of acute HCV infection. The authors note that when analysis was limited to specimens with viral load HCV RNA $>10^6$ IU/mL, there was an increase in sensitivity from 50% to 98%

(data not shown). Specificity could not be calculated. The El-Emshaty study is the smallest with 39 participants and reported a sensitivity of 91.3% (95% CI 71.9, 98.9), and specificity of 100% (95% CI 75.9, 100). Genotype distribution was unknown, though the study was performed in Egypt where genotype 4 is most prevalent. The Alzahrani study included 418 samples from 118 female adult participants and reported a sensitivity of 97.4% (95% CI 92.6%, 99.5%) and specificity of 100% (95% CI 98.4, 100). Finally, the Yang study conducted in Taiwan included 201 participants, 25% with HIV coinfection, and unknown genotype distribution. The reported sensitivity was 100% (95% CI 96.5, 100) and specificity 83.8% (95%CI 74.8, 90.2).

Quantitative data

Three studies provided quantitative data for analysis.[33, 41, 44] All used the Abbott ARCHITECT HCV Core Ag Assay in comparison with NAT. Non-parametric regression of these pooled quantitative data was used to visually assess the correlation between HCVcAg and RNA (Fig. 12). The few points with negative HCVcAg were shown to occur at RNA levels < 3000 IU/mL where loss of linearity was also noted. There were two outlier points between 10 000 and 100 000 IU/mL and an additional point on the threshold cut-off for positivity. No further data on genotype or coinfection information was provided to further characterize these points.

HCV core Ag for treatment monitoring

Two studies evaluated HCVcAg compared to NAT at SVR, both using the Abbott ARCHITECT index test.[65, 66] One additional study assessed the accuracy of HCVcAg compared to NAT at EVR only.[67] There were not enough studies to perform a meta-analysis. Results for sensitivity and specificity of the index test compared to NAT at baseline, EVR, and SVR were calculated and summarized in Table 4. These data do not evaluate the accuracy of HCVcAg at EVR to predict SVR but rather assess how the tests correlate at each specific time point and thus shed light on differences in the kinetics of core antigen and RNA. Two additional studies assessed timing of HCVcAg in EVR as a predictor of SVR using the Fujiribio Lumipulse test.[68,69] Descriptive statistics of each including demographic data, HIV and HBV coinfection, and HCV genotype distribution are presented in Tables 1b and 3b.

HCV core Ag performance at different time-points during treatment

The Feng study included 32 adults without HIV or HBV coinfection. All participants had genotype 1b chronic HCV infection with viral loads >2000 IU/mL. The sensitivity of HCVcAg at baseline, EVR, and SVR was reported to be 100%, though specificity of EVR was 88.9% (95% CI 68.4, 100). There were 21 patients who achieved SVR, and 11 whose HCVcAg and HCV RNA remained positive 24 weeks after completion of therapy. In all 11 patients, the HCV viral load was $>10^4$ IU/mL.

The Loggi-study enrolled 35 adult patients without HIV or HBV coinfection; 20% had genotype 1a, 80% had genotype 1b HCV infection. Seventeen patients achieved sustained

virological response. The baseline sensitivity of the HCVcAg was 100% without enough data to calculate specificity. Sensitivity at EVR was 73.5% (95% CI 58.7, 88.4) with 100% specificity. The false negatives occurred in samples with viral loads between 15 and 10 000 IU/mL. For SVR, the sensitivity was 100% with 94.1% specificity (95% CI 82.9, 100).

The Moscato study analysed samples from 23 patients with unknown demographic information and included 4% genotype 1a, 39.1% genotype 1b, 26.1% genotype 2, 21.7% genotype 3, and 8.7% genotype 4. Baseline and SVR data comparing HCVcAg to NAT were not reported. Direct comparison of HCVcAg compared to NAT at 4 weeks for 10 patients had 100% sensitivity with 70% specificity (95% CI 41.6, 98.4). Three false-positive HCVcAg results were obtained: for 1 of which HCVcAg turned negative 1 month later and 2 turned negative 2 months later (12 weeks into therapy). For three patients tested at 12 weeks, correlation between qualitative results of HCVcAg and HCV NAT was complete. In 9 additional patients with unknown demographic data, correlation between quantitative HCV RNA and HCVcAg was assessed in 54 serum specimens collected at various time points during treatment. Among these samples, authors reported 100% sensitivity of cAg, including 9 specimens with low-level viraemia between 100 and 1000 IU/mL.

HCV Core Ag as a predictor of SVR

Abbott ARCHITECT

Included in the Feng study presented above, was an assessment of measurement of HCVcAg for EVR as a predictor of SVR. The study found a sensitivity of 100% with a specificity of 28% of EVR to predict SVR. This translated into a positive predictive value (PPV) 72% and a negative predictive value (NPV) of 100%. At 4 weeks after therapy initiation (RVR), the performance was inversed with a sensitivity of 29% and a specificity of 100%. The best measure was identified to be a log10 reduction in HCVcAg (ΔHCV Ag) at 144 hours with 95% sensitivity and 73% specificity.

Fujirebio Lumipulse

The Takahashi study included 60 genotype 1b patients, and 30 genotype 2 patients with unknown HIV and HBV coinfection status. Serum HCV core Ag was measured at baseline and at 3 days, 1 week, 2 weeks, 4 weeks, and 12 weeks of treatment while qualitative NAT was performed at 12 weeks to assess EVR, and 24 weeks after completion of therapy for SVR. SVR was achieved in 50% of genotype 1b patients, and 90% of genotype 2 patients. In genotype 1b patients, HCVcAg was higher at each time point among the non-SVR group compared to the SVR group, while in genotype 2 patients there was no difference seen in HCVcAg quantity over time between the 3 non-SVR patients and those who achieved SVR, and HCVcAg was below detection limit in all genotype 2 patients by day 14. For genotype 1b, HCVcAg level on day 7 was found to be the best predictor for SVR with sensitivity 79.4%, specificity 88.5%, PPV 90%, NPV 76.7%, and accuracy of 83.3% (Table 5).

In the Fujino study, 49 adult genotype 1b patients were initially enrolled, though 44 completed protocol and were included in the analysis. Patients with HBV were excluded and HIV status was not described. SVR was achieved in 10 patients. HCVcAg and RNA were measured on days 1, 7, and 14 of therapy. Four of the SVR group had negative HCVcAg on day 1 of therapy while all had positive NAT. Negative HCVcAg on day 7 of therapy gave sensitivity 57.1%, specificity 93.3%, PPV 80%, NPV 82.4%, and accuracy of 81.8% in prediction of SVR (Table 5), while undetectable HCV RNA on day 7 yielded sensitivity 100%, specificity 87.2%, PPV 50%, NPV 100%, and accuracy of 88.6%.

6. Discussion

This systematic review addressed diagnostic accuracy of HCV core antigen tests for identification of active HCV infection among those with and without positive HCV antibody through an analysis of 50 published studies (PICO5a) that utilized 7 different index tests. Additionally, accuracy of HCV core antigen tests for treatment monitoring and as a test of cure was assessed in 5 published studies (PICO 9).

The Abbott ARCHITECT HCV Core Antigen test had the highest sensitivity (93.4%), while specificity was similar to that of the Ortho ELISA-Ag (98.7% vs 99.2%). The estimates for both sensitivity and specificity were more precise for the ARCHITECT assay. This was partly because the ARCHITECT was the most extensively studied, with 30 publications included in this review compared to 5 studies included for Ortho, but also partly because of the greater homogeneity among the ARCHITECT studies. The likelihood ratios for both tests were also very favourable with the positive LR >10 indicative of a large increase in probability of disease with a positive result and negative LR <0.1 indicative of a moderate decrease in the probability of disease with a negative result. The EIKEN Lumispot and Fujirebio Lumipulse were designed with the same principle of technology as the ARCHITECT and have similar sensitivity and specificity, though assessment was limited to 1 and 2 studies. Tests such as the Hunan Jynda assay have the lowest sensitivity (59.5%), which supports the notion that signal amplification (as with chemiluminesence) is necessary to achieve adequate detection limits.

Quantitative analysis of data available from 3 studies using the ARCHITECT demonstrated close correlation between HCVcAg and RNA, though the linearity declined around an HCV RNA level of 3000 IU/mL, which is consistent with the analytical limit of detection reported by Abbott.

All studies included with treatment monitoring and SVR data were in patients on interferon (IFN)-based therapies. Data was limited and a meta-analysis was not possible. Descriptive analysis found the sensitivity of ARCHITECT at EVR in comparison to RNA ranging from 74% to 100% with specificity from 70% to 100% and at SVR (only assessed in 2 studies) sensitivity was 100% and specificity ranged from 94% to 100%. HCVcAg predictive accuracy for SVR was described in only 3 studies, 1 using the ARCHITECT and 2 using the Lumipulse. All three included mostly genotype 1b patients, and results indicated best

predictive accuracy of core antigen for SVR from the decline or reversion to negative early on in therapy at 6–7 days.

There were limitations in the data summarized in this review. For several index tests, there were not enough studies to derive pooled estimates and descriptive analyses only could be completed. There was substantial heterogeneity among all index tests aside from the ARCHITECT, and there were not enough data to perform planned sensitivity analyses of covariates and meta-regression of subgroups; thus descriptive statistics were substituted. From the limited data available, it is clear that data on core antigen test performance in genotypes 4, 5 and 6 is largely lacking, which limits the conclusions. Most of the studies were performed in high-resource settings and might not be reflective of the population that will be tested if HCVcAg diagnostics are implemented in LMICs. Furthermore, most studies were performed in reference laboratories and test performance might be decreased if tests are applied in routine laboratories.

To assess treatment monitoring, only 2 studies measured HCVcAg in comparison with NAT at SVR and only descriptive analyses could be conducted. Several of the studies found in the search were designed to answer a different question from that of the PICO structure – whether an early decline in HCVcAg could predict SVR and at what time period this was most accurate. These data were also described, though again meta-analysis could not be performed. Additionally, there were no studies using DAA IFN-free treatment regimens, thus the results from this descriptive analysis might not be reflective of the results that are to be expected with DAAs. The timing of this review has also occurred during a rapidly changing landscape; the utility of viral load monitoring while on treatment with these highly effective therapies, and thus utility of HCVcAg as a surrogate of NAT, has been called into question.[9]

Strengths and limitations of the systematic review

Strengths of this review include the development of an a priori protocol for the literature search, article selection, data extraction, and assessment of methodological quality. The search was performed without language restriction, though ultimately 3 articles were excluded for inability to find appropriate translation for Russian, Korean and Polish. Nevertheless, studies may have been missed in the comprehensive search, and subsequent studies published after the search date could not be included. Article selection and standardized data extraction in accordance with the predefined protocol was ensured by independent reviewers. Authors were contacted for missing data and clarifications, though some studies were excluded due to lack of author response or inability to provide original data. In the analysis, bivariate random effects modeling was used when appropriate to derive pooled estimates and univariate analyses were performed in effort to utilize all available data.

Further research suggested

The data limitations in this review highlight a need for better surveillance data that will inform an understanding of how many patients are missed by assays that have higher limits

of detection (e.g. 3000 IU/mL for ARCHITECT). Furthermore, a better understanding is necessary on the outcomes of patients with low viral loads: are these patients more likely to resolve their infection or at least less prone to develop HCV disease, or do they still have notable disease progression that would make them eligible for treatment? Similarly, more information is necessary on patients with high viral loads and negative HCVcAg to inform the optimization of antigen detection. The fluctuation in RNA during the pre-seroconversion phase and correlation of core antigen is also poorly understood.

Additionally, research is required to determine how covariates such as HIV or HBV coinfection or genotype may impact the accuracy of HCVcAg for diagnosing active infection as well as for monitoring treatment outcomes. The kinetics of HCVcAg with treatment also need to be evaluated further, particularly in the context of new DAA regimens.

7. Summary

In summary, this systematic review showed that there are several HCVcAg assays associated with high sensitivity (>90%) and specificity (>98%) compared with NAT. While even those with the highest performance do not reach the sensitivity of NAT, well-performing HCVcAg tests with an analytical sensitivity reaching into the femtomolar range (~3000 IU/mL), which translates into diagnostic sensitivity of about 95%, could serve as a replacement for NAT for HCV detection. This is the case particularly if HCVcAg are more affordable than NAT, which is conceivable from the cost of goods for the test. Furthermore, HCVcAg tests could be applied for a one-step screening test as they turn positive earlier than antibody tests (1–2 days after HCV RNA appears) and have a high specificity, thus not requiring any further confirmatory testing.

For both core antigen tests and NATs to reach a larger population at risk in LMICs, tests with better point-of-care (POC) suitability need to be developed and sample processing and transport mechanisms need to be improved to optimize the use of platforms requiring reference laboratories. HCVcAg tests are possible on a POC platform; however, given the need for signal amplification (as suggested by this review), an instrument-free assay is not conceivable in the near future. Furthermore, sample processing is necessary.

The role for HCVcAg as a substitute for NAT in assessment for SVR remains less clear. While the two studies presented show excellent results, and the quantitative data from PICO5a supports close correlation of HCVcAg with RNA above 3000 IU/mL, the kinetics of HCVcAg with treatment are not fully understood. Particularly, data on the early kinetics of HCVcAg, and the appropriate timing of assessment for predicting SVR are limited.

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Tables

Table 1a. Characteristics of included studies for PICO 5a grouped alphabetically by index test type

Author, year	Country and income category	Study design	Study population	Age group	Number of subjects	Proportion with HIV infection	Proportion with HBV infection	Proportion female	Sample type	Sample condition
Abbott ARCHITECT HCV Ag										
Buket, 2014	Kazakhstan (B)	Cohort	Broad	Adults	115	Unknown	Unknown	56.5%	Serum	Unknown
Chevaliez, 2014	France (A)	Cross-sectional	Broad	Adults	514	Unknown	Unknown	36.6%	Serum	Unknown
Descamps, 2012	France (A)	Cross-sectional	Broad	Adults	22	Unknown	Unknown	40.1%	Serum	Frozen
Durante-Mangoni, 2013	Italy (A)	Cohort	Broad	Adults	114	0 %	0%	43%	Serum	Frozen
Duy Thong, 2015	Thailand (B)	Cohort	Broad	Adults	189	44.9%	0%	28.6%	Serum	Frozen
Ergünay, 2011	Turkey (A)	Cohort	Broad	Mixed	272	Unknown	Unknown	Unknown	Serum	Frozen
Florea, 2014	Romania (B)	Cross-sectional	Broad	Adults	76	0%	0%	75%	Serum	Frozen
Garbuglia, 2014	Italy (A)	Cohort	Broad	Adults	292	100%	3.8%	25.9%	Serum	Frozen
Gu, 2014	China (B)	Cross-sectional	Broad	Unknown	304	Unknown	Unknown	Unknown	Whole	Unknown
Hadziyannis, 2013	Greece (A)	Cross-sectional	Broad	Unknown	105	Unknown	Unknown	Unknown	Serum	Frozen
Heidrich, 2014	Germany (A)	Cohort	Broad	Adults	596	Unknown	Unknown	43%	Serum	Unknown
Kadkhoda, 2014	Canada (A)	Cross-sectional	Broad	Unknown	154	Unknown	Unknown	Unknown	Serum	Unknown
Kesli, 2011	Turkey (A)	Cohort	Healthy	Adults	212	Unknown	Unknown	57.5%	Serum	Unknown
Köroglu, 2012	Turkey (A)	Cohort	Broad	Unknown	32	Unknown	Unknown	45.5%	Serum	Unknown
Kuo, 2012	Taiwan (A)	Cohort	Broad	Adults	405	Unknown	Unknown	52.6%	Serum	Unknown
Li Cavoli, 2012	Italy (A)	Cohort	Broad	Adults	92	1.1%	2.2%	41.3%	Serum	Unknown

Mederacke. 2009	Germany (A)	Cohort	Broad	Unknown	118	0%	0%	Unknown	Serum	Unknown
Mederacke, 2012	Germany (A)	Cross-sectional	Broad	Unknown	237	49.50%	50.50%	Unknown	Serum	Unknown
Medici, 2011	Italy, Spain (A)	Cross-sectional	Broad	Unknown	1480	Unknown	Unknown	52.6%	Serum	Frozen
Miedouge, 2010	France (A)	Cohort	Broad	Unknown	2850	Unknown	Unknown	Unknown	Serum	Frozen
Murayama, 2012	Japan (A)	Cross-sectional	Broad	Unknown	80	Unknown	Unknown	Unknown	Plasma	Frozen
Ottiger, 2013	Switzerland (A)	Cross-sectional	Broad	Adults	97	6%	0%	38.1%	Plasma	Frozen
Park, 2010	South Korea	Cohort	Broad	Adults	282	Unknown	Unknown	49.3%	Serum	Unknown
Reyes-Méndez, 2014	Mexico (B)	Cross-sectional	Broad	Unknown	211	Unknown	Unknown	Unknown	Serum	Unknown
Rouet, 2015	Gabon (B)	Cross-sectional	Broad	Adults	54	100.00%	Unknown	70.1%	Plasma	Frozen
Russi, 2014	Italy (A)	Cohort	Broad	Adults	102	0%	0%	78.4%	Serum	Frozen
Tedder, 2013	UK (A)	Cohort	Broad	Unknown	54	0%	0%	Unknown	Plasma	Frozen
van Helden, 2014	Germany (A)	Cross-sectional	Broad	Unknown	3558	4.40%	6.60%	Unknown	Serum	Unknown
Vanhommerig. 2015	Netherlands (A)	Cohort	Broad	Unknown	93	100.00%	Unknown	0%	Serum	Unknown
Vermehren, 2012	Germany (A)	Cohort	Broad	Adults	160	0%	0%	54%	Serum	Frozen

Author, year	Country and income category	Study design	Study population	Age group	Number of subjects	Proportion with HIV infection	Proportion with HBV infection	Proportion female	Sample type	Sample condition
Bio-RAD Monolisa™ HCV Ag-Ab ULTRA										
Laperche, 2005	France (A)	Cohort	Broad	Unknown	35	Unknown	Unknown	Unknown	Plasma	Frozen
Nastouli, 2008	UK (A)	Cohort	Broad	Adults	25	100%	Unknown	0%	Serum	Frozen
Schnuriger, 2006	France (A)	Cohort	Broad	Adults	20	100%	Unknown	Unknown	Serum	Frozen
Tuke, 2008	UK (A)	Cross-sectional	Broad	Unknown	Unknown	Unknown	Unknown	Unknown	Plasma	Frozen
Vermeersch, 2010	Belgium (A)	Cross-sectional	Broad	Unknown	337	Unknown	Unknown	Unknown	Serum	Unknown
EIKEN Lumispot HCV Ag										
Saito, 2003	Japan (A)	Cross-sectional	Broad	Unknown	155	Unknown	Unknown	Unknown	Serum	Frozen
Murayama, 2012	Japan (A)	Cross-sectional	Broad	Unknown	80	Unknown	Unknown	Unknown	Plasma	Frozen
Fujirebio Lumipulse Ortho HCV Ag										
Murayama, 2012	Japan (A)	Cross-sectional	Broad	Unknown	80	Unknown	Unknown	Unknown	Plasma	Frozen
Hunan Jynda Bioengineering Group HCV Core Ag ELISA										
Lu, 2007	China (B)	Cohort	Broad	Unknown	191	Unknown	Unknown	Unknown	Serum	Unknown
Ouyang, 2006	China (B)	Cross-sectional	Broad	Unknown	149	Unknown	Unknown	Unknown	Serum	Unknown
Zhang, 2007	China (B)	Cohort	Healthy	Unknown	11	Unknown	Unknown	Unknown	Serum	Frozen
Zhu, 2010	China (B)	Cross-sectional	Broad	Mixed	173	Unknown	Unknown	Unknown	Serum	Unknown
DiaSorin S.A. Murex Ag/Ab EIA										
Alzahrani, 2008	Saudi Arabia (A)	Cohort	Broad	Adults	118	0.7%	6.1%	100%	Serum	Unknown
El-Emshaty, 2011	Egypt (B)	Cohort	Broad	Adults	39	Unknown	Unknown	69.2%	Serum	Frozen
Tuke, 2008	UK (A)	Cross-sectional	Broad	Unknown	unknown	Unknown	Unknown	Unknown	Plasma	Frozen
Yang, 2011	Taiwan (A)	Cohort	Broad	Adults	201	25%	0%	39%	Serum	Frozen

Ortho ELISA-Ag										
Agha, 2004	Egypt, Japan, Uzbekistan (AB)	Cohort	Broad	Unknown	246	Unknown	Unknown	Unknown	Serum	Unknown
El-Sayed, 2004	Egypt (B)	Cross-sectional	Broad	Unknown	50	Unknown	Unknown	Unknown	Serum	Frozen
Letowska, 2004	Poland (A)	Cohort	Healthy	Unknown	124	Unknown	Unknown	Unknown	Serum	Unknown
Nübling, 2002	USA (A)	Cohort	Broad	Unknown	52	Unknown	Unknown	Unknown	Plasma	Frozen
Ohta, 2004	Japan (A)	Cross-sectional	Broad	Unknown	225	Unknown	Unknown	Unknown	Serum	Unknown
Okazaki, 2008	Japan (A)	Cohort	Broad	Unknown	300	Unknown	Unknown	50.3%	Serum	Unknown

HCV: hepatitis C virus, HIV: human immunodeficiency virus, HBV: hepatitis B virus, Ag: antigen, Ab: antibody, ELISA: enzyme-linked immunosorbent assay, EIA: enzyme immunoassay, UK: United Kingdom, USA: United States of America, A: high-income countries, B: middle-income countries, C: low-income countries by World Bank List of Economies (July 2015)

Table 1b. Characteristics of included studies for PICO 9 grouped alphabetically by index test type

Author, year	Country and income category	Study design	Study population	Age group	Number of subjects	Proportion with HIV infection	Proportion with HBV infection	Proportion female	Sample type	Sample condition
Abbott ARCHITECT HCV Ag										
Feng, 2014	China (B)	RCT	Broad	Adults	32	0%	0%	50%	Serum	Unknown
Loggi, 2013	Italy (A)	Cohort	Broad	Adults	35	0%	0%	34.4%	Serum	Frozen
Moscato, 2010	Italy (A)	Cohort	Broad	Unknown	23	Unknown	Unknown	Unknown	Serum	Frozen
Fujirebio Lumipulse Ortho HCV Ag										
Fujino, 2009	Japan (A)	Cohort	Broad	Adults	90	Unknown	Unknown	24%	Serum	Unknown
Takahashi, 2005	Japan (A)	Cohort	Broad	Adults	44	Unknown	0%	31.8%	Serum	Unknown

HCV: hepatitis C virus, HIV: human immunodeficiency virus, HBV: hepatitis B virus, Ag: antigen, Ab: antibody, RCT: randomized controlled trial, A: high-income countries, B: middle-income countries, C: low-income countries by World Bank List of Economies (July 2015)

Table 2. Diagnostic accuracy by HCVcAg index test type for diagnosis of active HCV infection compared to nucleic acid testing as the reference standard. Results from bivariate, univariate, range of studies, and single studies are all reported.

Index Test	HCV Ab status	# Studies (# samples)	Sensitivity 95% CI	Specificity 95% CI	Positive LR 95% CI	Negative LR 95% CI	τ^2 [Covariance]
Abbott ARCHITECT[1]	All	20 (11,820)	93.4%[1] (88.7, 96.2)	98.7%[1] (96.9, 99.4)	71.8 (28.6, 160.3)	0.07 (0.04, 0.12)	Sens: 1.5 (SE 0.6) Spec: 2.3 (SE 1.0); [0.03]
Abbott ARCHITECT[2]	All	30 (12,788)	94.1%[2] (92.4, 95.7)	ND	NA	NA	Sens: 14.1
Abbott ARCHITECT[1]	Known Ab positive	16 (5,246)	92.5%[1] (86.9, 95.8)	97.8%[1] (94.7, 99.1)	42 (16.4, 106.4)	0.05 (0.03, 0.08)	Sens: 1.4 (SE 0.5) Spec: 1.7 (SE 1.0); [0.02]
Abbott ARCHITECT[2]	Known Ab positive	26 (6,214)	93.3%[2] (91.2, 95.3)	ND	NA*	NA*	Sens: 19.4
Abbott ARCHITECT[1]	Known Ab negative	4 (3,458)	74.4%[1] (6.2, 99.2)	98.8%[1] (97.2, 99.5)	62 (2, 198.5)	0.25 (0.003, 0.94)	Sens: 8.4 (SE 16.6) Spec: 0.2 (SE 0.6)
Ortho ELISA-Ag[1]	All	5 (1,177)	93.2%[1] (81.6, 97.7)	99.2%[1] (87.9, 100)	116.5 (6.7, 977)	0.06 (0.02, 0.07)	Sens: 1.4 (SE 1.0) Spec: 3.8 (SE 5.1); [–0.4]
Ortho ELISA-Ag[2]	All	6 (1,423)	90.8%[2] (83.5, 98.2)	ND	NA	NA	122.0
Bio-RAD Monolisa™ HCV Ag-Ab ULTRA*	All	5 (525)	28.6–95%*	94.9%** (89.9, 99.8)	NA	NA	NA
EIKEN Lumispot HCV Ag	All	2 (235)	97.5–98.1%*	ND	NA	NA	NA
Fujirebio Lumipulse Ortho HCV Ag[3]	All	1 (80)	95%** (90.2, 99.8)	ND	NA	NA	NA
Hunan Jynda Bioengineering Group HCV Core Ag ELISA[1]	All	4 (524)	59.5%[1] (46, 71.7)	82.9%[1] (58.6, 94.3)	3.5 (1.1, 12.6)	0.28 (0.2, 0.3)	NA+
Murex Ag/Ab EIA	All	4 (770)	50–100%*	83.8–100%*	NA	NA	NA

HCV: hepatitis C virus, cAg: core antigen, Ab: antibody, CI: confidence interval, LR: likelihood ratio, ELISA: enzyme linked immunosorbent assay, EIA: enzyme immunoassay, $\tau^{2:}$ Tau squared, SE: standard error**.** 1. Determined by bivariate meta-analysis – "metandi" command in STATA, 2. Determined by univariate meta-analysis – "metan" command in STATA**,** *: Meta-analysis not possible, range of results seen across studies reported, **: results from one study only, ND: no data, NA: not applicable – if sensitivity and specificity results were not available from meta-analysis, likelihood ratios were not calculated; +: output of τ^2 not interpretable given small number of studies

Table 3a. Available genotype information for studies included in PICO 5a grouped alphabetically by index test type

Author, year	Number of subjects	% G1	% G1a	% G1b	%G2	%G3	%G4	%G5	%G6	% Other or unknown
Abbott ARCHITECT HCV Ag										
Chevaliez, 2014	514	59.3%			5%	12.3%	19.2%	1%	1.9%	1.9%
Descamps, 2012	22	68.2%								31.8%
Durante-Mangoni, 2013	114	49%			31%	20%				
Duy Thong, 2015	189	35.4%			0%	44.9%			19.6%	
Ergünay, 2011	272	0.8%	2.2%	60.2%		0.4%	0.4%			35.8%
Garbuglia, 2014	292	17.1%	14.5%	9.4%	1%	27.6%	15.4%			15%
Hadziyannis, 2013	105	36%			6%	37%	21%			
Kesli, 2011	212			100%						
Li Cavoli, 2012	92	95%			5%					
Mederacke. 2009	118	45.8%			10%	19%				24.6%
Miedouge, 2010	2850	2%	8.2%	17.3%	15.3%	17.3%	11.2%	8.2%	3.1%	17.3%
Ottiger, 2013	97	30.9%	19.5%	10.3%	23.7%	15.5%				30.9%
Russi, 2014	102	50%			48.1%	1.9%				
Tedder, 2013	54		40.7%	22.2%	20.4%	16.7%				
Vermehren, 2012	160	19%	29%	51%						

Bio-RAD Monolisa™ HCV Ag-Ab ULTRA										
Laperche, 2005	35	5.7%	11.4%	34.3%	25.7%	14.3%	5.7%			2.9%
Nastouli, 2008	25		68%	4%		4%	16%			8%
Schnuriger, 2006	20	20%	15%		5%	30%	45%			
Tuke, 2008*	Unknown	62%			23%	15%				
EIKEN Lumispot HCV Ag										
Saito, 2003	155	65.2%			35.80%					
Ortho ELISA-Ag										
Agha, 2004	246	37%			9.8%	5.8%	47.3%			
Nübling, 2002	52	11.5%	42.3%	19.2%	11.5%	15.4%				

HCV: hepatitis C virus, Ag: antigen, Ab: Antibody, ELISA: enzyme linked immunosorbent assay, G1: genotype 1, G1a: genotype 1a, G1b: genotype 1b, G2: genotype 2, G3: genotype 3, G4: genotype 4, G5: genotype 5, G6: genotype 6

* Data are the same for DiaSorin S.A. Murex Ag/Ab EIA

Table 3b. Genotype information for studies included in PICO 9 grouped alphabetically

Author, year	Number of subjects	% G1	% G1a	% G1b	%G2	%G3	%G4	%G5	%G6	% Other or unknown
Abbott ARCHITECT HCV Ag										
Feng, 2014	32			100%						
Loggi, 2013	35	100%	20%	80%						
Moscato, 2010	23		4%	39.1%	26.1%	21.7%	8.7%			
Fujirebio Lumipulse Ortho HCV Ag										
Fujino, 2009	90			66.7%	33.3%					
Takahashi, 2005	44			100%						

HCV: hepatitis C virus, Ag: antigen, Ab: antibody, G1 = genotype 1, G1a = genotype 1a, G1b = genotype 1b, G2 = genotype 2, G3 = genotype 3, G4 = genotype 4, G5 = genotype 5, G6 = genotype 6

Table 4. Sensitivity and specificity of Abbott ARCHITECT HCV Ag assay compared to nucleic acid testing (NAT) assessed at baseline, at week 4 of interferon based therapy (early viral response), and at week 24 after completion of treatment (sustained viral response)

		Baseline		Early viral response (EVR)		Sustained viral response (SVR)	
Author, Year	**Number of subjects**	**Se (95% CI)**	**Sp (95% CI)**	**Se (95% CI)**	**Sp (95% CI)**	**Se (95% CI)**	**Sp (95% CI)**
Feng, 2014	32	100%	NA	100%	88.9% (68.4%, 100%)	100%	100%
Loggi, 2013	35	100%	NA	73.5% (58.7%, 88.4%)	100%	100%	94.1% (82.9%, 100%)
Moscato, 2010	23	NA	NA	100%	70% (41.6%, 98.4%)	NA	NA

HCV: hepatitis C virus, Ag: antigen, Se: sensitivity, Sp: specificity, CI: confidence interval, NA: not applicable as cannot be calculated from study data

Table 5. Sensitivity and specificity of HCV core antigen assay in prediction of sustained viral response (SVR) after initiation of interferon-based treatment

Author, Year	No. of subjects (no.to achieve SVR)	Index test	Timing of test after treatment start	Change in HCVcAg	Sensitivity	Specificity
Feng, 2014	32 (21)	Abbott ARCHITECT	6 days	Log 10	95.2%	70%
Fujino, 2009	90 (57)	Fujirebio Lumipulse	7 days	Absolute	79.4%	88.5%
Takahashi, 2005	44 (10)	Fujirebio Lumipulse	7 days	Absolute	57.1%	93.3%

HCV: hepatitis C virus, No.: number

Figures

Fig. 1a. PRISMA diagram of studies excluded from screen one, and those full papers retrieved for more detailed evaluation

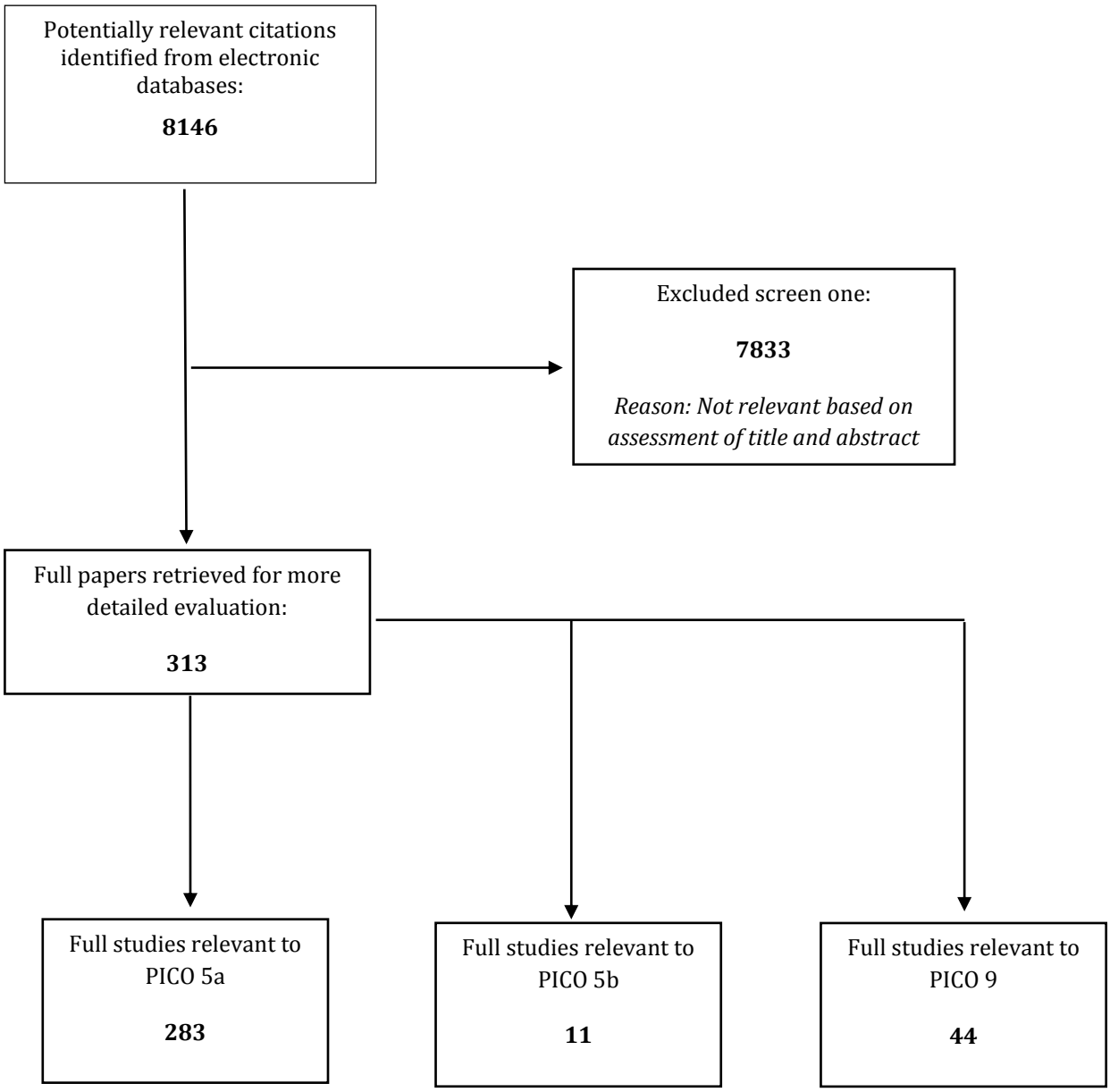


Fig. 1b. PRISMA diagram of studies included in the review for PICO 5a

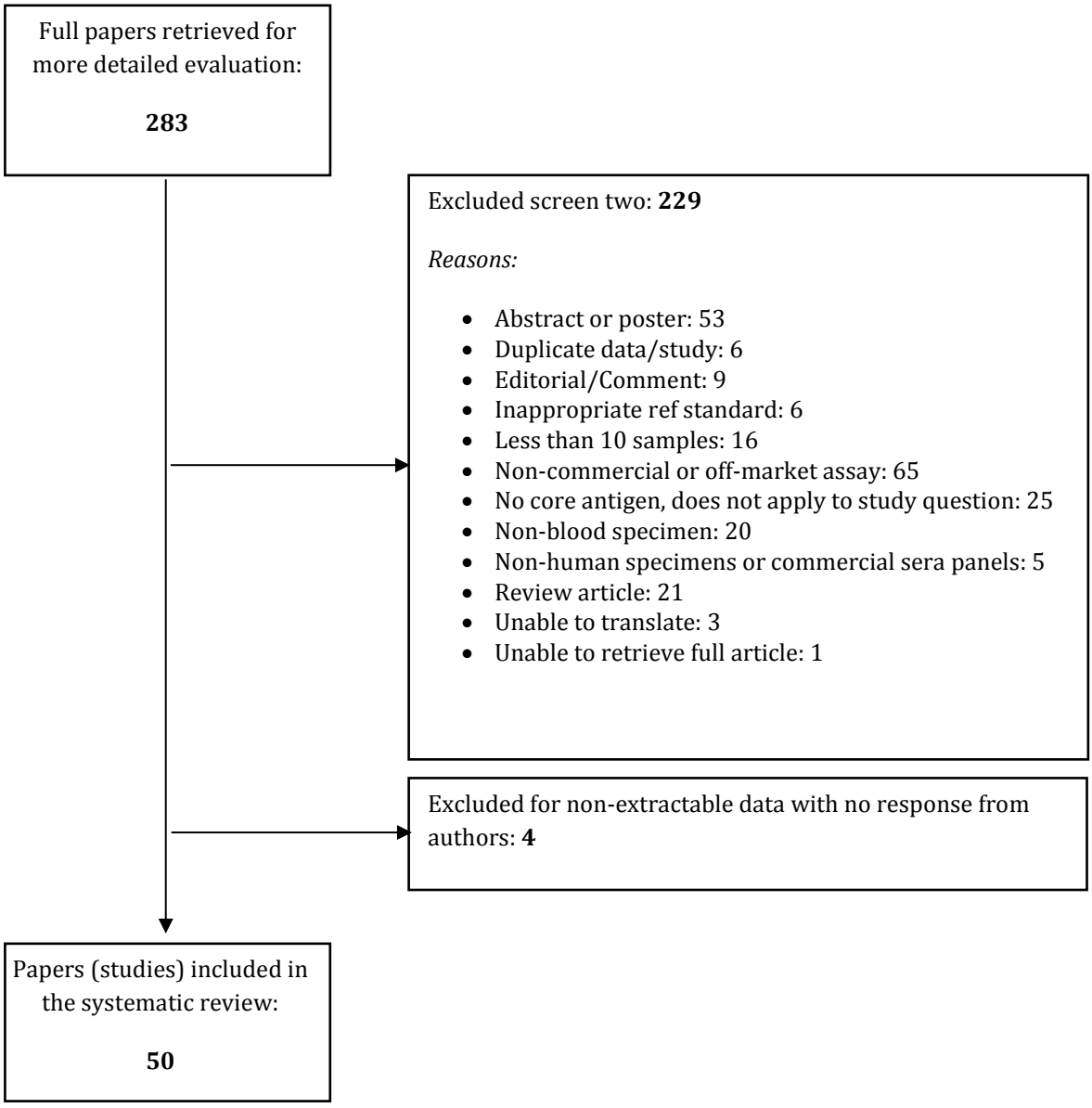


Fig. 1c. PRISMA diagram of studies included in the review for PICO 5b

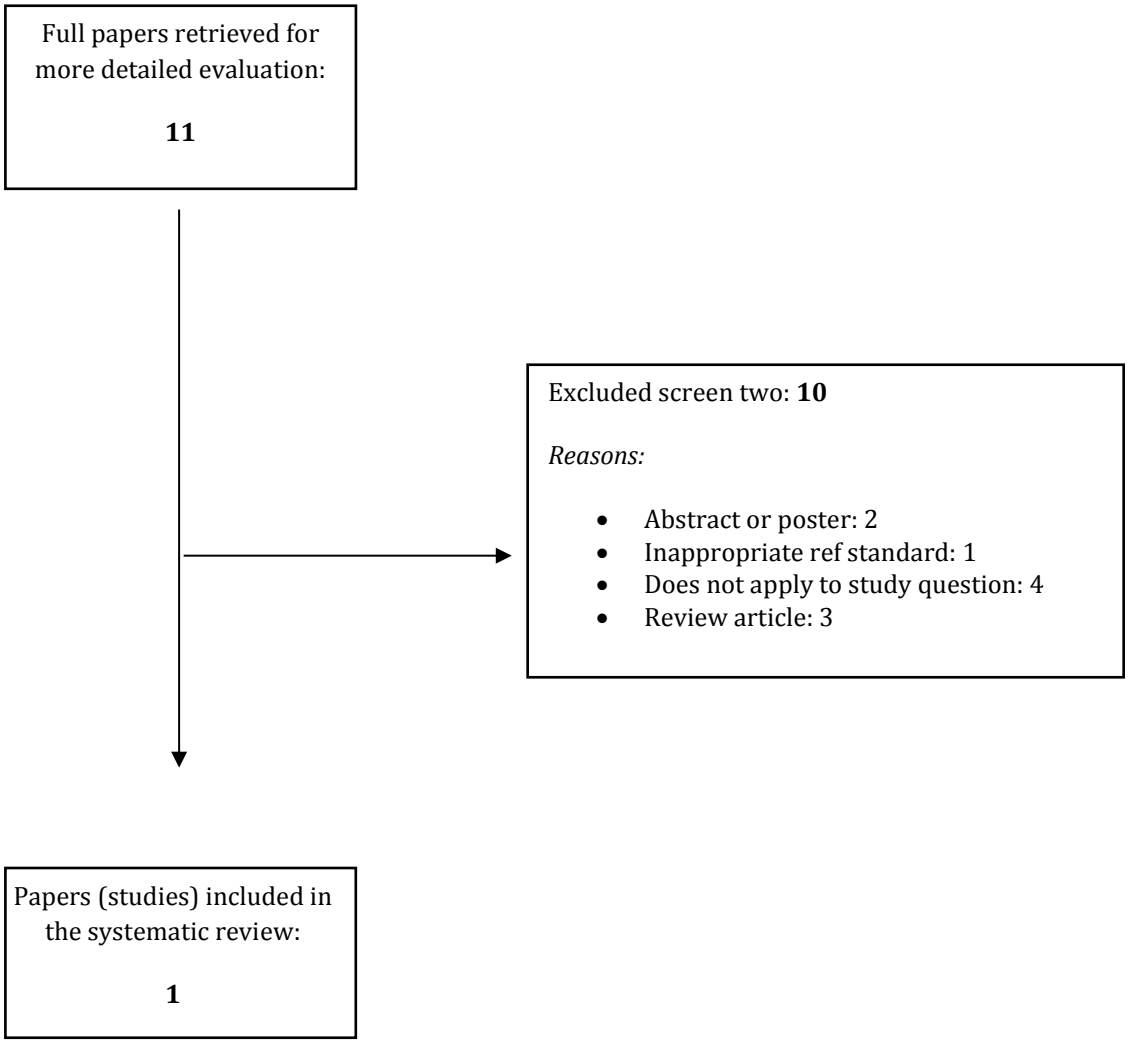
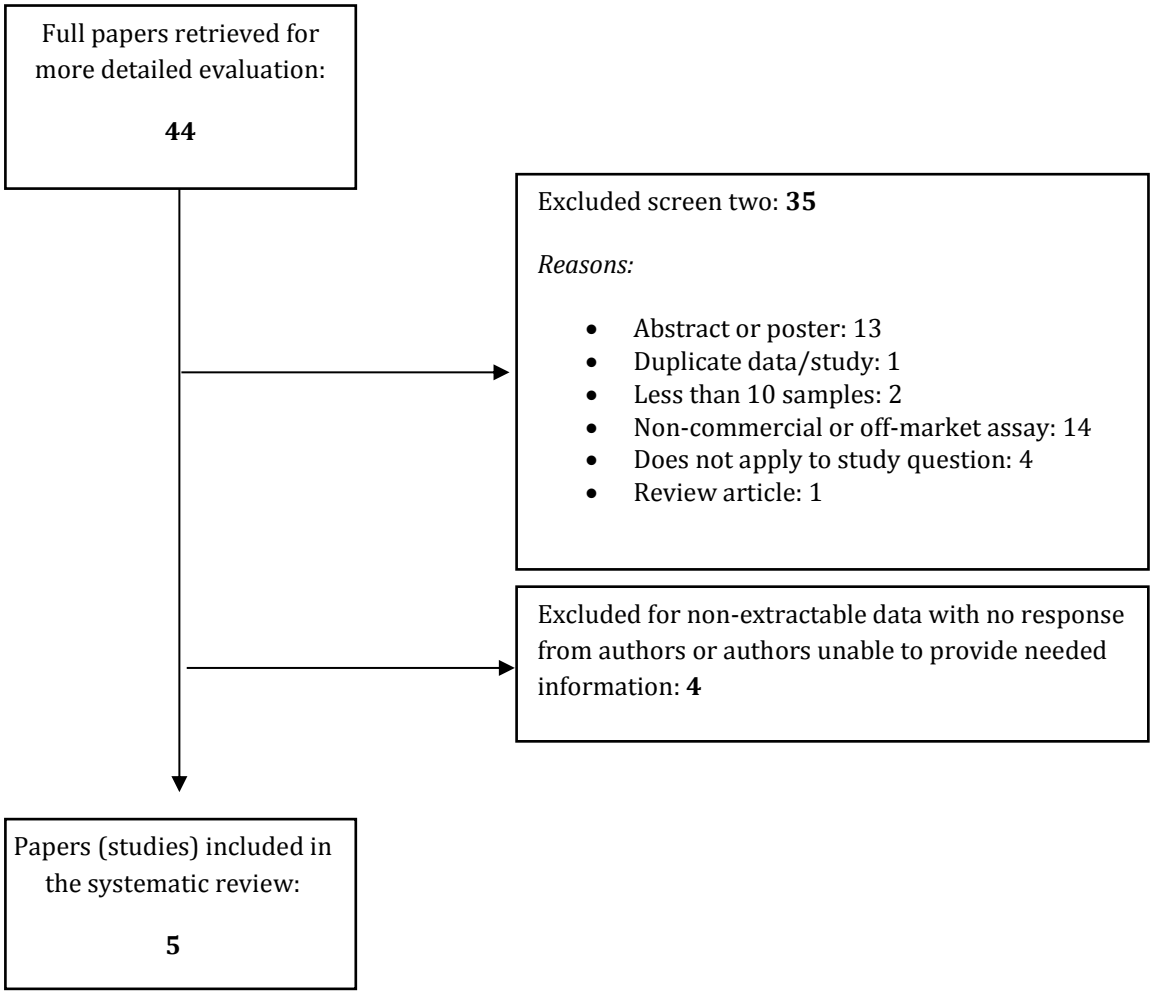


Fig. 1d. PRISMA diagram of studies included in the review for PICO 9



QUADAS figures

Fig. 2a. Risk of bias and applicability summary as judged by review authors about each QUADAS-2 domain presented as percentages across the 50 included studies for PICO 5a

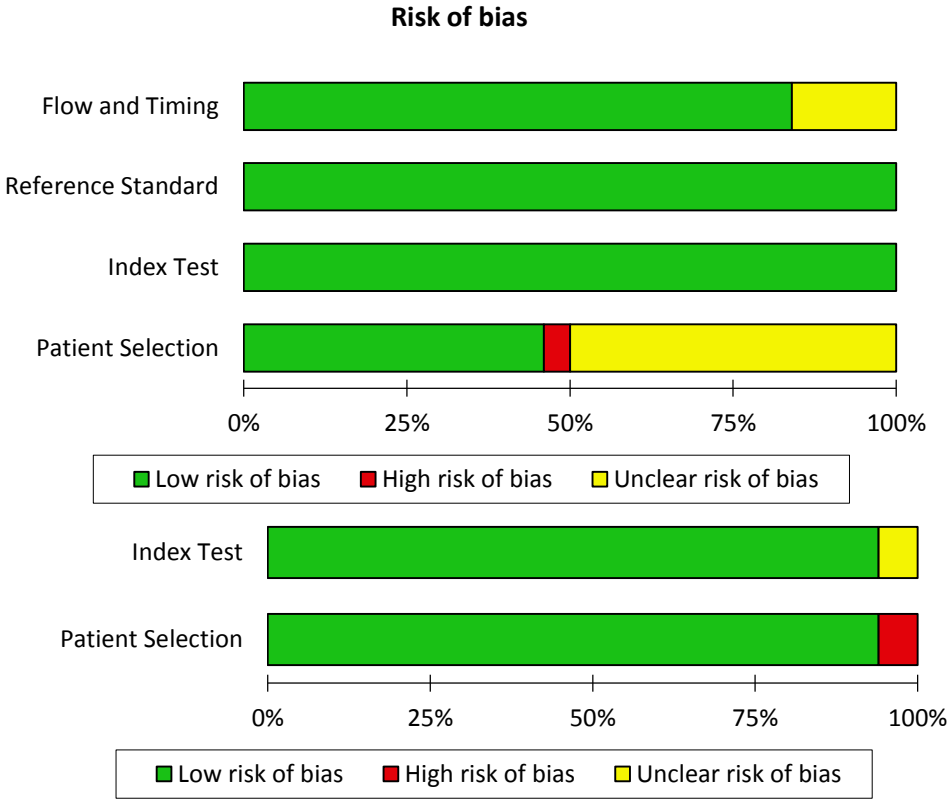
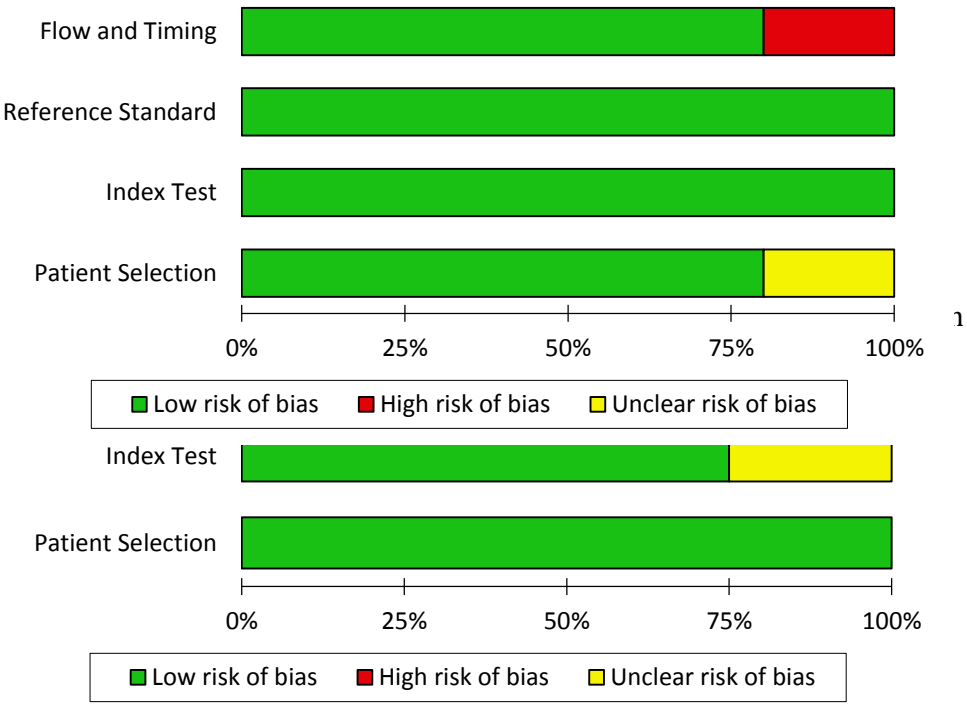


Fig. 2b. Risk of bias and applicability summary as judged by review authors about each QUADAS-2 domain presented as percentages across the 5 included studies for PICO 9

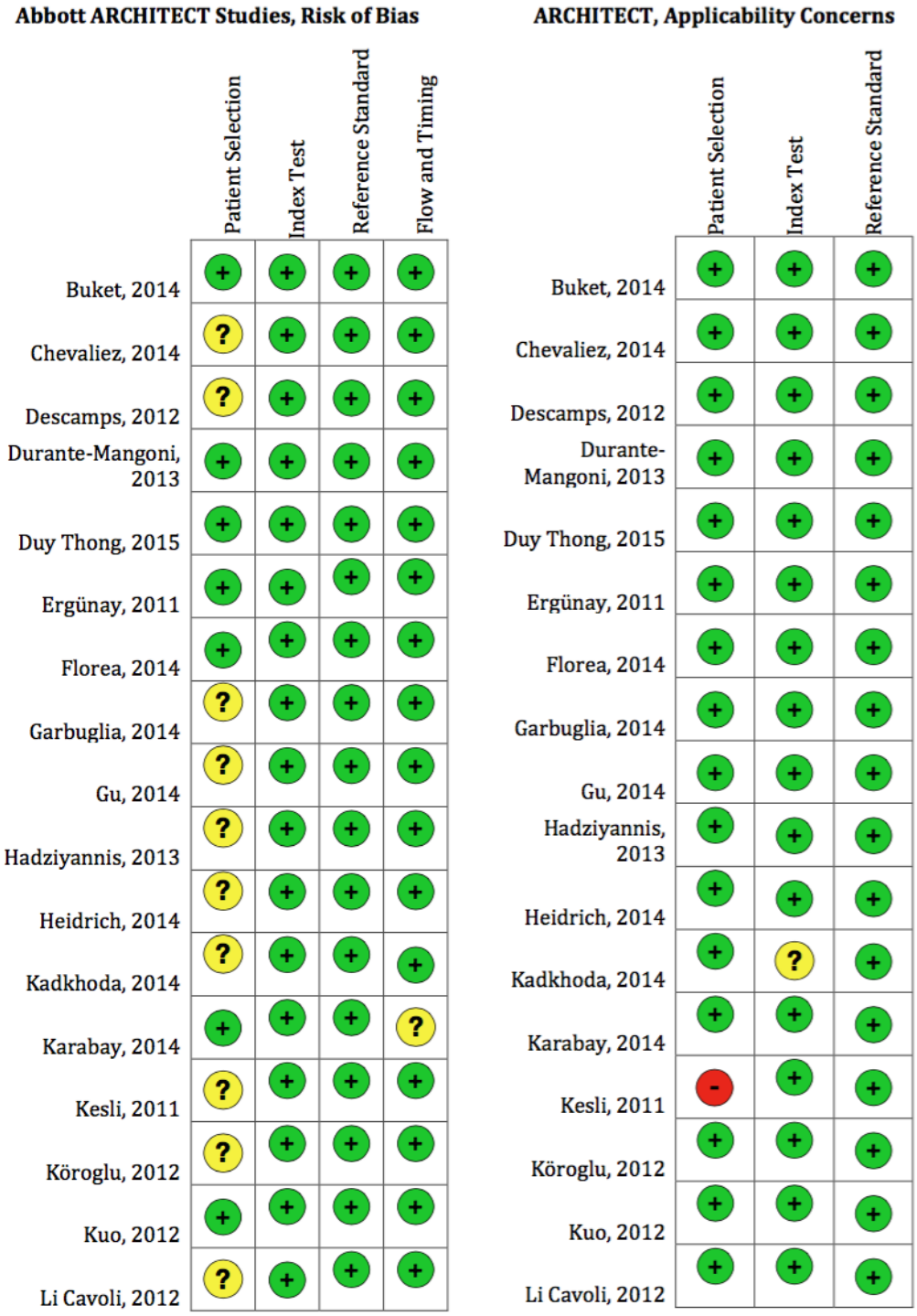
Risk of bias



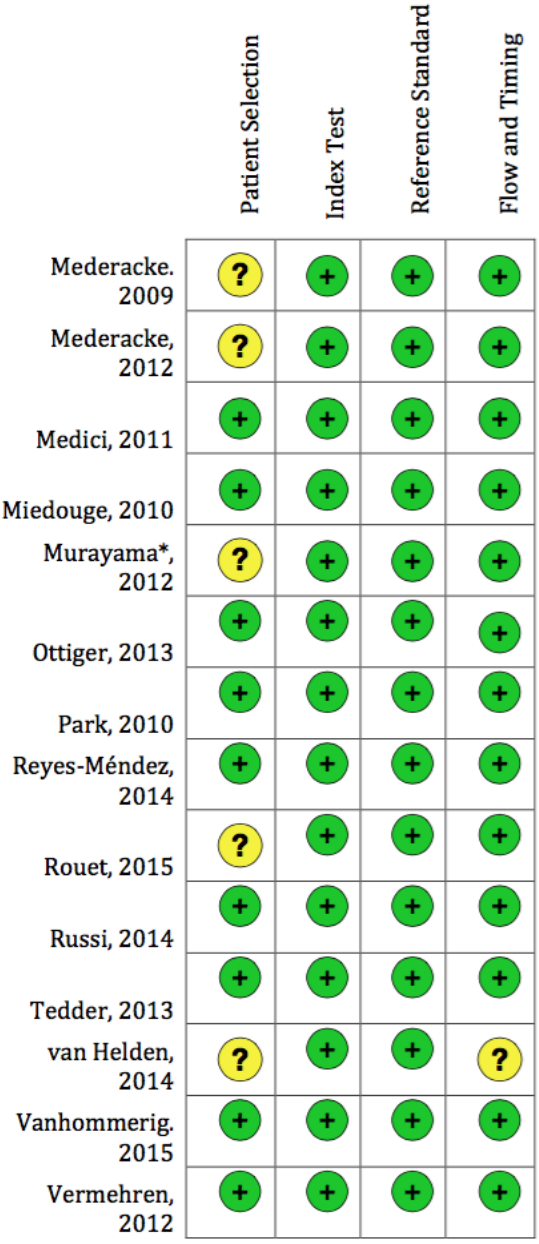
Applicability

Fig. 3a. Risk o
QUADAS-2 dom

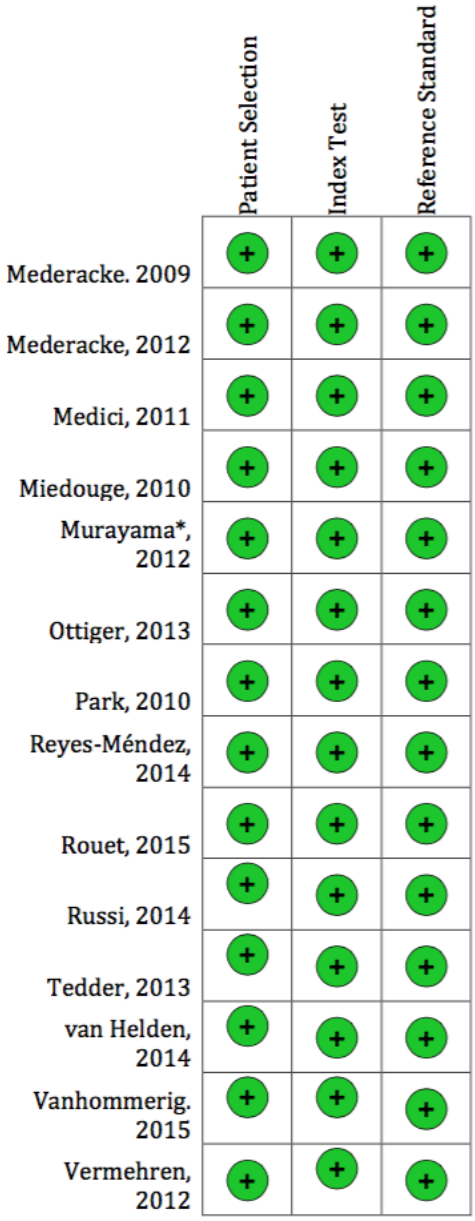
Fig. 3a (cont). Risk of bias and applicability summary as judged by review authors about each QUADAS-2 domain presented by individual study for PICO 5a



Risk of Bias, ARCHITECT Studies (Cont.)

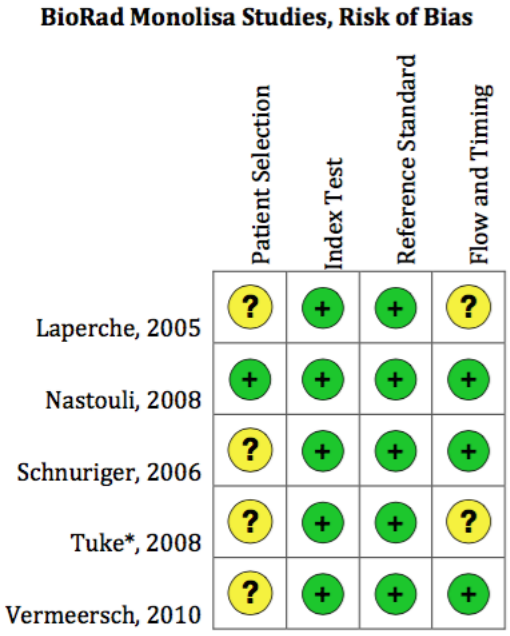


ARCHITECT, Applicability Concerns (Cont.)

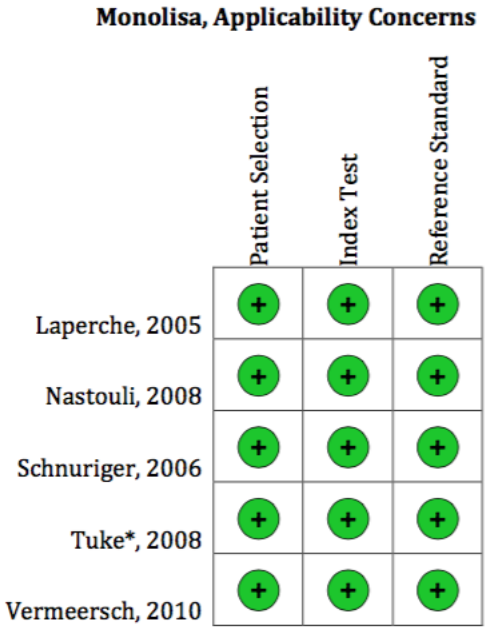


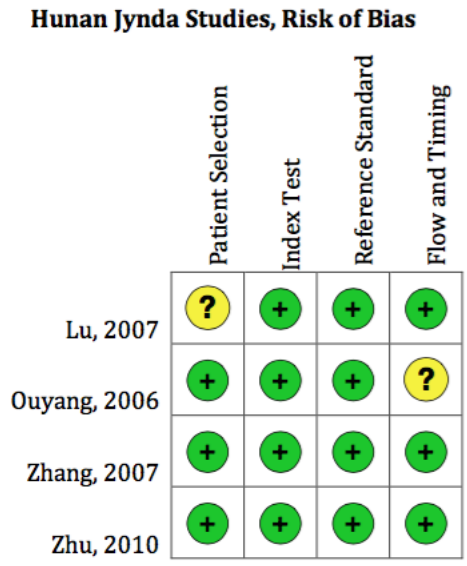
*Same data for Fujirebio Lumipulse Ortho HCV Ag and EIKEN Lumispot HCV Ag

Fig. 3a (cont). Risk of bias and applicability summary as judged by review authors about each QUADAS-2 domain presented by individual study for PICO 5a



*Same data for DiaSorin S.A. Murex Ag/Ab EIA





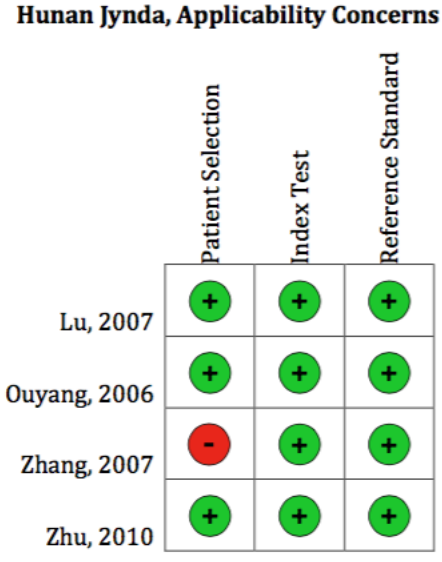


Fig. 3 (cont). Risk of bias and applicability summary as judged by review authors about each QUADAS-2 domain presented by individual study for PICO 5a

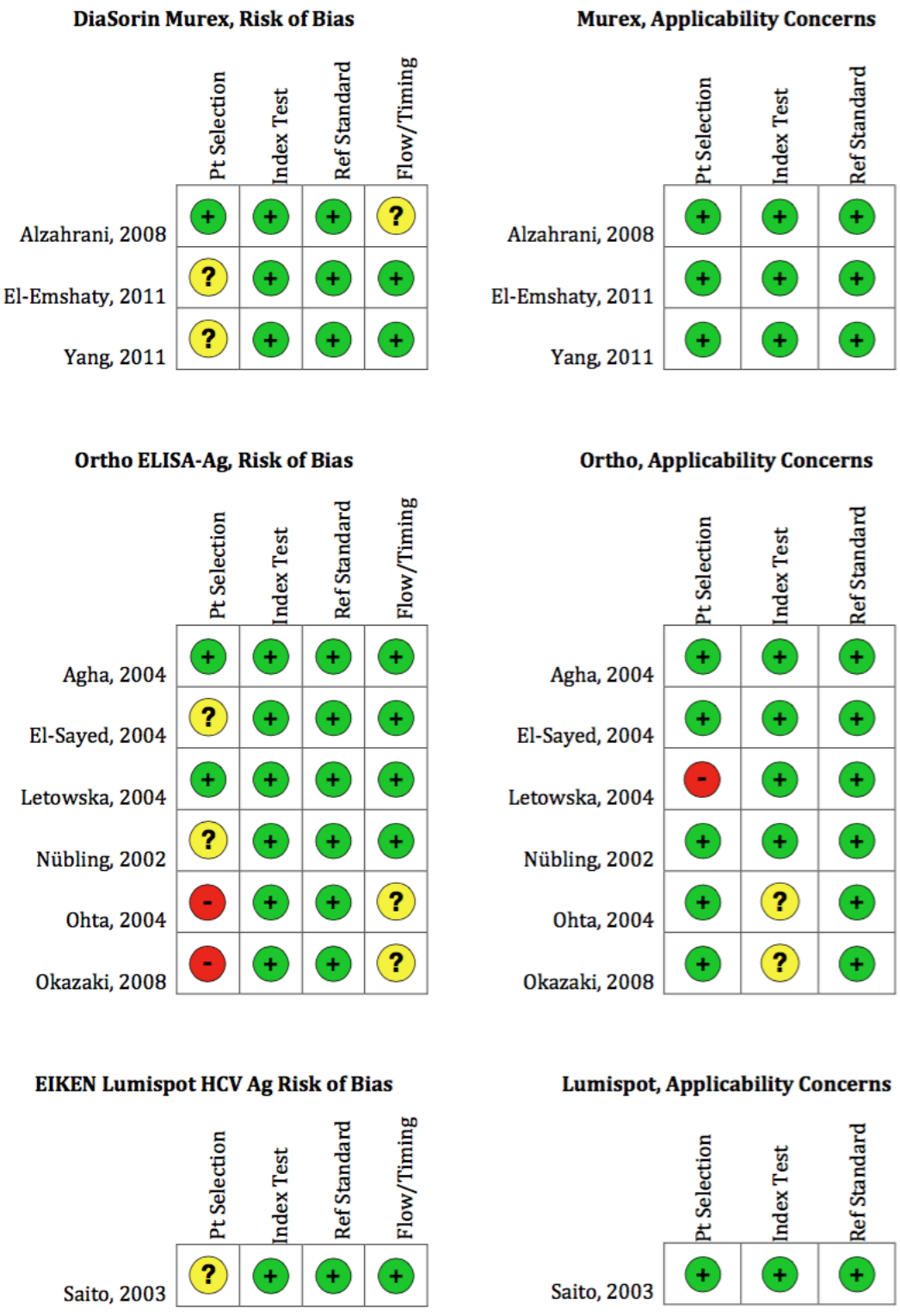


Fig. 3b. Risk of bias and applicability summary as judged by review authors about each QUADAS-2 domain presented by individual study for PICO .

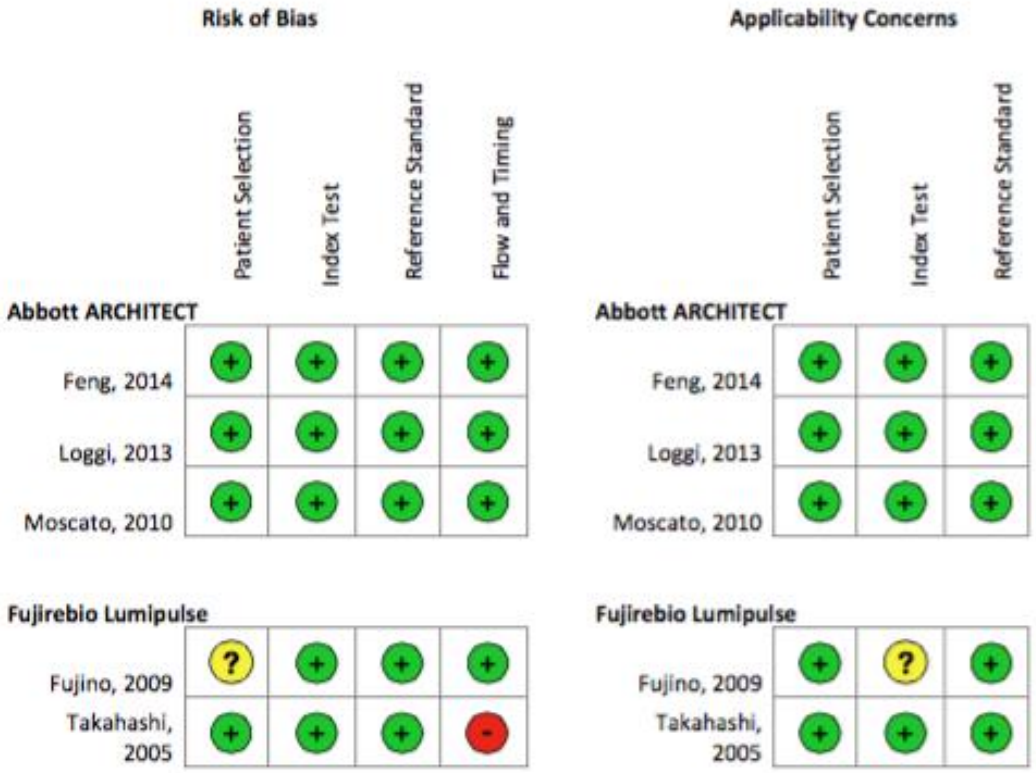
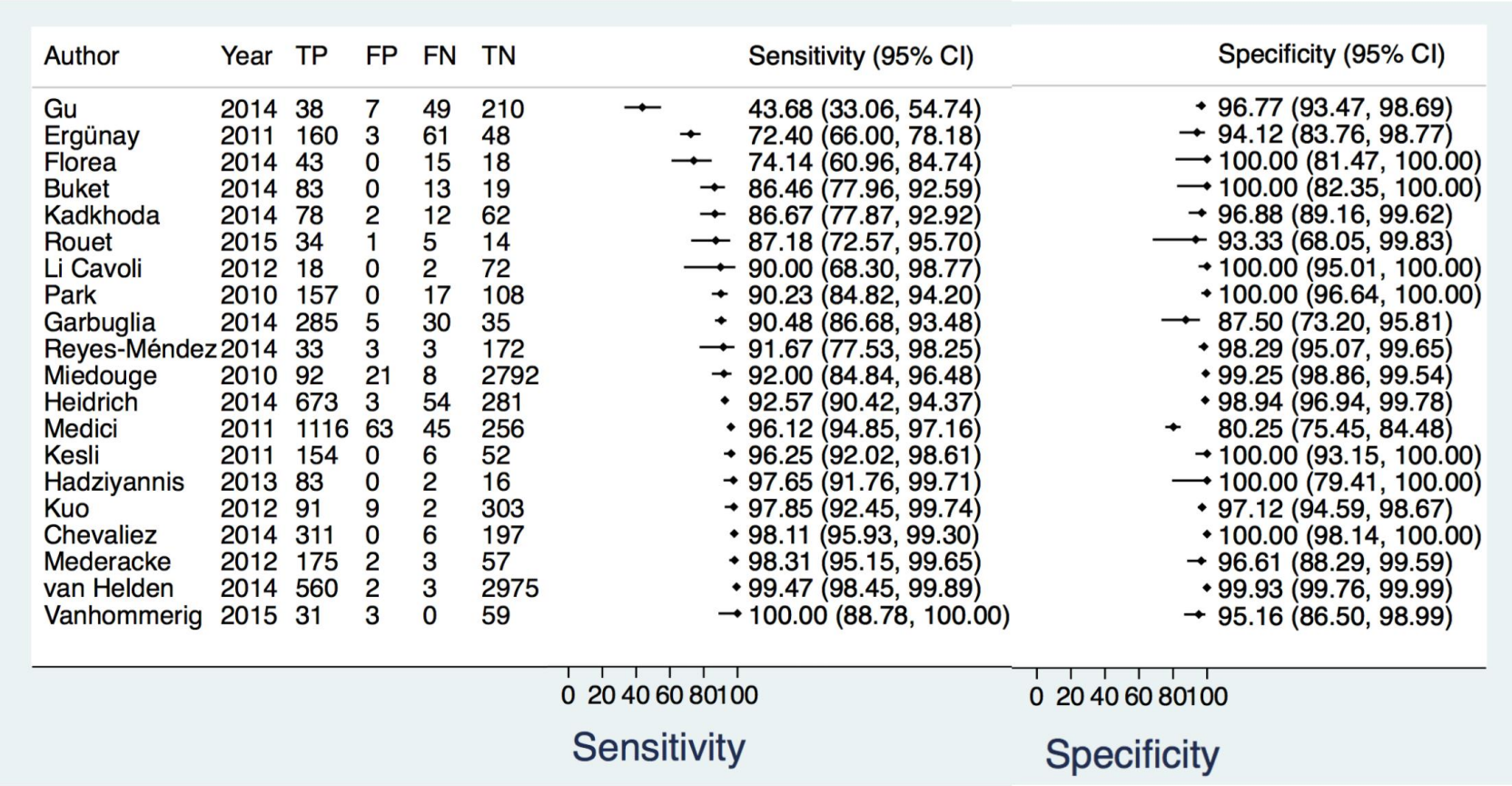
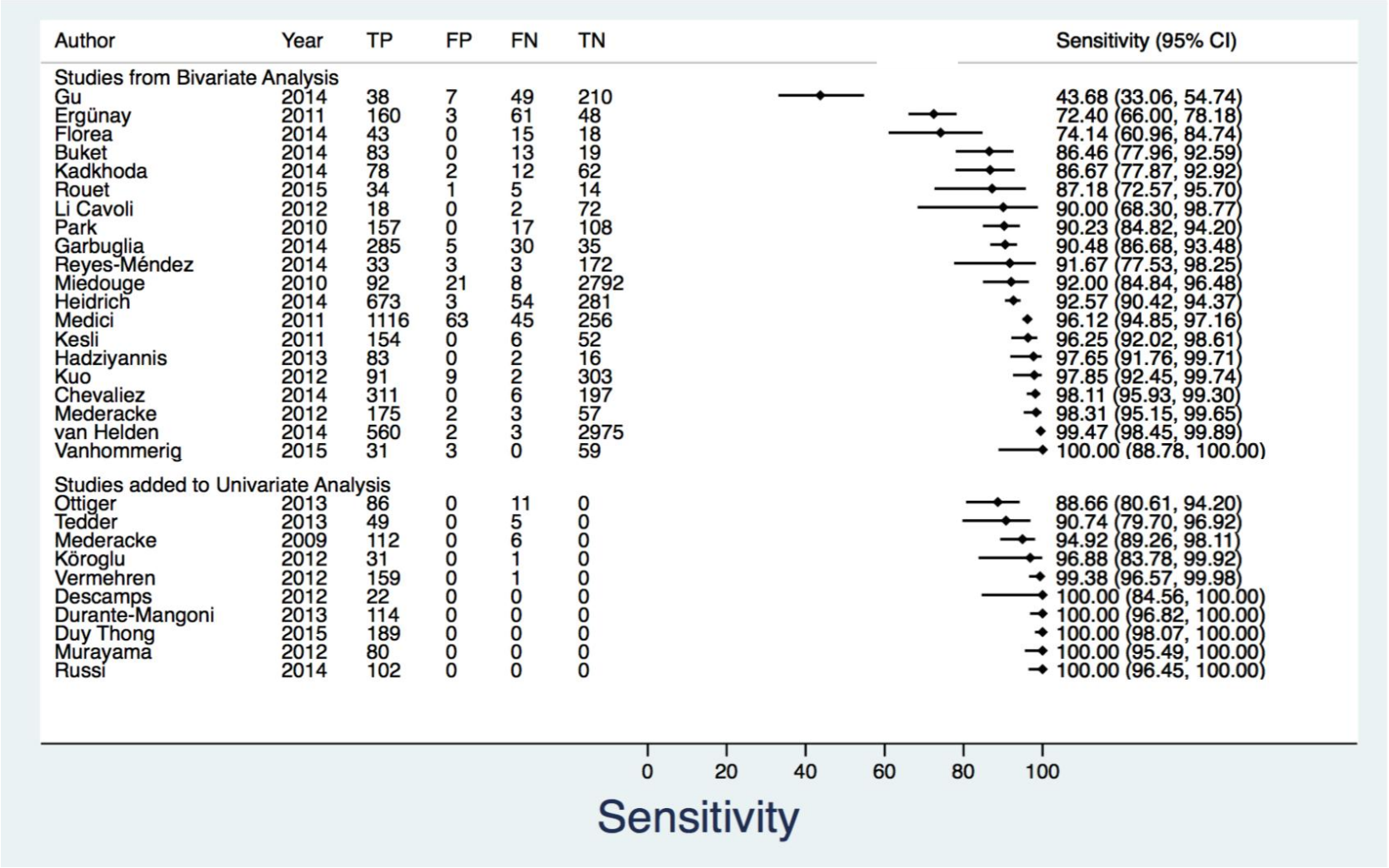


Fig. 4a. Forest plot of Abbott ARCHITECT HCV Ag assay sensitivity and specificity for the diagnosis of active HCV infection compared to NAT reference test for all samples regardless of HCV Ab status



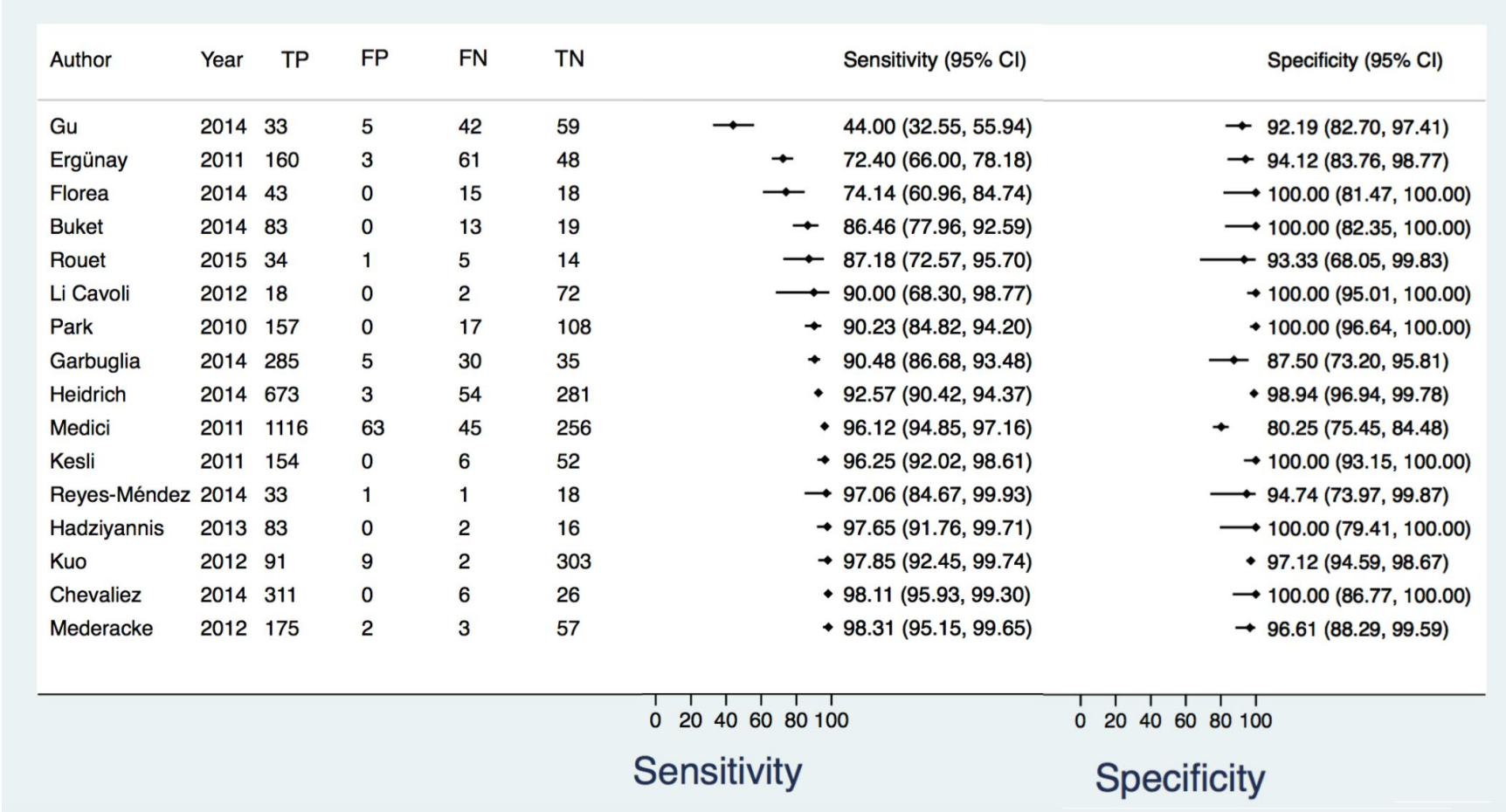
HCV: hepatitis C virus, Ag: antigen, NAT: nucleic acid testing, Ab: antibody, TP: true positive, FP: false positive, FN: false negative, TN: true negative, CI: confidence interval

Fig. 4b. Univariate analysis of Abbott ARCHITECT HCV Ag Assay sensitivity for the diagnosis of active HCV infection compared to NAT reference test for all studies with sensitivity data regardless of HCV Ab status



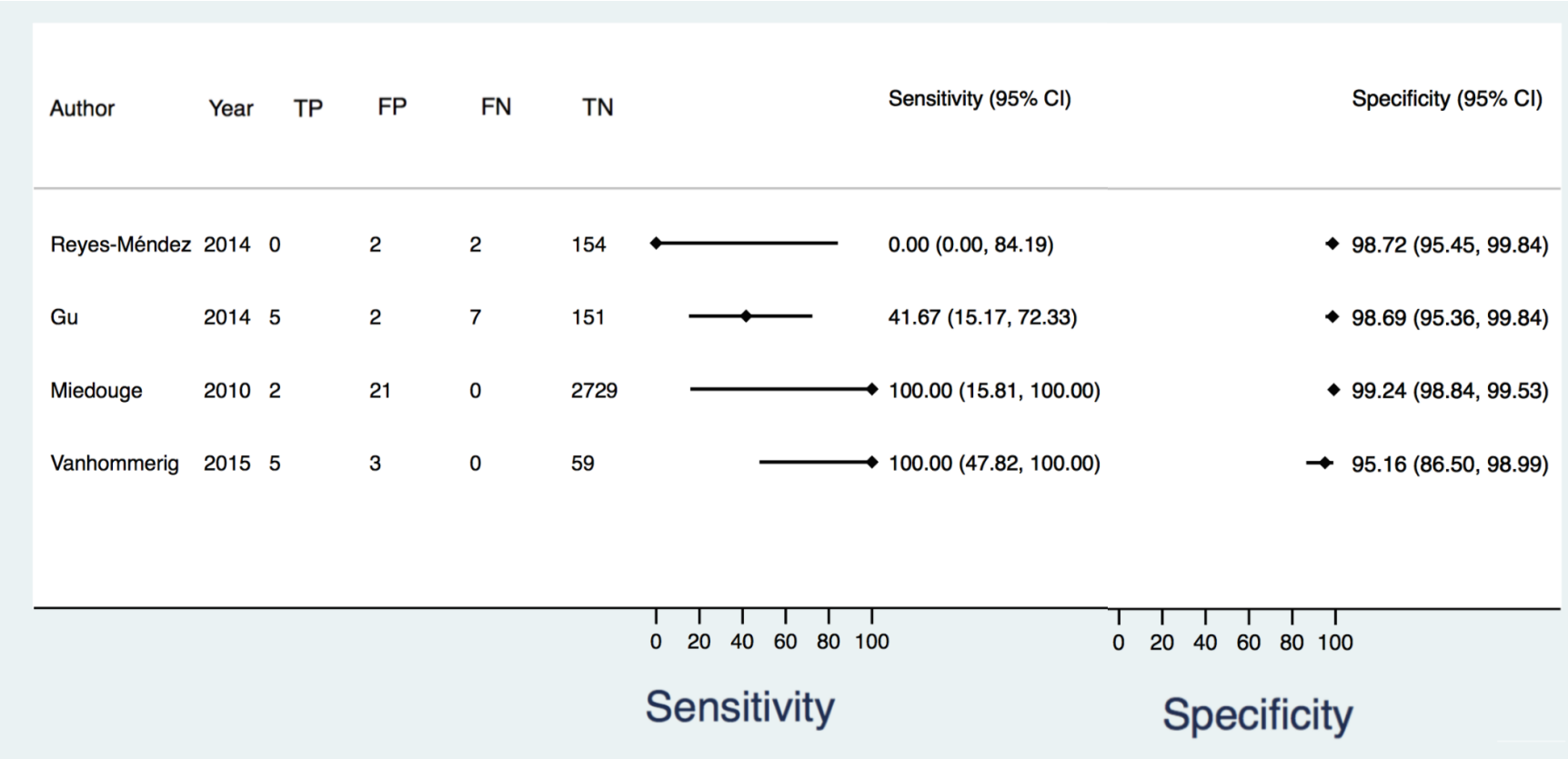
HCV: hepatitis C virus, Ag: antigen, NAT: nucleic acid testing, Ab: Antibody, TP: true positive, FP: false positive, FN: false negative, TN: true negative, CI: confidence interval

Fig. 4c. Forest plot of Abbott ARCHITECT HCV Ag assay sensitivity and specificity for the diagnosis of active HCV infection compared to NAT reference test for known HCV antibody-positive samples



HCV: hepatitis C virus, Ag: antigen, NAT: nucleic acid testing, Ab: antibody, TP : true positive, FP: false positive, FN: false negative, TN: true negative, CI: confidence interval

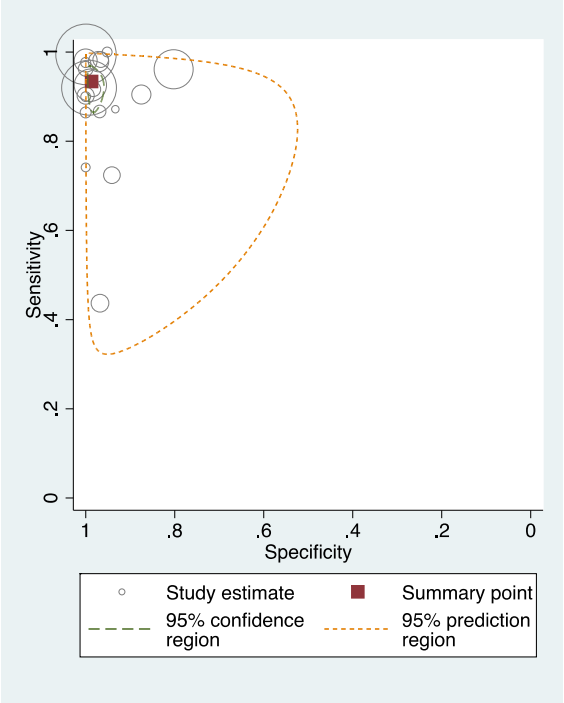
Fig. 4d. Forest plot of Abbott ARCHITECT HCV Ag assay sensitivity and specificity for the diagnosis of active HCV infection compared to NAT reference test for known HCV antibody-negative samples



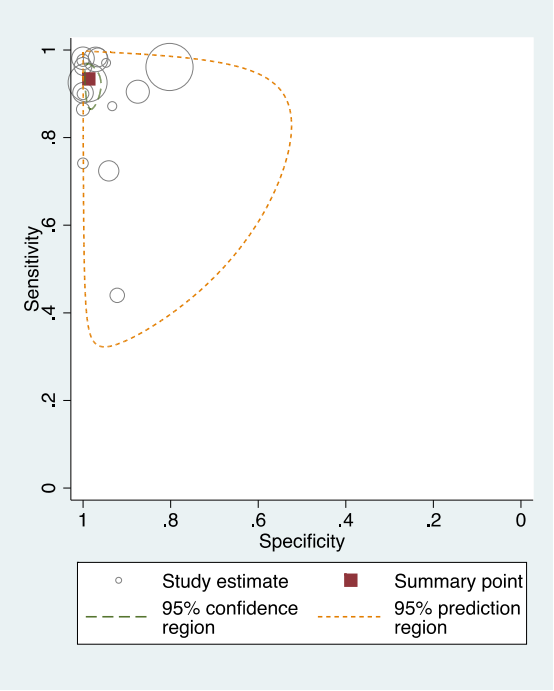
HCV: hepatitis C virus, Ag: antigen, NAT: nucleic acid testing, Ab: antibody, TP: true positive, FP: false positive, FN: false negative, TN: true negative, CI: confidence Interval

Fig. 5. Bivariate analysis of Abbot ARCHITECT HCV antigen assay sensitivity and specificity for diagnosis of active HCV infection compared to gold standard nucleic acid testing in (a) all samples regardless of HCV antibody (Ab) status, (b) HCV Ab-positive samples (c) HCV Ab-negative samples. These plots show pooled summary estimates (red squares), the dashed red line represents the 95% confidence region and the dashed green line represents the 95% prediction region. The individual circles represent each study and the size of the circle is proportional to the total sample size.

(a)



(b)



c)

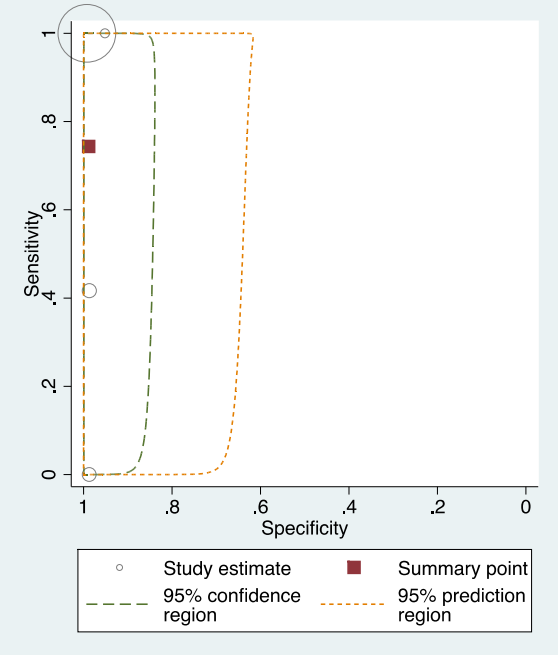
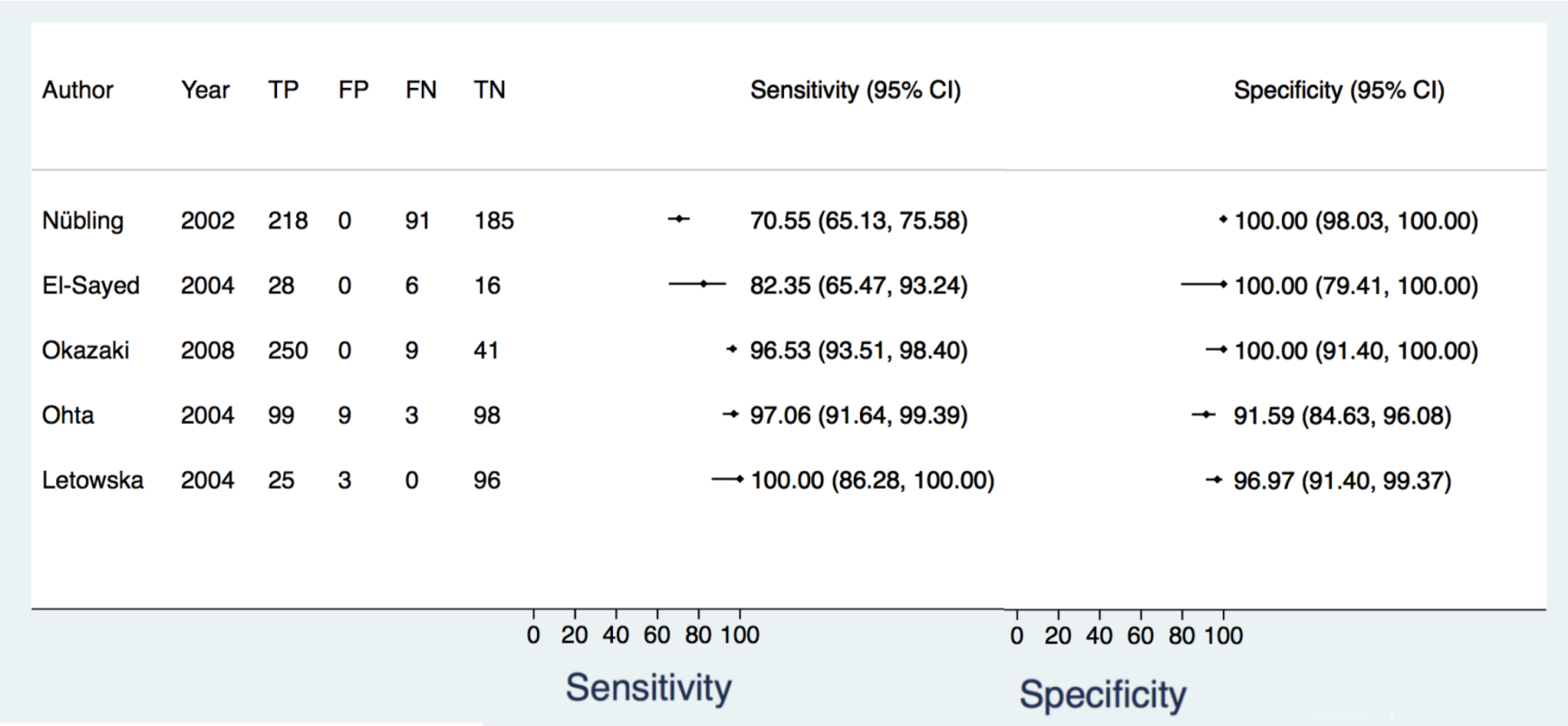


Fig. 6. Forest plot Ortho ELISA-Ag sensitivity and specificity for diagnosis of active HCV infection compared to NAT reference test for all samples regardless of HCV Ab status



ELISA: enzyme linked immunosorbent assay, Ag: antigen, HCV: hepatitis C virus, NAT: nucleic acid testing, Ab: antibody, TP: true positive, FP: false positive, FN: false negative, TN: true negative, CI: confidence interval

Fig. 7. Bivariate analysis of Ortho ELISA-Ag sensitivity and specificity for diagnosis of active HCV infection compared to gold standard nucleic acid testing in all samples regardless of HCV antibody status. This plot shows pooled summary estimates (red squares), the dashed red line represents the 95% confidence region and the dashed green line represents the 95% prediction region. The individual circles represent each study and the size of the circle is proportional to the total sample size.

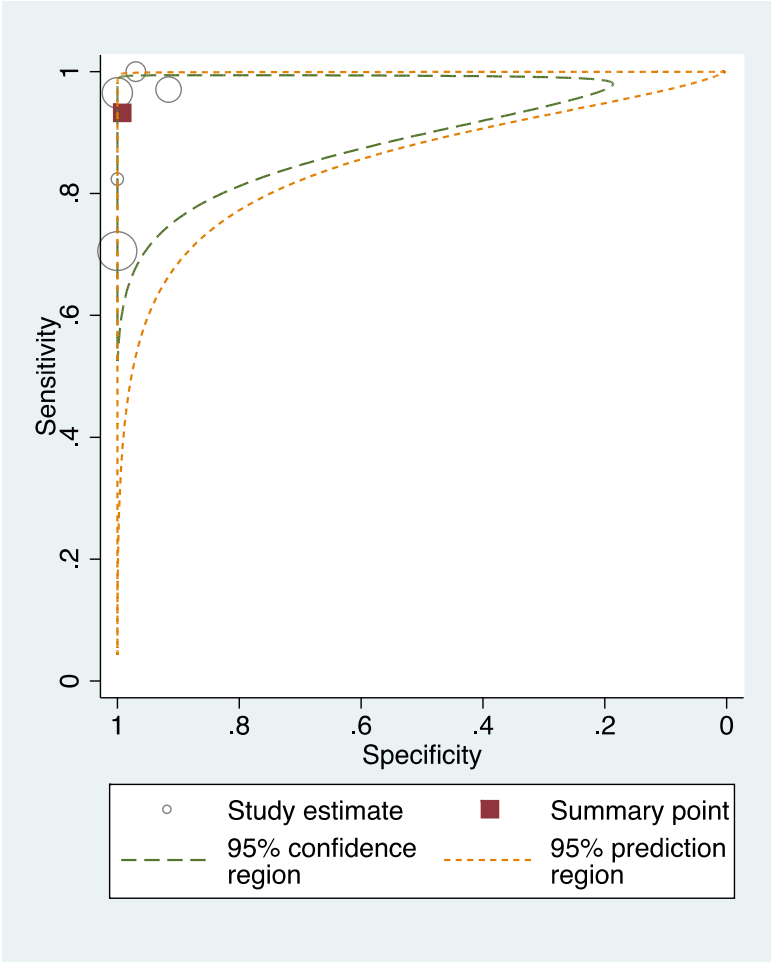
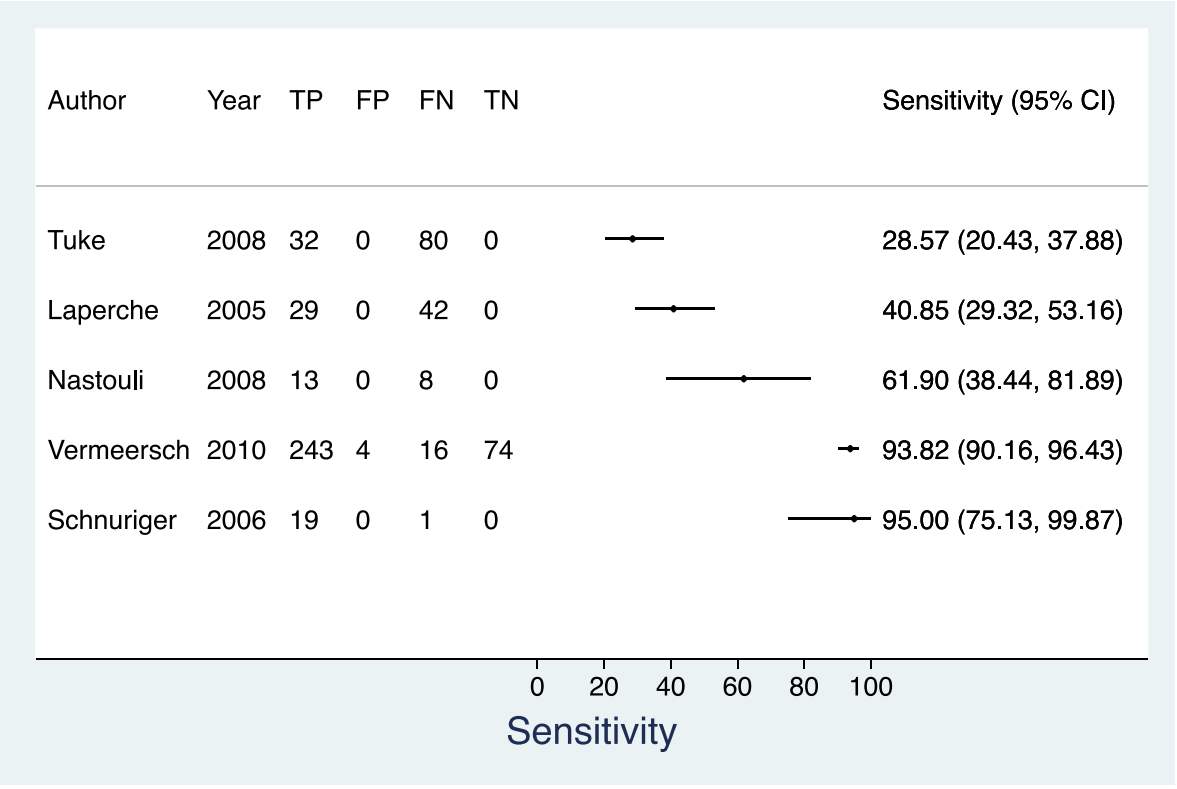


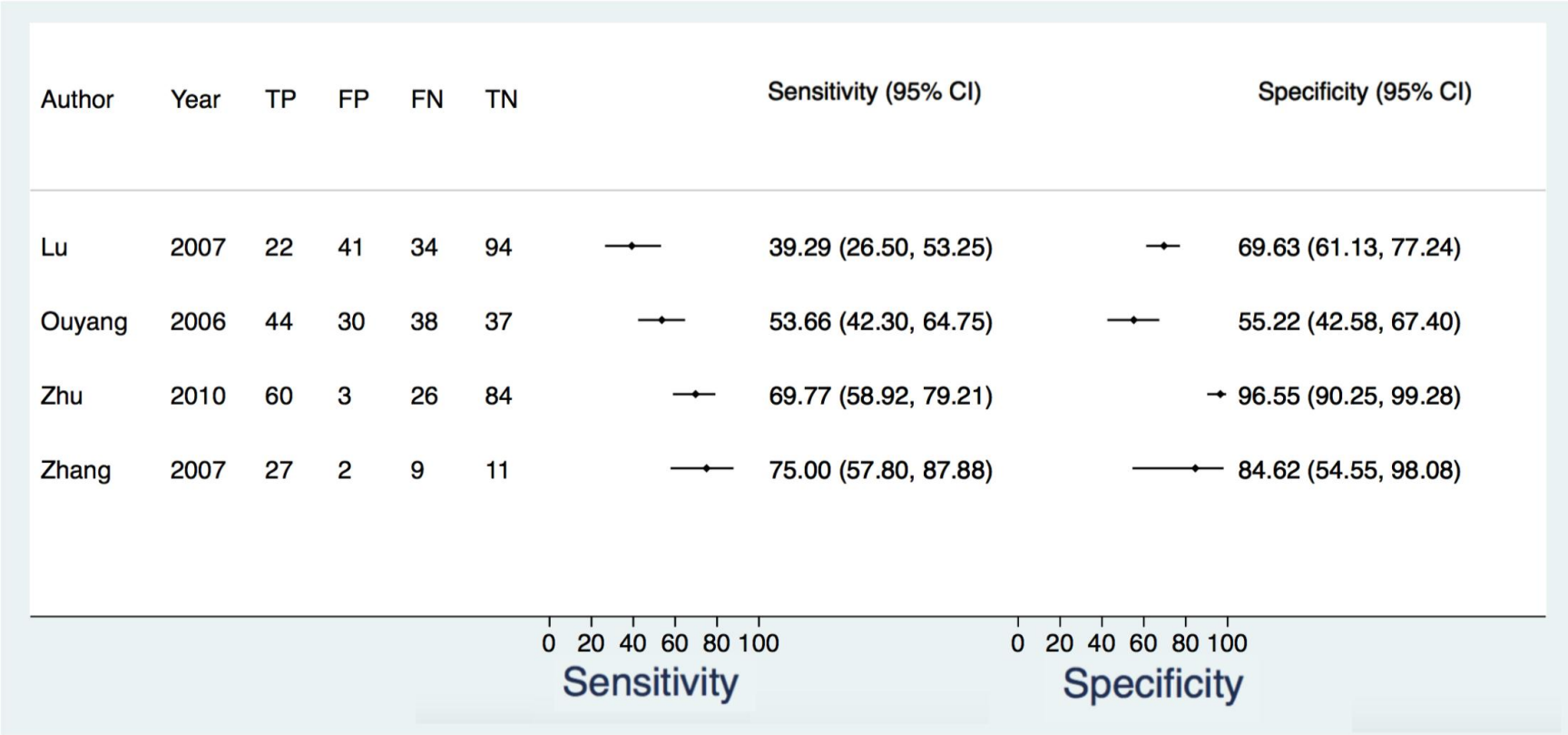
Fig. 8. Forest plot of Bio-RAD Monolisa™ HCV Ag-Ab ULTRA sensitivity for diagnosis of active HCV infection compared to NAT reference test for all samples regardless of HCV Ab status

HCV: hepatitis C virus, Ag: antigen, Ab: antibody, NAT: nucleic acid testing, TP: true positive, FP: false positive,



FN: false negative, TN: true negative

Fig. 9. Forest plots of Hunan Jynda HCV Core Ag ELISA sensitivity and specificity for diagnosis of active HCV infection compared to NAT reference test for all samples regardless of HCV Ab status



HCV: hepatitis C virus , ELISA: enzyme linked immunosorbent assay, Ag: antigen, NAT: nucleic acid testing, Ab: Antibody, TP: true positive, FP: false positive, FN: false negative, TN: true negative, CI: confidence interval

Fig. 10. Bivariate analysis of Hunan Jynda Bioengineering Group HCV Core Ag ELISA sensitivity and specificity for diagnosis of active HCV infection compared to gold standard nucleic acid testing for all samples regardless of HCV antibody status. This plot shows pooled summary estimates (red squares), the dashed red line represents the 95% confidence region and the dashed green line represents the 95% prediction region. The individual circles represent each study and the size of the circle is proportional to the total sample size.

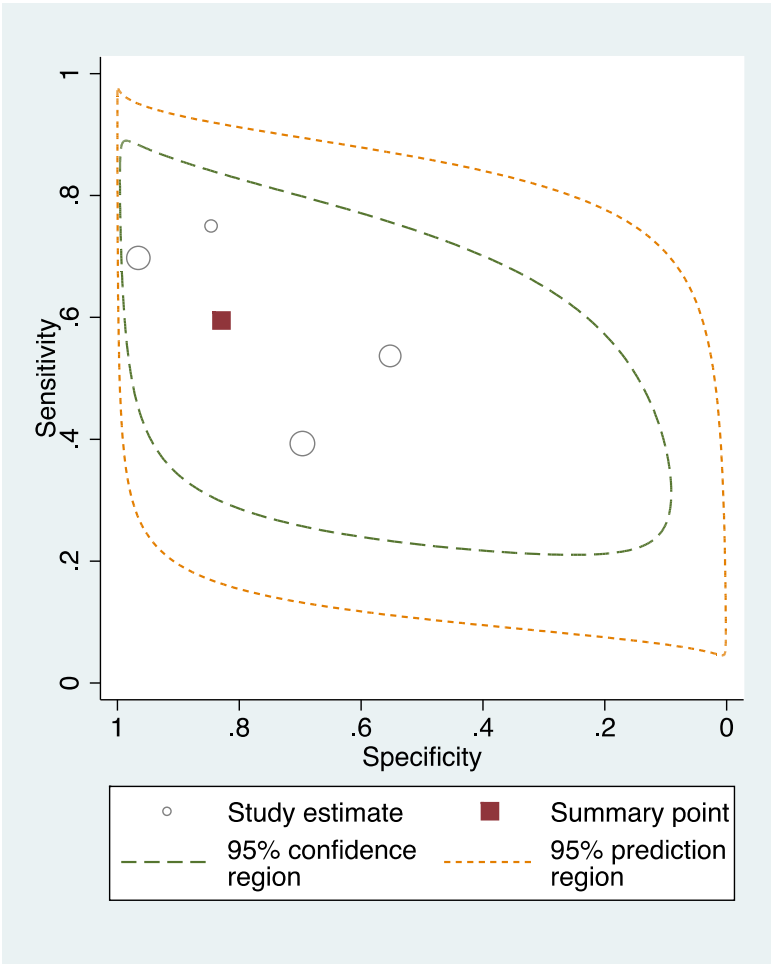
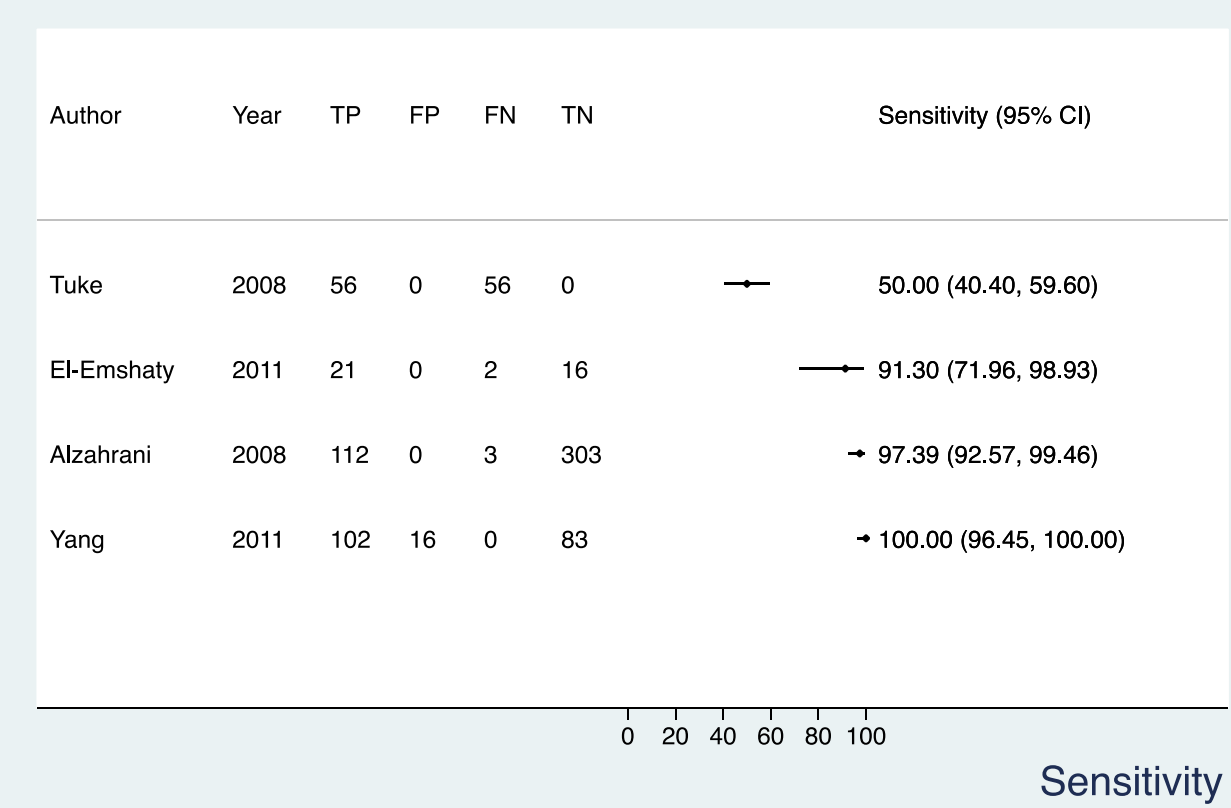
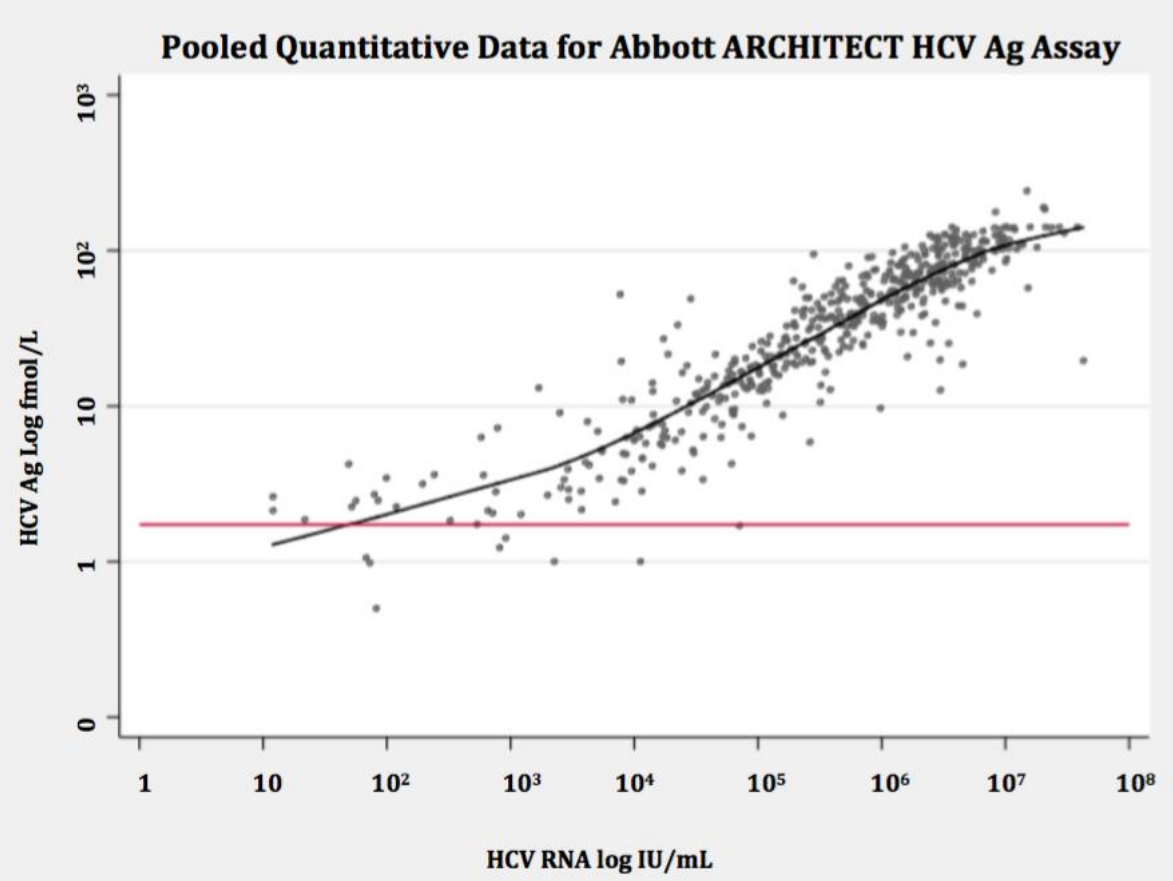


Fig. 11. Forest plot of DiaSorin S.A. Murex Ag/Ab EIA sensitivity for diagnosis of active HCV infection compared to NAT reference test for all samples regardless of HCV Ab status



Ag: antigen, Ab: antibody, EIA: enzyme immunoassay, HCV: hepatitis C infection, NAT: nucleic acid testing, TP: true positive, FP: false positive, FN: false negative, TN: true negative

Fig. 12. Non-parametric regression smoother of pooled quantitative data assessing correlation between Abbott ARCHITECT HCV core Ag measured in log fmol/L and HCV RNA measured in log IU/mL. The red line indicates the positivity threshold of the core antigen index test corresponding to 3 fmol/L.



HCV = hepatitis C virus, Ag = antigen, RNA = ribonucleic acid

Appendices

Appendix A. Search report for systematic review on HCV Antigen use for diagnostics as well as treatment monitoring

Date of search = March 2015. The following tables shows the sources that have been searched and the hits retrieved from those searches

Source	Date range searched	Hits retrieved (before duplicate removal)
Electronic databases		
Medline (PubMed)	All available	2820
Cochrane	All available	127
Embase	All available	5501
Web of Science	All available	2635
Scopus	All available	3549
Final number of records in EndNote database **after deleting duplicates**		**8146**

Search strategy Embase

1. 'hepatitis c antigen'/exp OR 'hepatitis c antigen' OR 'hepatitis c'/exp OR 'hepatitis c virus'/exp OR 'hepatitis c virus'
2. 'hepatitis c antigen' OR 'hepatitis C' OR hepatitis c virus' or 'hcv'
3. #1 OR #2
4. 'antigen'/exp OR 'antigen' OR 'virus antigen'/exp OR 'virus antigen'
5. 'antigen' OR 'virus antigen'
6. #4 OR #5
7. 'nucleic acid amplification'/exp OR 'nucleic acid amplification' OR 'virus rna'/exp OR 'virus rna' OR 'rna'/exp OR 'rna'
8. 'nucleic acid amplification' OR 'virus rna' OR 'rna' OR 'nucleic acid test'
9. #7 OR #8
10. #3 AND #6 AND #9

'hepatitis c antigen'/exp OR 'hepatitis c'/exp OR 'hepatitis c virus'/exp OR 'hepatitis c antigen' OR 'hepatitis c' OR 'hepatitis c virus' OR 'hcv' AND ('antigen'/exp OR 'virus antigen'/exp OR 'antigen' OR 'virus antigen') AND ('nucleic acid amplification'/exp OR 'virus rna'/exp OR 'rna'/exp OR 'nucleic acid amplification' OR 'virus rna' OR 'rna' OR 'nucleic acid test'

Search strategy Web of Knowledge (SCI-expanded, SSCI, Conference Proceedings science, BIOSIS previews)

1. Hepatitis C OR HCV (topic)
2. Antigen* OR core antigen* (topic)
3. RNA OR NAT or nucleic acid test* (topic)
4. #1 AND #2 AND #3

Search strategy PubMed

((hepatitis C[MeSH Terms] OR "Hepacivirus"[Mesh] OR "HCV" OR "hepatitis C") AND (antigen* OR antigens, viral/blood[MeSH Terms] OR hepatitis c antigens[MeSH Terms]) AND ("Nucleic Acid Amplification Techniques"[Mesh] OR nucleic acid test*[tw] OR nucleic acid amplification[tw] OR RNA OR RNA, viral/blood[MeSH Terms]))

Search strategy SCOPUS

(TITLE-ABS-KEY ("Hepatitis C"OR hcv)AND TITLE-ABS-KEY (antigen*OR "core antigen*")AND TITLE-ABS-KEY (rna OR"nucleic acid test"))AND(LIMIT-TO (DOCTYPE ,"ar")OR LIMIT-TO (DOCTYPE ,"ip"))

Search strategy Cochrane

1. MeSH descriptor: Hepatitis C
2. MeSH Descriptor: Hepacivirus
3. MeSH Descriptor: Hepatitis C Antigens
4. Hepatitis C
5. HCV
6. #1 or #2 or #3 or #4 or #5
7. MeSH Descriptor: Nucleic Acid Amplification Techniques
8. RNA
9. Nucleic acid test
10. #7 or #8 or #9
11. Antigen
12. “Core antigen”
13. #11 or #12
14. #6 AND #10 AND #13

Appendix B. Data extraction form

ID	
First Author	
Corresponding author and email	
Was author contacted?	1 – Yes 2 – No If yes, dates(s)
Title	
Year (of publication)	
Year (study start date)	
Language	1 – English 2 – Other If other, specify:
HCV Genotypes specified	1 – *n*, % Genotype 1 __ Genotype 1a __ Genotype 1b __ 2 – *n*, % Genotype 2 __ 3 – *n*, % Genotype 3 __ 4 – *n*, % Genotype 4 __ 5 – *n*, % Genotype 5 __ 6 – *n*, % Genotype 6 __
% HIV positive	
% HBsAg + (chronic HBV infection)	
% Adults/children	
Age (mean SD, median IQR, range)	
Gender, % Female	
Country where study was conducted	
Country World Bank Classification (at time of study start date)	1 – Middle/Low 2 – High 3 – Both middle/low and high
Study design	1 – Randomized trial 2 – Cross-sectional 3 – Cohort 4 – Case Control 5 – Other, specify 9 – Unk/NR If other, specify:

Participant selection	1 – Consecutive 2 – Random 3 – Convenience 4 – Other 9 –Unk/NR
Study population	1 – Broad 2 – Healthy persons only 3 – Unk/NR Comments: _____
Direction of study data collection	1 – Prospective 2 – Retrospective 9 – Unk/NR
Comments about study design	
Were samples excluded based on prior testing of the sample?	1 – yes (specify below) 2 – no 9 – Unk/NR Comments: _____
Number after screening by exclusion and inclusion criteria	_____ 9 – Unk/NR
Sample size (total number included in 2/2 table)	_____ 9 – Unk/NR
Unit of analysis	1 – One specimen per patient 2 – Multiple specimens per patient 3 – Unknown number of specimens per patient 9 – NR/Unclear Describe as in paper, if unclear:
Types of specimen and number	1 – Serum ___ 2 – Plasma ___ 3 – Whole blood____
HCV NAT method used	1 – PCR 2 – bDNA 3 – TMA 9 – Unk/NR
HCV NAT method quantitative?	1 – Yes 2 – No 9 – Unk/NR
HCV Ag test manufacturer	1 – Abbott ARCHITECT

	2 – Bio-RAD Mono Lisa 3 – Other: please specify 9 – Unk/NR
HCV Ag method quantitative?	1 – Yes 2 – No 9 – Unk/NR
Were reference NAT test and HCV Ag test performed on specimen within 30 days	1 – Yes 2 – No 3 – Unk/NR
Was Ag test obtained/repeated while subject was on treatment for HCV infection or after treatment completed?	1 – yes (Specify below) 2 – No Specify timing of Ag Collection 1 – Baseline, prior to treatment 2 – EVR 3 – SVR 12 weeks 4 – SVR 24 weeks What treatment regimen was used? 1 – Interferon based therapy 2 – Interferon free direct acting antivirals 3- Unk/NR
Did all patients NAT within the study?	1 – yes 2 – no 9 – Unk/NR Comments: _____
Was index test performed per recommendation of the manufacturer?	1 – Yes 2 – No 9 – Unk/NR Comments: _____

Appendix C. QUADAS-2 protocol

Domain 1. Patient selection

Risk of bias: could the selection of patients have introduced bias?

- Signalling question 1: Was a consecutive or random sample of patients or specimens enrolled? Score "yes" if the study enrolled a consecutive or random sample of eligible patients; "no" if the study selected patients by convenience, and "unclear" if the study did not report the manner of patient selection or unable to tell.
- Signalling question 2: Was a case–control design avoided? Rate "no" if case–control study, "yes" if prospective or cross-sectional study.
- Signalling question 3: Did the study avoid inappropriate exclusions? Score "no" if the study excluded samples based on prior testing of the sample and "unclear" if unable to tell.

Risk of Bias is scored as "low risk" if selection was done in a random or consecutive manner and the study was prospective and did not exclude samples based on prior testing; "high risk" if selection was by convenience, from case-control study or excluded samples; and "unclear risk" if the manner of participant selection is unclear and no clinical information is provided.

Applicability: Are there concerns that the included patients and setting do not match the review question?

We are interested in how HCV AG test performs across HCV genotypes and among HIV-infected (immunocompromised) persons. If a study includes only very selected persons, only healthy or blood donors, it would not be relevant to the study question. Setting of testing is not relevant to the review question. We will score "low risk" if broad study population, "high risk" if population is blood donors or healthy persons only, and "unclear risk" if the population is not well characterized.

Domain 2. Index test

Risk of bias: could the conduct or interpretation of the index test have introduced bias?

- Signalling question 1: Were the index test results interpreted without knowledge of the results of the reference standard? Rate "yes" if results of reference standard were blinded. Rate "no" if reference standard results were unblinded.
- Signalling question 2: If a threshold was used, was it pre-specified? Answer "yes" for all studies as limit of detection for all commercially available HCV Ag tests are pre-specified.

Score "low risk" for all tests interpreted with blinded results of reference standard. Score "high risk" for antigen tests interpreted with results from reference standard available. Score "unclear risk" if availability of reference test is not specified.

Applicability: Are there concerns that the index test, its conduct, or its interpretation differ from the review question? Variations in test technology, execution, or interpretation may affect estimates of the diagnostic accuracy of a test.

- Score “low concern” if the test was done as per recommendation of the manufacturer. Score “high concern” if additional processing steps were added. Score “unclear” if not discussed in the study.

Domain 3. Reference standard

Risk of bias: could the reference standard, its conduct, or its interpretation have introduced bias?

- Signalling question 1: Is the reference standard likely to correctly classify the target condition?

  There are multiple methods of NAT, each with slightly varying sensitivity, however overall the tests are highly sensitive and the verification should be minimal. We will score “yes” for all studies

  Signalling question 2: Were the reference standard results interpreted without knowledge of the results of the index test?

  The reference standard in this case also does not allow for interpretation. Therefore it is unlikely to introduce bias even if reference standard was resulted with knowledge of the index test result.

For risk of bias, score “low risk” for all studies.

Applicability: Are there concerns that the target condition as defined by the reference standard does not match the question?

Judge applicability to be of “low risk” for all studies as circulating virus is by definition associated with active infection and the specificity of the reference standard is high. While the reference standard is not able to differentiate between acute or chronic infection, the core antigen is also not expected to do so. The differentiation will be done based on the constellation of NAT results with serology results. This will be assessed in a stratified analysis.

Domain 4. Flow and timing

Risk of bias: Could the patient flow have introduced bias?

- Signalling question 1: Was there an appropriate interval between the index test and reference standard? We will limit time between reference and index testing to <1 month. Score “yes” if time between tests is <1 month, score “no” if time between tests is more than 1 month.
- Signalling question 2: Did all patients in the study receive the same reference standard? Answer “yes” if all patients had NAT, answer “no” if reference standard NAT was not used for all patients, answer “unclear” if it is not specified.
- Signalling question 3: Were all patients included in the analysis? Determined the answer to this question by comparing the number of patients enrolled with the number of patients included in the two-by-two tables.

For risk of bias, score "low risk" if the number of participants enrolled was clearly stated and corresponded to the number presented in the analysis or if exclusions were adequately described. Score "high risk" if there were participants missing or excluded from the analysis and there was no explanation given; and "unclear risk" if not enough information was given to assess whether participants were excluded from the analysis; usually this means that the number of participants originally enrolled in the study was not explicitly stated.

Appendix D. List of excluded studies and reasons for exclusion organized by PICO.

PICO 5a:

1. Alados-Arboledas JC, Calbo-Torrecillas L, Lopez-Prieto MD, de Francisco-Ramirez JL, de Miguel-Sastre C. Clinical assessment of Monolisa HCV ag-ab ULTRA (Bio-Rad) in a general hospital. Enferm Infecc Microbiol Clin. 2007;25(3):172–6. *Inappropriate reference test*
2. Allain JP, Coghlan PJ, Kenrick KG, Whitson K, Keller A, Cooper GJ, et al. Prediction of hepatitis C virus infectivity in seropositive Australian blood donors by supplemental immunoassays and detection of viral RNA. Blood. 1991;78(9):2462–8. *No HCV core antigen performed*
3. Alzahrani AJ. Analysis of hepatitis C virus core antigenemia in Saudi drug users. Saudi Med J. 2005;26(10):1645–6. *Editorial or comment*
4. Alzahrani AJ, Obeid OE, Al-Ali A, Imamwardi B. Detection of hepatitis C virus and human immunodeficiency virus in expatriates in Saudi Arabia by antigen-antibody combination assays. J Infect Dev Ctries.2009;3(3):235–8. *Less than 10 independent samples*
5. Aoyagi K, Iida K, Ohue C, Matsunaga Y, Tanaka E, Kiyosawa K, et al. Performance of a conventional enzyme immunoassay for hepatitis C virus core antigen in the early phases of hepatitis C infection. Clin Lab. 2001;47(3–4):119–27. *Non-commercial or off-market assay*
6. Aoyagi K, Ohue C, Iida K, Kimura T, Tanaka E, Kiyosawa K, et al. Development of a simple and highly sensitive enzyme immunoassay for hepatitis C virus core antigen. J Clin Microbiol. 1999;37(6):1802–8. *Non-commercial or off-market assay*
7. Araujo AC, Astrakhantseva IV, Fields HA, Kamili S. Distinguishing acute from chronic hepatitis C virus (HCV) infection based on antibody reactivities to specific HCV structural and nonstructural proteins. J Clin Microbiol. 2011;49(1):54–7. *No HCV core antigen performed*
8. Arrojo IP, Pareja MO, Orta MDR, Luque FN, Lamas MCH, Gordo FS, et al. Detection of a healthy carrier of HCV with no evidence of antibodies for over four years. Transfusion. 2003;43(7):953–7. *Less than 10 independent samples*
9. Attallah AM, Ismail H, Tabll AA, Shiba GE, El-Dosoky I. A novel antigen detection immunoassay for field diagnosis of hepatitis C virus infection. J Immunoassay Immunochem. 2003;24(4):395–407. *Non-commercial or off-market assay*
10. Attallah AM, Omran MM, Nasif WA, Ghaly MF, El-Shanshoury AERR, Abdalla MS, et al. Diagnostic performances of hepatitis C virus-NS4 antigen in patients with different liver pathologies. Arch Med Res. 2012;43(7):555–62. *Non-commercial or off-market assay*
11. Attallah AM, Shiha GE, Malak CAA, Hagras HE, Abdel-Razik WS, Ismail H. Utility of a novel HCV-NS4 antigen detection immunoassay for monitoring treatment of HCV-infected individuals with pegylated interferon alpha-2a. Hepatol Res. 2004;28(2):68–72. *Non-commercial or off-market assay*

12. Attia MA, Zekri AR, Goudsmit J, Boom R, Khaled HM, Mansour MT, et al. Diverse patterns of recognition of hepatitis C virus core and nonstructural antigens by antibodies present in Egyptian cancer patients and blood donors. J Clin Microbiol. 1996;34(11):2665–9. *No HCV core antigen performed*
13. Baggett DW, Moroney S, Saewert M, Jaczko B, Zelechowski J, Bahi C, et al. Dynamics of circulating HCV core antigen and HCV RNA in the early phase of HCV infection. Transfusion. 2000;40(10):26S. *Abstract or poster*
14. Ballardini G, Manzin A, Giostra F, Francesconi R, Groff P, Grassi A, et al. Quantitative liver parameters of HCV infection: relation to HCV genotypes, viremia and response to interferon treatment. J Hepatol. 1997;26(4):779–86. *Non-blood specimen*
15. Baranov AV, Maleev VV. Association between HCV RNA level and anti-HCV antibodies during chronic hepatitis C. Zh Mikrobiol Epidemiol Immunobiol. 2009(5):19–22. *No HCV Core antigen performed*
16. Bdour S. Hepatitis C virus infection in Jordanian haemodialysis units: serological diagnosis and genotyping. J Med Microbiol. 2002;51(8):700–4. *No HCV core antigen performed*
17. Beer N, Shinar E, Novack L, Safi J, Soliman H, Yaari A, et al. Accuracy of hepatitis C virus core antigen testing in pools among seroconverters. Transfusion. 2006;46(10):1822–8. *Non-commercial or off-market assay*
18. Berger A. Recent developments in hepatitis C infection (epidemiology, diagnosis and therapy). Laboratoriums Medizin. 2001;25(7–8):218–22. *Review article*
19. Berger A, Doerr HW, Preiser W, Weber B. Lack of correlation between different hepatitis C virus screening and confirmatory assays. J Virol Methods. 1996;59(1–2):141–6. *No HCV core antigen performed*
20. Bochkova G, Fomina S, Puzyrev V, Obriadina A, Burkov A, Ulanova T. The evaluation of the ELISA kit nullDS-EIA-anti-HCVSPECTR-GMnull as supplemental assay for confirmation of anti-HCV screening positive results. Clin Microbiol Infect. 2011;17:S664. *Abstract or poster*
21. Bochkova G, Fomina S, Puzyrev V, Obriadina A, Burkov A, Ulanova T. The evaluation of the new ELISA kit "EIA-anti- HCV-SPECTRUM-M" intended for separate detection of anti-IgM to different HCV antigens. J Viral Hepat. 2012;19:5–6. *Abstract or poster*
22. Bouvier-Alias M, Patel K, Dahari H, Beaucourt S, Larderie P, Blatt L, et al. Clinical utility of total HCV core antigen quantification: a new indirect marker of HCV replication. Hepatology. 2002;36(1):211–8. *Non-commercial or off market assay*
23. Bouzgarrou N, Fodha I, Ben Othman S, Achour A, Grattard F, Trabelsi A, et al. Evaluation of a total core antigen assay for the diagnosis of hepatitis C virus infection in hemodialysis patients. J Med Virol. 2005;77(4):502–8. *Non-commercial or off market assay*
24. Brandao CPU, Marques BLC, Marques VA, Villela-Nogueira CA, Do O KMR, de Paula MT, et al. Simultaneous detection of hepatitis c virus antigen and antibodies in dried blood spots. J Clin Virol. 2013;57(2):98–102. *Inappropriate reference test*
25. Brody RI, Eng S, Melamed J, Mizrachi H, Schneider RJ, Tobias H, et al. Immunohistochemical detection of hepatitis C antigen by monoclonal antibody TORDJI-22 compared with PCR viral detection. Am J Clin Pathol. 1998;110(1):32–7. *Non-blood specimens*
26. Brojer E, Gronowska A, Medyńska J, Grabarczyk P, Mikulska M, Lętowska M, et al. The hepatitis C virus genotype and subtype frequency in hepatitis C virus RNA-positive, hepatitis C virus antibody-negative blood donors identified in the nucleic acid test screening program in Poland. Transfusion. 2004;44(12):1706–10. *No HCV core antigen performed*

27. Brojer E, Liszewski G, Niznik A, Rosiek A, Letowska M, Peterson JE, et al. Detection of HCV core antigen in HCV RNA positive, anti-HCV negative blood donations from Polish blood donors. Transfusion. 2001;41(2):304. *Editorial or comment*
28. Brojer E, Gronowska A, Rosiek A, Mikulska M, Letowska M. HCV core antigen detection and quantification of viremia in the "window period" of donors identified by routine HCV RNA screening in Poland. Vox Sang. 2005;89:92. *Poster or abstract*
29. Burek V. Hepatitis C viral infection – news in diagnostics. Infektoloski Glasnik. 2002;22(1):27–9. *Review article*
30. Busch MP, Wright DJ, Hirschkorn DF, Baggett D, Maret S, Lee SR, et al. Sensitivity of 1(st) and 2(nd) generation HCV antigen assays versus nucleic acid testing (NAT) for detection of ramp-up phase of HCV infection. Transfusion. 2001;41(9):3S. *Abstract or poster*
31. Cagnon L, Wagaman P, Bartenschlager R, Pietschmann T, Gao TJ, Kneteman NM, et al. Application of the trak-C (TM) HCV core assay for monitoring antiviral activity in HCV replication systems. J Virol Methods. 2004;118(1):23–31. *Non-human subjects, non-commercial or off-market assay*
32. Cano H, Candela MJ, Lozano ML, Vicente V. Application of a new enzyme-linked immunosorbent assay for detection of total hepatitis C virus core antigen in blood donors. Transfus Med.. 2003;13(5):259–66. *Non-commercial or off-market assay*
33. Cao H, Zhang K, Shu X, Li G. The effect of hepatitis B virus infection on detection of hepatitis C virus core antigen. Hepatol Int 2012;6(1):199. *Abstract or poster*
34. Cao H, Zhang K, Shu X, Xu QH, Li G. Detection of core antigen of hepatitis virus C in patients infected with hepatitis virus C and B. Zhonghua Gan Zang Bing Za Zhi. 2011;19(10):726–8. *Non-commercial or off-market assay*
35. Cao H, Zhang K, Shu X, Xu QH, Li G. Detection of hepatitis C core antigen in intravenous drug addictions. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi. 2011;25(4):304–6. *Non-commercial or off-market assay*
36. Carabaich A, Ruvoletto M, Bernardinello E, Tono N, Cavalletto L, Chemello L, et al. Profiles of HCV core protein and viremia in chronic hepatitis C: possible protective role of core antigen in liver damage. J Med Virol. 2005;76(1):55–60. *Non-commercial or of- market assay*
37. Carney R, Maranao D, Sudra R, Chaytor S, Labbett W, Johnson M, et al. A hepatitis C virus core antigen assay is a cost-effective, sensitive and specific test in the detection of acute hepatitis C in HIV infected subjects. HIV Med. 2014;15:8. *Abstract or poster*
38. Cavazza S, Lagging M. Indeterminate third-generation hepatitis C recombinant immunoblot assay and HCV RNA analysis: isolated reactivity against NS5 associated with HCV viraemia in clinical patients but not blood donors. Scand J Infect Dis. 2005;37(6–7):488–92. *No HCV core antigen performed*
39. Cetinić Balent N, Mikulić R, Đaković Rode O. Enzyme immunoassay for separate detection of anti-HCV antibodies to individual HCV antigens as a confirmatory assay in diagnostics of HCV infection. Infektoloski Glasnik. 2013;33(3):109–15. *No HCV core antigen performed*
40. Chakravarti A, Chauhan MS, Dogra G, Banerjee S. Hepatitis C virus core antigen assay: can we think beyond convention in resource limited settings? Braz J Infect Dis. 2013;17(3):369–74. *Non-commercial or off market assay*
41. Challine D, Dameron G, Laperche L, Larderie P, Rigot P, Claquin J, et al. Does HCV core antigen or nucleic acid testing in graft donors improve organ transplantation viral safety? Transfusion. 2001;41(9):3S–4S. *Abstract or poster*

42. Chaudhary R, Burres E. Detection of antibody to envelope (E2) antigen of hepatitis C virus. Can J Infect Dis. 1997;8(4):229–31. *No HCV core antigen performed*
43. Choi YJ, Hu Y, Goris J, Kim E. Histopathology, viral antigen and RNA in hepatitis C virus-associated hepatitis. Lab Invest. 1997;76(1):803.
44. Cividini A, Cerino A, Muzzi A, Furione M, Rebucci C, Segagni L, et al. Kinetics and significance of serum hepatitis C virus core antigen in patients with acute hepatitis C. J Clin Microbiol. 2003;41(5):2144–6. *Less than 10 independent samples*
45. Courouce AM, Barin F, Botte C, Lunel F, Maisonneuve P, Maniez M, et al. A comparative evaluation of the sensitivity of 7 anti-hepatitis-C virus screening tests. Vox Sang. 1995;69(3):213–6. *No HCV core antigen performed*
46. Couroucé AM, Le Marrec N, Bouchardeau F, Razer A, Maniez M, Laperche S, et al. Efficacy of HCV core antigen detection during the preseroconversion period. Transfusion. 2000;40(10):1198–202. *Less than 10 independent samples*
47. Craxi A, Valenza M, Fabiano C, Magrin S, Fiorentino G, Diquattro O, et al. Third generation hepatitis C virus tests in asymptomatic anti-HCV-positive blood donors. J Hepatol. 1994;21(5):730–4. *No HCV core antigen performed*
48. Cresswell F, Shaw S, Hughes D, Youssef E, Homer G, Hassan-Ibrahim M, et al. Hepatitis C antigen testing: a reliable alternative for diagnosing acute hepatitis C infection. HIV Med. 2014;15:137. *Abstract or poster*
49. Cucchietti A, Parker S, Oldfield L, Limas C, Garrod A, Mc Bryne L. Seroconversion sensitivity of elisa screening assays for HCV, HIV and HBV antigens and/or antibodies. Vox Sang. 2011;101:87. *Abstract or poster*
50. Daniel HD, Vivekanandan P, Raghuraman S, Sridharan G, Chandy GM, Abraham P. Significance of the hepatitis C virus (HCV) core antigen as an alternative plasma marker of active HCV infection. Indian J Med Microbiol. 2007;25(1):37–42. *Non-commercial or off-market assay*
51. Daniel HDJ, Chandy GM, Abraham P. Quantitation of hepatitis C virus using an in-house real-time reverse transcriptase polymerase chain reaction in plasma samples. Diagn Microbiol Infect Dis. 2008;61(4):415–20. *No HCV core antigen performed*
52. Dawson G. The potential role of HCV core antigen testing in diagnosing HCV infection. Antivir Ther. 2012;17(7 PARTB):1431–5. *Review article*
53. Dawson GJ. HCV core antigen and combination (antigen/antibody) assays for the detection of early Seroconversion. J Med Virol. 2007;79:S54–S8. *Review article*
54. Dawson GJ. HCV core antigen detection in seropositive samples. J Med Virol. 2007;79:S52–S3. *Review article*
55. De Almeida Ponde RA. Enzyme-linked immunosorbent/chemiluminescence assays, recombinant immunoblot assays and nucleic acid tests in the diagnosis of HCV infection. Eur J Clin Microbiol. Infect Dis 2013;32(8):985–8. *Review article*
56. Dickson RC, Mizokami M, Orito E, Qian K, Lau JYN. Quantification of serum HCV core antigen by a fluorescent enzyme immunoassay in liver transplant recipients with recurrent hepatitis C – clinical and virologic implications. Transplantation. 1999;68(10):1512–6. *Non-commercial or off-market assay*
57. Dow BC, Munro H, Buchanan I, Ferguson K, Davidson F, Lycett C, et al. Acute hepatitis C virus seroconversion in a Scottish blood donor: HCV antigen is not comparable with HCV nucleic acid amplification technology screening. Vox Sang. 2004;86(1):15–20. *Less than 10 independent samples*

58. Dubrous P, Hugard L, Terrier F. Screening for HCV core antigen among HIV-positive patients infected by sexual transmission. Med Mal Infect. 2004;34(5):236–8. *Less than 10 independent samples*
59. Durante-Mangoni E, Vallefuoco L, Perna E, Iossa D, Andini R, Caianiello C, et al. Correlates and prognostic value of hepatitis C virus core antigen serum levels during chronic infection and treatment. Clin Microbiol Infect. 2010;16:S317. *Abstract or poster*
60. Dwyer E, Riley P, Pakianathan M. Analysis of hepatitis C antigen testing in an urban sexual health clinic. HIV Med. 2014;15:111–2. *Abstract or poster*
61. Eiras A, Franco E, Montoro JA, Planelles D, Villaescusa R. HCV NAT (minipool RT-PCR) and HCV core antigen ELISA. Transfusion. 2003;43(1):118; author reply -9. *Editorial or comment*
62. El Ekiaby M, Laperche S, Moftah F, Burnouf T, Lelie N. The impact of different HCV blood screening technologies on the reduction of transfusion transmitted HCV infection risk in Egypt. Vox Sang. 2009;96:23–4. *Abstract or poster*
63. Ellethy AT, Sliem HA, Hassan GMA. Updated molecular diagnosis of chronic hepatitis C. J Gastroenterol Hepatol Res. 2012;1(8):147–52. *Review article*
64. Ergunay K, Sener B, Alp A, Karakaya J, Hascelik G. Quantitative determination of HCV core antigen: A marker for monitoring hepatitis C viraemia. Clin Microbiol Infect. 2011;17:S663. *Abstract or poster*
65. Fabrizi F, Lunghi G, Aucella F, Mangano S, Barbisoni F, Bisegna S, et al. Novel assay using total hepatitis C virus (HCV) core antigen quantification for diagnosis of HCV infection in dialysis patients. J Clin Microbiol. 2005;43(1):414–20. *Non-commercial or off-market assay*
66. Frank K, Karl A. Comparison of different confirmation test methods to anti-HCV used for repeatedly HCV-reactive samples. Infusionsther Transfusionsmed. 2001;28(Suppl. 1):47. *No HCV core antigen performed*
67. Galli C. New strategies for the identification of active hepatitis C virus (HCV) infections. Biochim Clin. 2013;37:S137. *Abstract or poster*
68. Galli C. New strategies for the screening of hepatitis C virus infection. Vox Sang. 2013;105:181. *Abstract or poster*
69. Gaudy C, Thevenas C, Tichet J, Mariotte N, Goudeau A, Dubois F. Usefulness of the hepatitis C virus core antigen assay for screening of a population undergoing routine medical checkup. J Clin Microbiol. 2005;43(4):1722–6. *Non-commercial or off-market assay*
70. Gavarro A, Cebollero A, Martinez S, Veraguas A, Acosta A. Evaluation of a new method to determine core antigen of hepatitis C virus and its comparison with the determination of RNA-HCV. Clin Chem Lab Med. 2011;49:S522–S. *Abstract or poster*
71. Grant PR, Sims CM, Tedder RS. Quantification of HCV RNA levels and detection of core antigen in donations before seroconversion. Transfusion. 2002;42(8):1032–6. *Non-commercial or off-market assay*
72. Gu SJ, Liu J, Zhang HJ, Gu BL, Lai HJ, Zhou HL, et al. Core antigen tests for hepatitis C virus: a meta-analysis. Mol Biol Rep. 2012;39(8):8197–208. *Review article*
73. Hadziyannis E, Vassilopoulos D, Georgiou A, Spanou F, Koskinas J. Comparison of a new HCV core antigen automated immunoassay to quantitative HCV RNA measurement in patients with chronic hepatitis C. Hepatology. 2010;52(4):1230A. *Abstract or poster*
74. Hayashi K, Hasuike S, Kusumoto K, Ido A, Uto H, Kenji N, et al. Usefulness of a new immuno-radiometric assay to detect hepatitis C core antigen in a community-based population. J Viral Hepat. 2005;12(1):106–10. *Non-commercial or off-market assay*

75. Heidrich B, Pischke S, Kirschner J, Schneider J, Mederacke I, Raupach R, et al. Performance of HCV-core antigen testing in liver transplanted patients. J Hepatol. 2011;54:S223. *Abstract or poster*
76. Higashimoto M, Takahashi M, Jokyu R, Syundou H, Saito H. Improvement of sensitivity in the second generation HCV core antigen assay by a novel concentration method using polyethylene glycol (PEG). Rinsho Byori. 2007;55(11):1008–14. *Less than 10 independent samples*
77. Hmaïed F, Ben Mamou M, Arrouji Z, Slim A, Ben Redjeb S. Use of combined detection of hepatitis C virus core antigen and antibodies to reduce the serological window-phase. Pathol Biol (Paris). 2007;55(2):121–6. *Inappropriate reference test*
78. Hosseini-Moghaddam SM, Iran-Pour E, Rotstein C, Husain S, Lilly L, Renner E, et al. Hepatitis C core Ag and its clinical applicability: potential advantages and disadvantages for diagnosis and follow-up? Rev Med Virol. 2012;22(3):156–65. *Review article*
79. Icardi G, Ansaldi F, Bruzzone BM, Durando P, Lee S, de Luigi C, et al. Novel approach to reduce the hepatitis C virus (HCV) window period: clinical evaluation of a new enzyme-linked immunosorbent assay for HCV core antigen. J Clin Microbiol. 2001;39(9):3110–14. *Less than 10 independent samples*
80. Icardi G, Bruzzone B, Gota F, Torre F, Giannini E, Massone L, et al. A new assay for hepatitis C virus (HCV) core antigen detection: an alternative to nucleic acid technologies in positive or indeterminate anti-HCV subjects? Ann Ig. 2003;15(6):863–70. *Non-commercial or off market assay*
81. Irshad M, Dhar I, Joshi YK. Significance of hepatitis C virus core protein in the diagnosis of hepatitis C virus infection in different liver diseases. J Investig Med. 2006;54(8):478–83. *Duplicate data*
82. Irshad M, Dhar I, Khushboo, Singh S, Kapoor S. Comparison of serological and nucleic Acid based assays used to diagnose hepatitis C virus (HCV) infection in acute and chronic liver diseases. Int J Health Sci (Qassim). 2007;1(1):3–10. *No extractable data, no response from author*
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Annex 5.9.1

PICO 7 - Dried blood spots

Dried blood spots as a sample collection method for hepatitis B surface antigen serological testing

A systematic review and meta-analysis

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1. Abstract

Introduction: Several expert organizations recommend screening of individuals living in high or even intermediate prevalence of hepatitis B. Despite this, few countries have or implement such screening recommendations. In high-prevalence low- and middle-income countries, there is a need for improved HBV screening, especially in decentralized settings. The use of dried blood spots (DBS) sent to centralized lab facilities may be useful in certain contexts. A systematic review and meta-analysis were performed to address the question: Among persons identified for hepatitis B testing, what is the diagnostic accuracy and impact of detecting HBsAg from DBS samples versus venous sample?

Methods: Following an *a priori* protocol, PubMed, MEDLINE, WHO Global Index Medicus, Web of Science, MSF, Cochrane, EMBASE, CABS Abstracts and LILACS databases were searched by two reviewers in duplicate. Data were extracted with the primary outcome of HBsAg DBS test accuracy using the gold standard of a venous sample. For analysis of sensitivity and specificity, a bivariate analysis using a maximum likelihood estimate and 95% confidence intervals was used. Likelihood ratios were calculated directly from the pooled sensitivity and specificity. QUADAS-2 was used to assess bias and a GRADE evaluation was performed to evaluate the quality of included studies.

Results: Two hundred forty studies were obtained for consideration, of which 10 met the criteria for inclusion in the review and 9 provided sufficient data for inclusion in the meta-analysis. The meta-analysis revealed a sensitivity of 92.9% (upper bound–lower bound 86.2–96.5) and specificity of 99.0% (upper bound–lower bound 96.2–99.7). From the pooled sensitivity and specificity, the positive likelihood ratio is 92.9 and the negative likelihood ratio is 0.072. As heterogeneity was identified on the forest plots, stratified analyses were done by storage temperature and test cut-off values. The analysis stratified by storage temperature revealed a sensitivity of 78.7% (upper bound–lower bound 70.3–85.2) and 96.1% (upper bound–lower bound 91.9–98.2) for cold chain versus ambient temperature or higher, and a specificity of 98.6% (upper bound–lower bound 68.0–100) and 99.7% (upper bound–lower bound 98.3–100) for cold chain versus ambient temperature or higher. The analysis stratified by test cut-off revealed a sensitivity of 88.0% (upper bound–lower bound 74.0–95.0) and 95.6% (upper bound–lower bound 91.2–97.8) for standard and lowered cut-off, respectively, and a specificity of 98.6% (upper bound–lower bound 89.5–99.8) and 99.1% (upper bound–lower bound 96.6–99.8), respectively. The assessment for risk of bias revealed some risk due to patient selection and interpretation of index test. The GRADE analysis showed the included studies to provide evidence of moderate quality to assess sensitivity and specificity.

Discussion: This review includes evidence of moderate quality that supports acceptable accuracy of DBS for testing HBSAg as compared to use of plasma samples and suggests that DBS may be used where there is limited access to venepuncture or inadequate technology to prepare and transport plasma samples. It is important to note that use of DBS may require changing cut-offs to

determine test positivity. As there are relatively little data on accuracy of DBS in real-life conditions (including high humidity), operational research will be needed in real-life conditions.

2. Introduction

According to the World Health Organization's latest data, an estimated 240 million people are chronically infected with the hepatitis B virus (HBV) and more than 780 000 people die every year due to complications of hepatitis B.[1] In 20–30% of chronically infected individuals, hepatitis B progresses to liver cancer and/or cirrhosis.[2] For some individuals, acute hepatitis shortly after infection can cause fatal liver failure. Hepatitis B disproportionately affects populations in low- and middle-income countries: adult chronic infection prevalence in sub-Saharan Africa and East Asia is 5–10% (similar high rates affect populations in the Amazon and south-east and south-central Europe), and of 2–5% in the Middle East and India.[3]

Several organizations such as the US Centers for Disease Control and Prevention (CDC), the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) recommend screening of individuals living in high or even intermediate prevalence. Despite this, few countries have or implement such screening recommendations and a very large number of individuals living with HBV are not diagnosed. In high-prevalence low- and middle-income countries, there is a need for improved HBV screening, especially in decentralized settings. While commercial rapid tests exist, the use of dried blood spots (DBS) sent to centralized lab facilities may be useful in certain contexts.

There are a number of HBsAg serological tests used, including rapid diagnostic tests (RDTs) and enzyme immunoassays (EIAs). Among RDTs, there are numerous options, such as the Determine HBsAg RDT. A recent meta-analysis of accuracy of hepatitis B RDTs demonstrated that the pooled sensitivity was 94.76% (95% credible interval [CrI] 90.08–98.23%) and specificity was 99.54% (95% CrI 99.03–99.95%).[4]

Use of DBS

Hepatitis B surface antigen (HBsAg) is the screening test for HBV. Use of DBS for HBV screening may simplify sample collection and preparation (e.g. through collection of finger-prick blood samples) and improve the ability to store and transport samples for testing.[5,6] Starting with the sample collection method, blood can easily be collected from pricking a finger or a heel, thus reducing the need for more highly-trained health-care workers. For DBS, less blood volume is required than in conventional venepuncture, and sample preparation is simple (it does not require electrical power, or a centrifuge), and inexpensive. Furthermore, once collected, the handling of the samples is rendered more easy: samples are less cumbersome, and can be transported in little space and at room temperature thus reducing or eliminating need for cold chain. Finally, individuals conducting the tests on the samples have a reduced risk of

contamination once the blood has dried. This technique has already had success in diagnosing other infections such as HIV and is being developed for screening of other diseases such as hepatitis C.[7] It has also been used since decades for the mass screening of congenital disorders and neonatal diseases, such as hypothyroidism and phenylketonuria (Guthrie test).

In March 2015, WHO published the first guidelines for the prevention, care, and treatment of individuals with chronic HBV infection. These guidelines focused on assessment for treatment eligibility, initiation of first-line therapies, switching, and monitoring, and did not include screening recommendations. WHO is now undertaking guidelines for testing for chronic hepatitis B and C infection in low- and middle-income settings. A topic for consideration in these guidelines is the potential use of DBS for serological and molecular testing for HBV and HCV to facilitate access to and uptake of testing.

In order to better evaluate the sensitivity and specificity of DBS for testing for HBsAg, the following PICOT question was developed for HBsAg. Among persons identified for hepatitis B testing, what is the diagnostic accuracy and impact of detecting HBsAg from DBS samples versus venous sample?

Population: Samples for serology (HBsAg) for HBV

Intervention: Using DBS samples

Comparisons: Using plasma or serum from venous samples

Outcomes: Diagnostic accuracy (Sensitivity, Specificity, Positive likelihood ratio, Negative likelihood ratio, TN, TP, FN, and FP)

3. Methods

A protocol was prepared for the literature search, article selection, data extraction and assessment of methodological quality.

a. Search strategy and selection criteria

Types of studies

Case–control, cohort, and cross-sectional and randomized trials were included and articles were selected that compared DBS HBsAg testing against the gold standard of HBsAg testing using serum, and reported specificity and sensitivity or sufficient data to calculate sensitivity and specificity.

Participants

No date, geographical or population demographic exclusions were used. Patients of all age groups were included.

Target conditions

For use in screening for or diagnosing hepatitis B

Reference standard

Testing for HBsAg in serum using any commercially available test

Outcome measures

Sensitivity refers to the proportion of samples with true HBV infection diagnosed with a positive HBsAg test using DBS confirmed with a positive HBsAg in serum.

Specificity refers to the proportion of samples with negative HBsAg using DBS and no evidence of HBV infection confirmed with a negative HBsAg in serum.

Search methods

We searched English language manuscripts from PubMed, MEDLINE, WHO Global Index Medicus, Web of Science, MSF, Cochrane, EMBASE, CABS Abstracts and LILACS databases using the search terms contained in Annex 1. The search was conducted between April and June 2015.

Title, abstract and full-text review was done in duplicate using pre-defined eligibility criteria with a third reviewer serving as a tie-breaker for inclusion disagreements. The reference lists for articles selected for inclusion were also reviewed for additional manuscripts to review. Additional data and clarifications were sought by contacting study authors. Two articles were excluded because of inability to locate the full text of these articles.

b. Data extraction

All the studies were subject to the same data extraction procedure and form based on the following parameters: author, publication and study dates, country and their World Bank economic category (high, middle/low income, or both high and middle/low income), percentage of HIV-positive participants, percentage of children and adults, age range, gender distribution, study design, participant selection, direction of study (prospective vs retrospective), type of specimen used for DBS, specimen used as gold standard (plasma or serum), test used, and whether or not the tests were administered following the recommendations from the manufacturer. Two reviewers independently extracted data. Studies without extractable sensitivity or specificity data were excluded from the meta-analysis.

c. Statistical data analysis

Statistical analysis of the data was performed using OpenMeta [Analyst]. For analysis of sensitivity and specificity, a bivariate analysis using maximum likelihood estimate and 95% confidence intervals was used. Likelihood ratios were calculated directly from the pooled sensitivity and specificity. We used forest plots to visually assess heterogeneity. Stratified analysis was performed by studies that used the standard manufacturer-recommended cut-off value and those that used a lower cut-off, and by studies that stored DBS in the cold chain (refrigerated or frozen) and those that stored the DBS samples at ambient temperatures or higher.

d. Risk of bias and quality assessment

The QUADAS-2 tool was used to assess risk of bias. A GRADE assessment performed by two reviewers in parallel to assess the quality of included studies.

4. Results

a. Summary of included studies

A search yielded 240 studies for consideration of which 10[8–17] met the criteria for inclusion in the review (Fig. 1 and Table 1) and 9 provided sufficient data for inclusion in the meta-analysis.[8–11, 13, 14, 16, 17] The studies provided 370 positive samples among 1516 total samples. Four studies were from high-income countries,[10, 14–16] four from middle-income countries[8, 9, 12,17] and two from low-income countries.[11, 13] Five studies drew samples from broad populations, including pregnant women,[8, 9, 13–15] two from inpatient populations,[10, 12] two from attendees at liver clinics[16, 17] and one from attendees of an HIV testing centre.[11] Storage conditions including time and temperature varied among the studies, with 3 studies keeping samples in refrigerated or frozen storage,[8, 9, 12] six studies with some or all samples at ambient temperature or higher[10, 11, 13, 14, 16, 17] and one not specified.[15] Finally, some studies lowered the test cut-off value for DBS samples while others used the manufacturer-recommended cut-off level.

b. Diagnostic performance

In general, testing for HBsAg using DBS maintained good accuracy as compared with the reference test using plasma or serum. The meta-analysis revealed a sensitivity of 92.9% (upper bound–lower bound 86.2–96.5) and specificity of 99.0% (upper bound–lower bound 96.2–99.7) (Table 4 and

Figs 2 and 3). From the pooled sensitivity and specificity, the positive likelihood ratio is 92.9 and the negative likelihood ratio is 0.072.

c. Impact of storage conditions

A range of storage conditions were evaluated in the included papers, including storage temperature ranging from –20 to 33°C and storage time ranging from overnight to 180 days. In general, storage at room temperature or higher (30–33°C) did not affect accuracy of testing as compared to storage in the cold chain. However, the two studies that evaluated storage at room temperature or higher with prolonged storage time did note a decrease in sensitivity when samples were stored for >15 days at room temperature[16] or a decrease in sensitivity and specificity for samples stored at room temperature for more than 63 days.[17] A stratified analysis of studies that stored samples in the cold chain (refrigerated at 2–8°C or frozen)[8, 9] versus those that stored samples at ambient temperature or above (30–33°C)[11, 13, 14] was performed for 5 studies (2 cold chain and 3 ambient temperature or higher). Other studies were excluded as they examined DBS samples in a range of temperature conditions, but did not provide sufficient information to determine sensitivity or specificity at a given temperature. The analysis revealed a sensitivity of 78.7% (upper bound–lower bound 70.3–85.2) and 96.1% (upper bound–lower bound 91.9–98.2) for cold chain versus ambient temperature or higher and a specificity of 98.6% (upper bound–lower bound 68.0–100) and 99.7% (upper bound–lower bound 98.3–100) for cold chain versus ambient temperature or higher (Figs 4 and 5).

d. Impact of cut-off

Several papers noted that the ideal cut-off (e.g. as suggested by receiver operating characteristic [ROC] curves) for determining test positivity should be lower for DBS samples as compared to plasma or serum samples (Table 1).[11–12, 15] Authors postulated this was due to the small blood volume used in DBS (commonly 50 µL for a circle of 12 mm diameter). Stratified analysis by cut-off value (standard versus raised) revealed a sensitivity of 88.0% (upper bound–lower bound 74.0–95.0) and 95.6% (upper bound–lower bound 91.2–97.8) for standard[8, 9, 13, 16] and lower cut-off,[10, 11, 14, 17] respectively, and a specificity of 98.6% (upper bound–lower bound 89.5–99.8) and 99.1% (upper bound–lower bound 96.6–99.8), respectively (Figs 6 and 7).

e. Assessment of bias and quality assessment

The assessment for risk of bias revealed that several studies did not use a random or consecutive sampling method or used a study population that is not consistent with the target screening

population.[8, 13, 14, 16, 17] As there are no current standards for test cut-offs for HBsAg using DBS, several studies used results of ROC curves to change the cut-off used for the DBS samples, adjusting the cut-off downward to achieve better sensitivity.[10–12, 14, 15, 17] This adjustment may introduce bias and thus, these studies were judged to have a high risk of bias on the index test domain. The included studies had a low risk of bias on the reference test and flow and timing domains (Table 2). The GRADE analysis showed the included studies to provide evidence of moderate quality to assess sensitivity and specificity (Table 3).

5. Discussion

Overall conclusions

This systematic review and meta-analysis includes evidence of moderate quality that supports acceptable accuracy of DBS for testing HBsAg as compared to use of plasma samples, and suggests that DBS may be used for screening for HBV using HBsAg DBS where there is limited access to venepuncture or inadequate technology to prepare and transport plasma samples. Although studies are limited, DBS is likely stable and maintains good accuracy in conditions with higher temperatures and with higher humidity, although accuracy may be negatively affected when storing for prolonged durations (>14 days) at higher temperatures (room temperature and above). In a stratified analysis that examined studies that stored samples in a cold chain versus those that stored samples at ambient or higher temperatures did not reveal a detriment in accuracy for DBS stored at ambient or higher temperatures. As there are relatively little data on accuracy of DBS in real-life conditions (including high humidity), operational research will be needed in real-life conditions.

It is important to note that use of DBS may require changing cut-offs to determine test positivity. As DBS uses a small sample of blood, in order to maintain sensitivity, a lower cut-off may be required as compared to when using plasma samples. In the stratified analysis performed examining standard or lower cut-off values, DBS that used a lower-cut off had a higher sensitivity than those that used the manufacturer-recommended cut-off without sacrificing significant specificity. Each individual test kit needs to be evaluated separately for its own ideal cut-off for DBS based on a ROC curve. This will usually differ from the serum test cut-off and usually be a lower cut-off to increase sensitivity. It will be important to validate use of DBS with commercially available HBsAg tests and determine cut-off values.

Key limitations

This review also has a number of limitations. Overall, the number of studies was small. In particular, there is a dearth of studies that systematically examine the effects of storage conditions on the accuracy of DBS and of those that did assess this, several did not specify the exact conditions (e.g. exactly what is room temperature). Thus, should DBS be adopted for screening for HBV, it will be important to pursue further operational research using field specimens in prepared and stored in real-life conditions. Ideally, to fit operational needs, these

studies should use capillary whole blood to prepare the DBS, use a commonly available type of filter paper while comparing several commercial test kits and conditions of storage.

Future work

Expanded use of HBsAg testing will be critical to improving the diagnosis and treatment of HBV globally. While commercial HBsAg rapid diagnostic tests may be useful in certain contexts, the ability to use DBS for improved preparation, storage and transport of samples for decentralized testing may help to expand the reach of this important diagnostic test.

Fig. 1. Study flow diagram

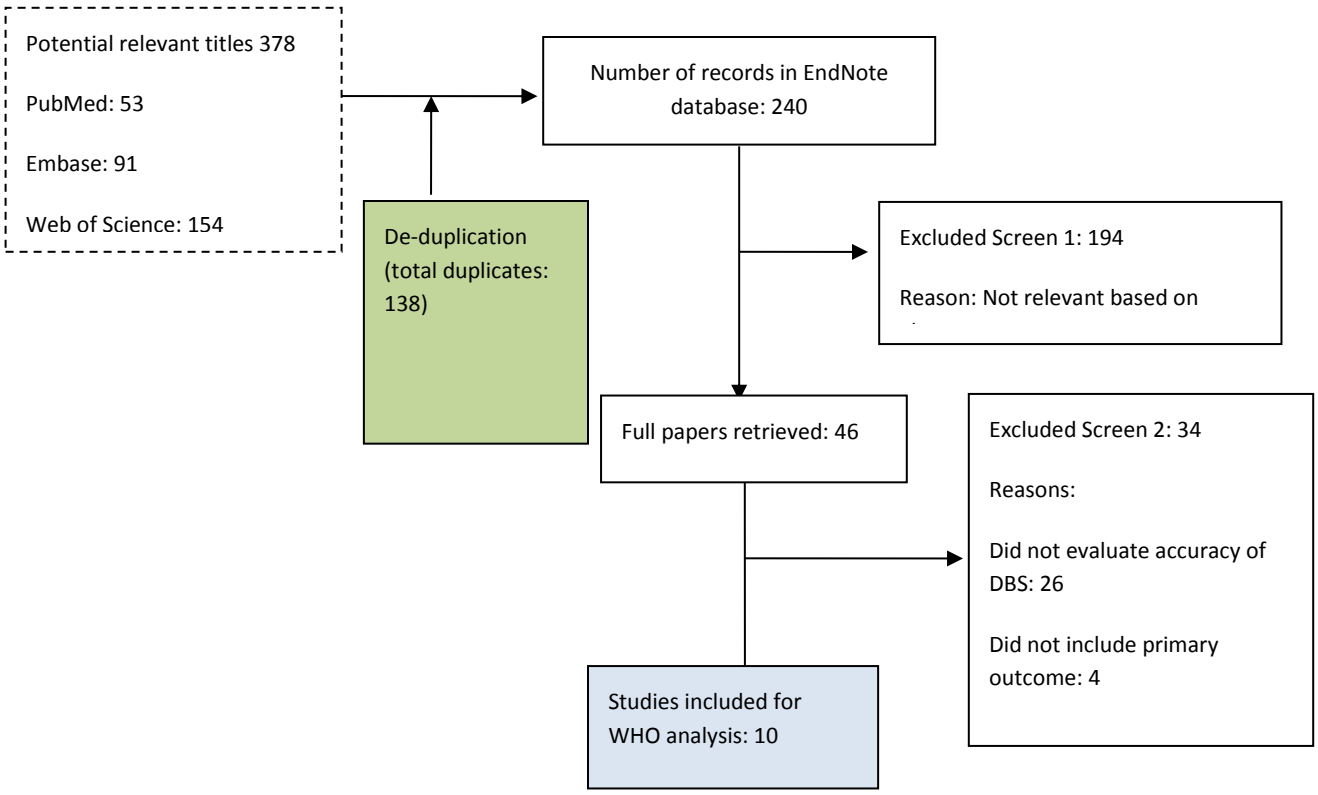


Table 1. Study-level characteristics of included studies

Author	Country and income category	Study design	Study pop	Sample size	Storage conditions	Reference test	Sample for DBS	Filter paper	Specificity	Sensitivity	Cut-off value	Effect of storage conditions
Boa-Sorte 2014	Brazil Upper–middle income	Cross-sectional	Pregnant women	692	Temperature: refrigerated Time: less than 5 days	IMUNOSCREENHBSAG–SS (Mbiolog Diag.) and Murex HBsAg (MurexBioTechUnlmtd)	Venous	Schleicher and Schuell 903	100	100	Unchanged	
Forbi 2010	Nigeria Lower–middle income	Cross-sectional	Broad	300	Temperature: 4 °C Time: overnight	Shantest TM- HBsAg ELISA	Venous	Whatman no. 3	88.6	78.6	Unchanged	
Gruner 2015	Germany High income	Cross-sectional	Inpatients	299	Temperature: 20 °C, 4 °C or ambient temperature Time: up to 14 days	HBsAg assay ARCHITECHT system (Abbott Diagnostics)	Venous or capillary	PerkinElmer 226 and Whatman no. 3	99.8	91.7	0.15 IU/mL	
Kania 2013	Burkina-Faso Low income	Cross-sectional	Attendees of HIV testing centre	218	Temperature: ambient temperature Time: not specified	ETI-MAK-4 HBsAg EIA (DiaSorin S.p.A.)	Venous	Whatman 903	100	96	Optical density: MAPC (mean absorbance of positive control)/2 + 0.3 standard deviations= 0.825.	
Lee 2011	Malaysia Upper middle income	Cross-sectional	Patients at a tertiary hospital	150	Temperature: 20 °C Time: not specified	Abbott, kit not specified (Abbott Laboratories)	Venous	Whatman 903	97.8	96.5	Cut-off point of 1.72 Relative light units	
Mendy 2005	The Gambia Low income	Cohort	Broad	166	Temperature: 30–33 °C (humid conditions) Time: up to 4 weeks	Determine HBsAg (Abbott Laboratories)	Venous	Whatman, grade BFC 180	100	96	Unchanged	

Mohamed 2013	France High income	Cohort	Broad	200	Temperature: Room temperature Time: 1, 3, 7 and 14 days	Abbott ARCHITECT HBsAg assay (Abbott Laboratories)	Venous	Whatman FTA DMPK-C	100	98	0.30 +/-0.81 IU/mL	No significant change
Ross 2013	Germany High income	Cross-sectional	Broad	299	Time and temperature not specified	Abbott ARCHITECT HBsAg (Abbott Laboratories)	Venous	Not specified	100	98.6	15 IU/mL (HBsAg)	
Villa 1981	Italy High income	Cross-sectional	Patients attending liver clinic	24	Temperature: -20 °C, 4 °C and room temperature. Time: 1,7, 15, 30, 60, and 180 days	Ausria II, RIA kit (Abbott Laboratories)	Capillary	Not specified	100	100	Unchanged	Temperature: storage at room temperature resulted in no significant change compared to samples stored at 4 °C or -20 °C Time: storage longer than 15 days negatively affected sensitivity.
Villar 2011	Brazil Upper middle income	Cross sectional	Patients attending hepatitis clinic	133	Temperature: –20 °C, 4–8 °C, 22–25 °C Time: 1, 7, 14, 21, 42, 63, 112, and 183 days	ETI-MAK-4 HBsAg (Diasorin)	Venous or capillary	Whatman 903	96.7	97.62	Absorbance value 0.115	Accuracy of DBS samples was stable over 63 days at all temperatures evaluated but after 63 days, accuracy diminished when stored at 22–25 °C

Table 2. QUADAS-2 risk of bias assessment

Author	Patient selection	Index test	Reference standard	Flow and timing
Boa-Sorte	LR	LR	LR	LR
Forbi	LR	LR	LR	LR
Gruner	LR	HR	UR	UR
Kania	LR	HR	LR	LR
Lee	LR	HR	LR	LR
Mendy	HR	LR	LR	LR
Mohamed	HR	HR	LR	LR
Ross	LR	HR	LR	LR
Villa	HR	LR	LR	LR
Villar	HR	HR	LR	LR

HR: high risk of bias; UR: unknown risk of bias; LR: low risk of bias

Table 3. GRADE table

Number of studies	Type of study	Directness	Precision	Consistency	Risk of bias	Overall quality
Sensitivity 92.9% (95% CI 86.2–96.5)						
10 studies (370 HBsAg positive among 1516 samples)	Cross-sectional or cohort	No significant indirectness	No significant imprecision	Significant inconsistency (one paper reported lower sensitivity)	Significant risk of bias (patient enrolment not consecutive or random in some studies; pre-specified cut-off not used in some studies)	Moderate
Specificity 99.0% (95% CI 97.6–100%)						
10 studies (370 HBsAg positive among 1516 samples)	Cross-sectional or cohort	No significant indirectness	No significant imprecision	No significant inconsistency	Significant risk of bias (patient enrolment not consecutive or random in some studies; pre-specified cut-off not used in some studies)	Moderate

Table 4. Meta-analysis: sensitivity and specificity

	Estimate	Upper bound–lower bound
Sensitivity	92.98	6.2–96.5
Specificity	99.09	6.2–99.7

Fig. 2. Forest plot sensitivity

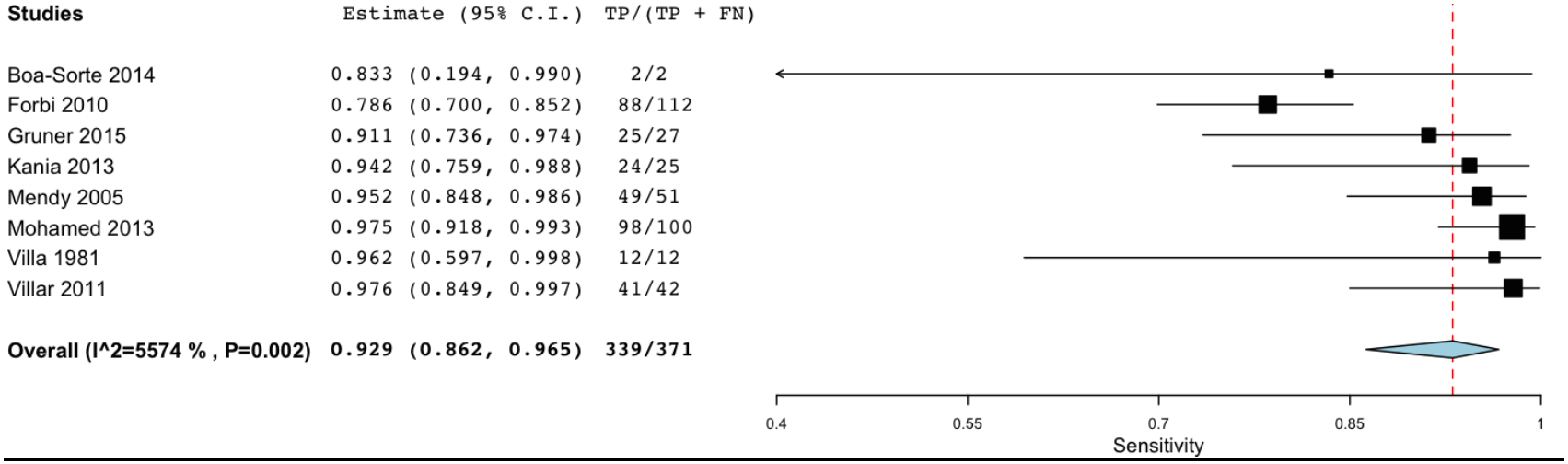


Fig. 3. Forest plot specificity

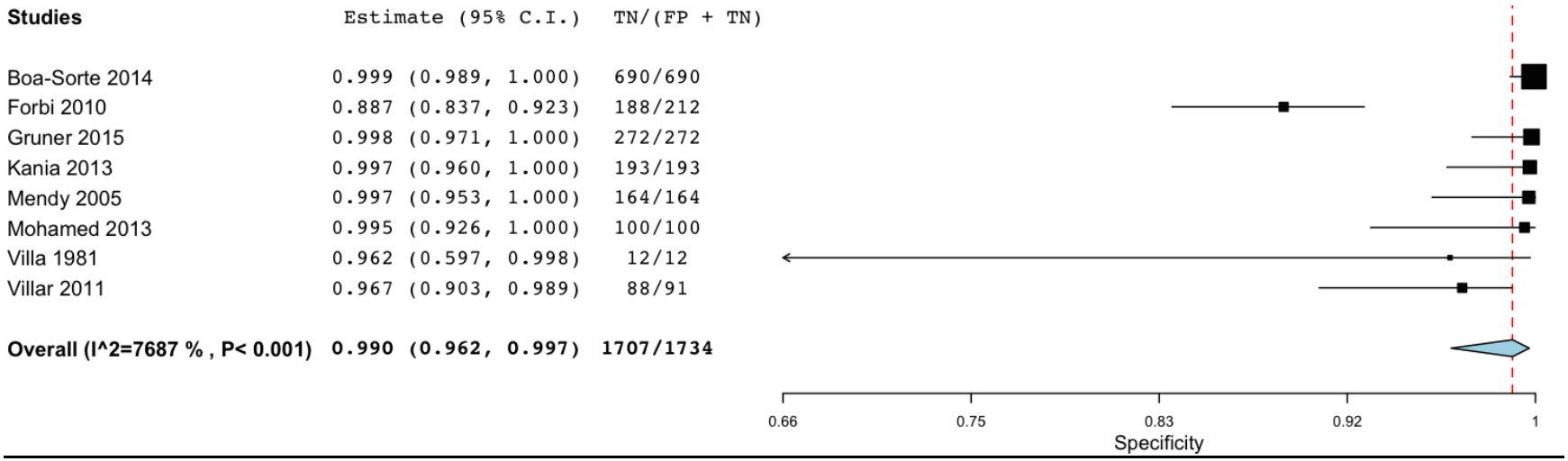
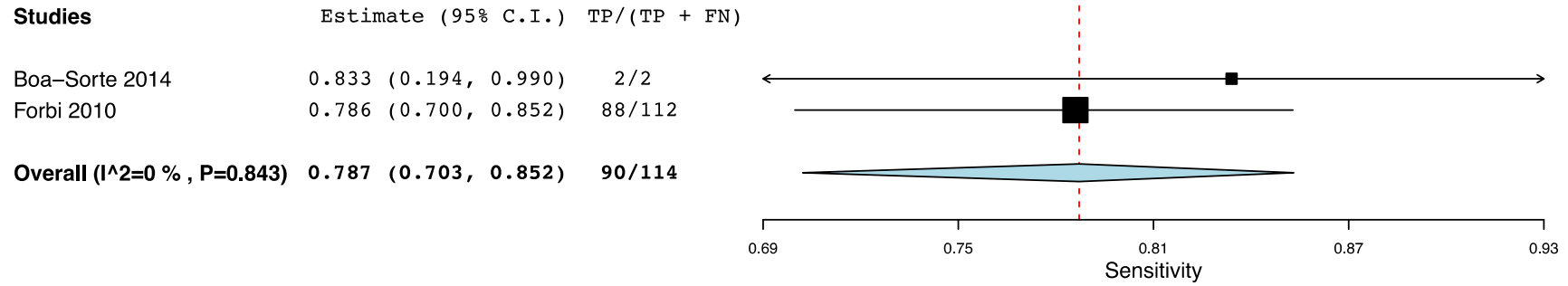


Fig. 4. Forest plot, sensitivity at differing storage temperatures

Maintained in cold chain (refrigerated or frozen)



Maintained at room temperature or higher

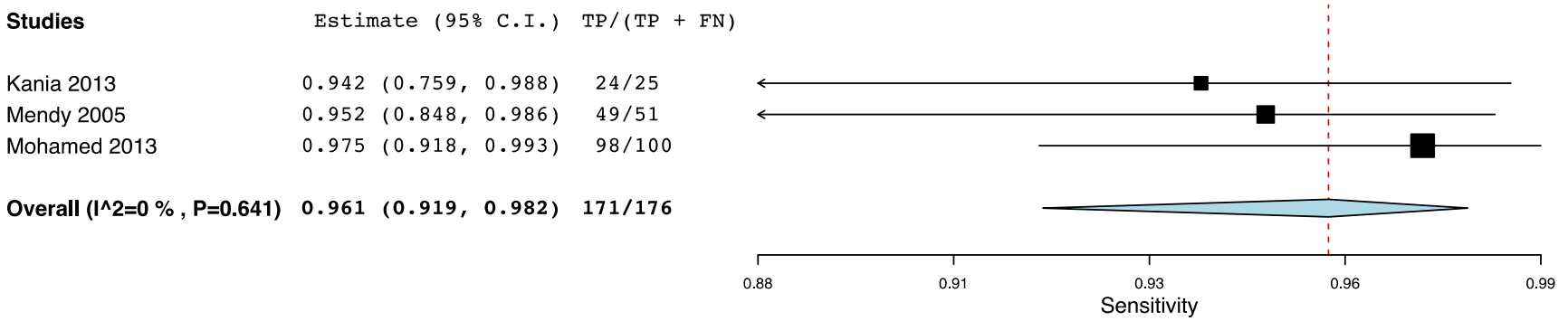
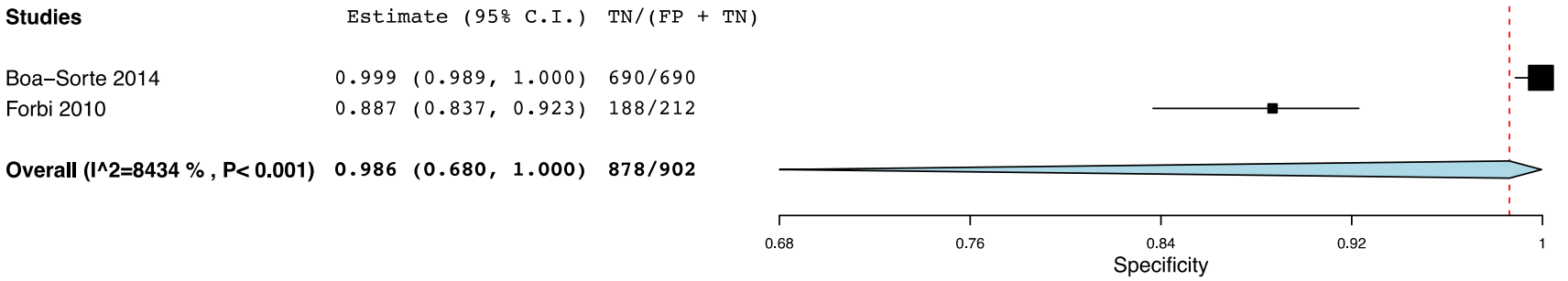


Fig. 5. Forest plot, specificity at differing storage temperatures.

Maintained in cold chain (refrigerated or frozen)



Maintained at room temperature or higher

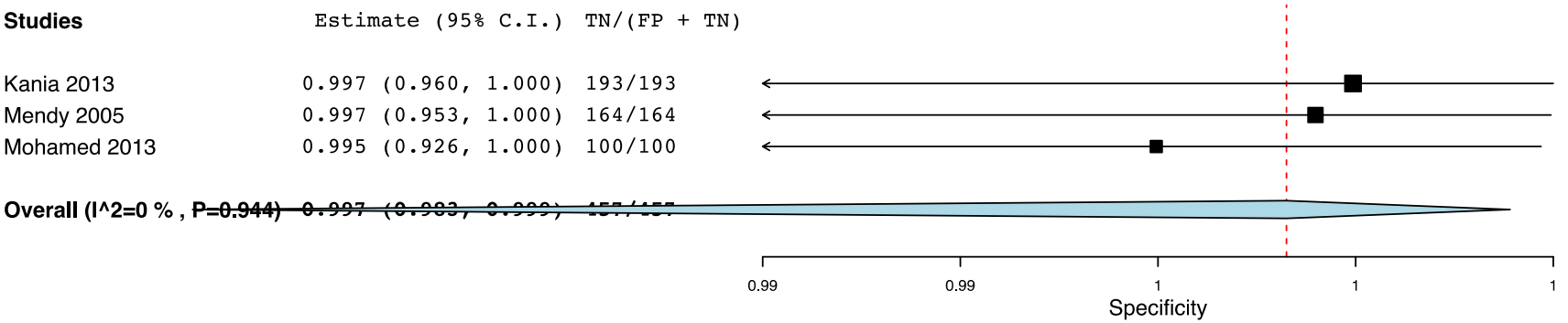
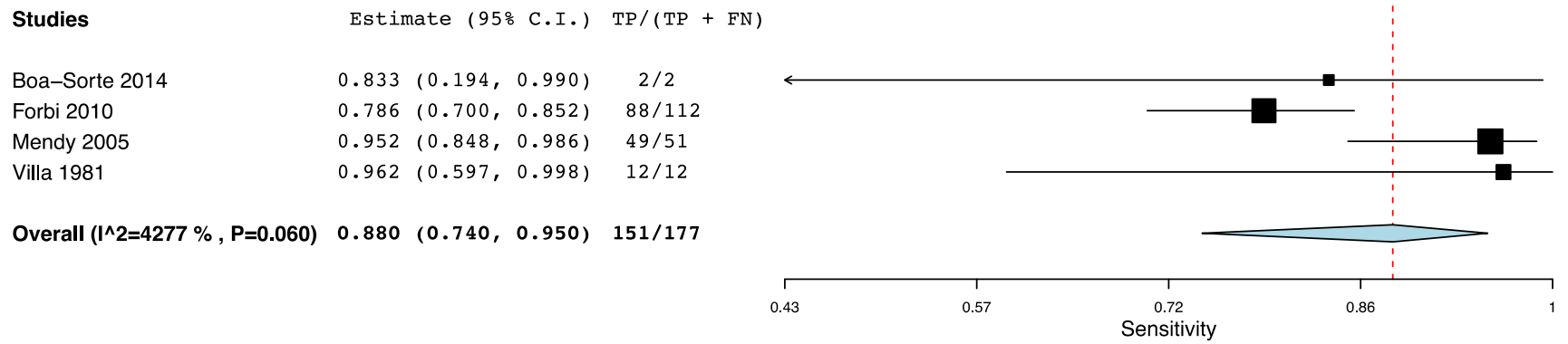


Fig. 6. Forest plot sensitivity, standard and lowered cut-off

Standard cut-off



Lowered cut-off

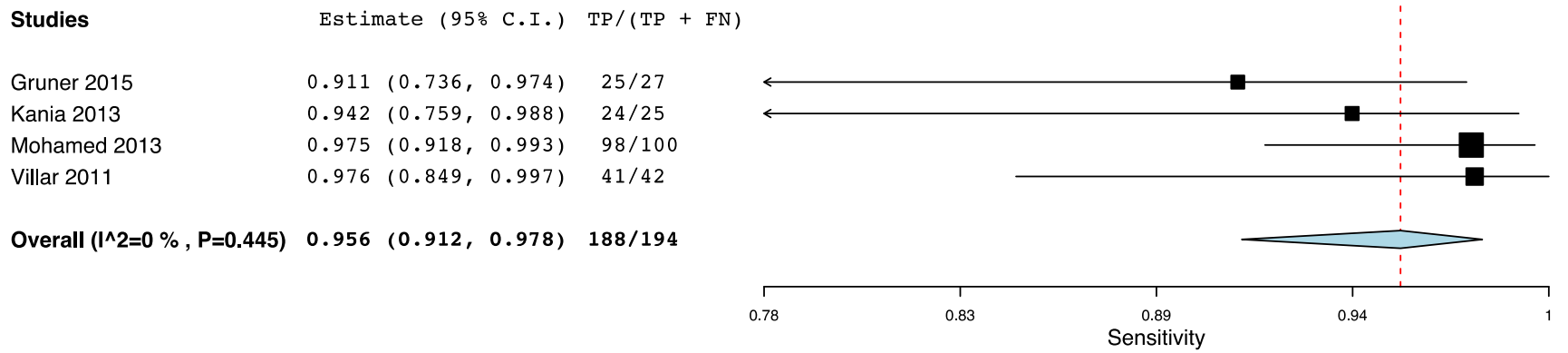
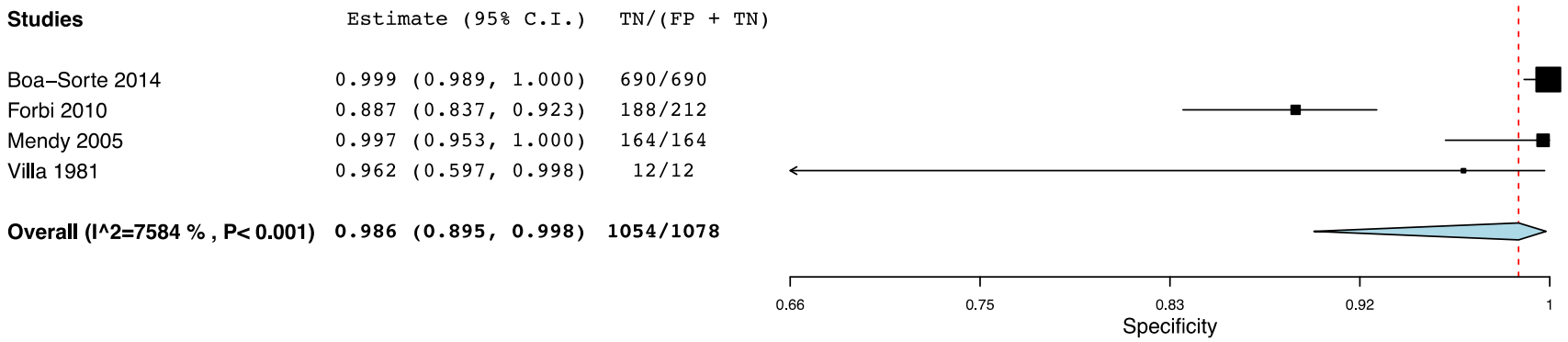
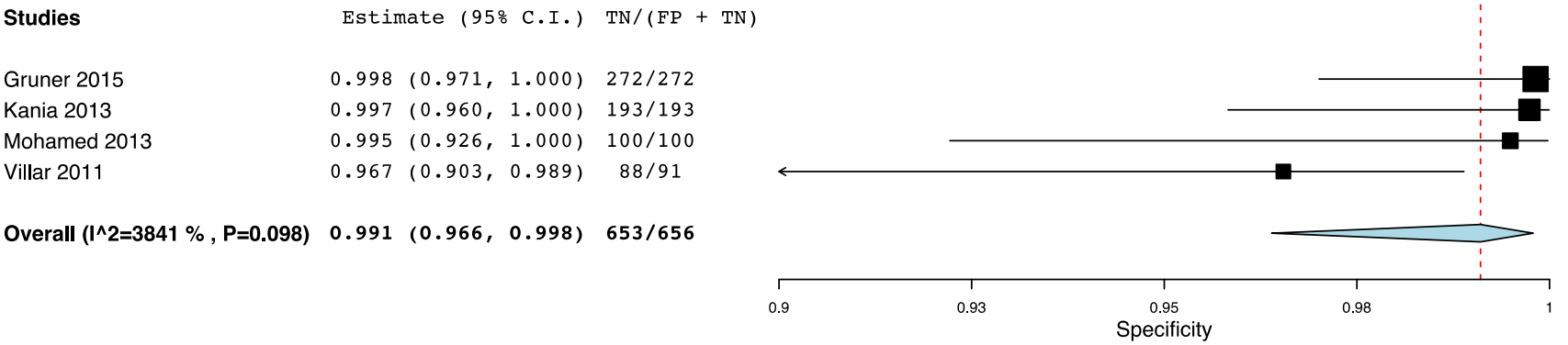


Fig. 7. Forest plot specificity, standard and lowered cut-off

Standard cut-off



Lowered cut-off



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Appendix 1: Search strategies

Pubmed

(("Hepatitis B" [Mesh] OR "Hepatitis B virus" [Mesh] OR “hepatitis B” OR “hepatitis B virus” OR “HBV”) OR (“Hepatitis B Antigens” [Mesh] OR “Hepatitis B Surface Antigens” [MESH] OR hepatitis b antigen* OR hepatitis b virus antigen* OR hepatitis b surface antigen* OR “HBsAg” OR “hbv sag” OR "hb sag" OR "HBsAg") AND (“Dried Blood Spot Testing”[Mesh] OR “dried blood spot testing” OR dried blood spot* OR dried plasma spot* OR “DBS” OR “dried blood” OR “dried plasma”))

Embase

1. ‘hepatitis b’/exp OR ‘hepatitis b antigen’/exp OR ‘hepatitis b surface antigen’/exp OR ‘hepatitis b virus’/exp
2. ‘hepatitis b’ OR ‘hepatitis b virus’ OR ‘hepatitis b surface antigen’ OR ‘hepatitis b antigen’ OR ‘hbv’ OR ‘HBsAg’ OR ‘hepatitis b sag’ OR ‘hbv sag’ OR ‘hbsag’ OR ‘hb sag’
3. ‘dried blood spot testing’/exp OR ‘dried blood spot testing’ OR ‘dried blood spot’ OR ‘dried blood’ OR ‘dried plasma spot testing’ OR ‘dried plasma spot’ OR ‘dried plasma’ OR ‘dbs’
4. #1 OR #2
5. #3 AND #4

Web of Knowledge (SCI-expanded, SSCI, Conference Proceedings science, BIOSIS previews)

1. **TOPIC**: (hepatitis b) *OR* **TOPIC**: (hepatitis b surface antigen) *OR***TOPIC:** (hepatitis b antigen) *OR* **TOPIC:** (hepatitis b virus) *OR* **TOPIC:** (hbv) *OR* **TOPIC:** (HBsAg) *OR* **TOPIC:** (hbv sag) *OR* **TOPIC:** (hbsag) *OR* **TOPIC:** (hb sag)
   Indexes = SCI-EXPANDED, SSCI, A&HCI Timespan=All years
2. ((((((**TOPIC:** (dried blood spot testing) *OR***TOPIC:** (dried blood spot)) *OR***TOPIC:** (dried blood)) *OR***TOPIC**: (dried plasma spot testing)) *OR***TOPIC**: (dried plasma spot)) *OR***TOPIC**: (dried plasma)) *OR***TOPIC:** (dbs))
   Indexes = SCI-EXPANDED, SSCI, A&HCI Timespan=All years
3. #2 AND #1
   Indexes= SCI-EXPANDED, SSCI, A&HCI Timespan=All years

Cochrane

1. MeSH descriptor: [Hepatitis B] explode all trees
2. MeSH descriptor: [Hepatitis B Antigens] explode all trees
3. MeSH descriptor: [Hepatitis B Surface Antigens] explode all trees
4. MeSH descriptor: [Hepatitis B virus] explode all trees
5. hbv or hepatitis b or hepatitis b virus or hepatitis b antigen or hepatitis b surface antigen or HBsAg or hbv sag or hbsag or hb sag
6. MeSH descriptor: [Dried Blood Spot Testing] explode all trees

7. dried blood spot testing or dried blood spot or dried blood or dried plasma spot testing or dried plasma spot or dried plasma or dbs
8. #1 or #2 or #3 or #4 or #5
9. #6 or #7
10. #8 and #9

Medline (OVID)

1. exp Hepatitis B/ or exp Hepatitis B virus/ or hepatitis B.mp. or hepatitis b virus.mp. or hbv.mp.
2. exp Hepatitis B Antigens/ or exp Hepatitis B Surface Antigens/ or hepatitis b antigen*.mp. or hepatitis b virus antigen*.mp. or hepatitis b surface antigen*.mp. or hbsag.mp. or hbv sag.mp. or HBsAg.mp. or hb sag.mp.
3. exp Dried Blood Spot Testing/ or dried blood spot testing.mp. or dried blood spot*.mp. or dried plasma spot*.mp. or dbs.mp. or dried blood.mp. or dried plasma.mp.
4. 1 or 2
5. 3 and 4

Annex 5.9.2

PICO 7 - Dried blood spots

Dried blood spots as sample collection method for HCV antibody: a systematic review and meta-analysis

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22 September 2015

1. Executive summary

Introduction: Dried blood spots are a convenient diagnostic for viral diseases due to transport and logistical advantages over venous blood sampling. Their diagnostic accuracy for the detection of HCV antibody is not known.

Methods: We conducted a systematic review and meta-analysis on the diagnostic accuracy of HCV antibody from DBS samples compared to venous samples in those persons identified for HCV testing. MEDLINE, EMBASE, Global Health and Cochrane library were searched with a sensitive search strategy and data was extracted based on a predefined extraction scheme. We described ranges of diagnostic accuracy outcomes as well as agreement of DBS against venous blood samples. For pooled analysis of sensitivity and specificity, a bivariate analysis using maximum likelihood estimate and 95% confidence interval was used and heterogeneity of results was assessed. PRISMA guideline was followed and the QUADAS tool was used to assess for risk of bias.

Results: Eighteen studies of 485 abstracts were included in the qualitative review; 14 of those contributed to the quantitative analysis. Overall quality of studies was moderate. A pooled bivariate analysis of sensitivity and specificity revealed an overall sensitivity of 98% (CI95% 94–99) and an overall specificity of 99% (CI95% 97–100). Positive likelihood ratio was 171 and negative likelihood ratio 0.02. Heterogeneity was moderate with a tau^2 of 0.1. In a separate univariate analysis, storage conditions did not explain heterogeneity of sensitivity or specificity, with all samples contributing to the quantitative analysis stored less than 24 hours at room temperature. However, two studies reported on false positive samples when DBS samples were stored at room temperature for longer than 3 days. Many different tests and cut-offs were used, so no stratified analysis on type of test or cut-off was performed.

Discussion: This systematic review of diagnostic accuracy of HCV antibody in DBS compared to venous blood shows good diagnostic accuracy with a body of evidence of moderate quality. Manufacturers should validate their tests for the use of DBS and include instructions for this in their manuals. Future studies on diagnostic accuracy should focus on storage conditions common to field situations to be able to assess pragmatic use of this sample method.

2.Introduction

i. Epidemiology

More than 150 million people are infected with hepatitis C (HCV) worldwide but only a proportion of these are aware of their infection.[1] Diagnosing HCV is done via serology by detecting antibodies to the core and non-structural antigens. Many guidelines from high-income settings recommend screening major high-risk groups including injecting drug users and persons living in high-prevalence settings.[1]

ii. Approach to HCV antibody testing

HCV testing is traditionally done by serology using enzyme-linked immunosorbent assay (ELISA) and many validated commercially available tests exist for this, two of which are WHO prequalified. Confirmation of active infection is gained by doing a polymerase chain reaction (PCR) of HCV RNA, as 15–45% spontaneously clear the virus. A previous WHO guideline based on a systematic review performed found low-quality evidence that confirmation of chronic infections should directly follow and not be delayed.[1]

iii. Use of dried blood spot sampling

Dried blood spots (DBS) are another way of obtaining blood samples, not requiring patients to undergo venous blood sampling if sourced from capillary blood. Storage and transportation are easier and risk of biohazard during transportation is reduced.[2] That is why DBS has been used increasingly in recent years to diagnose viral diseases, including HIV and viral hepatitis.[3] Disadvantages of using DBS include the fact that the commercial assays existing are not validated or regulatory approved for this method. The actual work in the laboratory is also more laborious (in terms of manual sample processing) than using serum samples.[4]

Some studies show the use of DBS increases uptake of hepatitis testing among several vulnerable risk groups,[5-7] while others were not able to confirm this.[8] The advantages of transport and storage make DBS a good choice for diagnosis of HCV in low-resource settings.[3] Several programmes and studies have used DBS for HCV antibody screening without validation,[9–11] and several recent studies have attempted to validate the use of DBS in diagnostic accuracy studies.[12,13] Recent systematic reviews have been published on HCV RNA detection with DBS,[14] on the uptake of interventions for HCV screening[15] and the use of point-of-care tests in viral hepatitis testing.[16] However, to our knowledge no attempt has been made to summarize the evidence on diagnostic accuracy for HCV antibody testing on DBS.

iv. Systematic review as preparation for a new WHO guideline

In March 2015, WHO published the first guidelines for the prevention, care and treatment of individuals with chronic HBV infection. These guidelines focused on assessment for treatment eligibility, initiation of first-line therapies, switching and monitoring. They did not include

screening recommendations. WHO is now undertaking guidelines for testing for chronic hepatitis B and C infection in low- and middle-income settings. A topic for consideration in these guidelines is the potential use of DBS for serological and molecular testing for HBV and HCV to facilitate access to and uptake of testing.

We conducted a systematic review and meta-analysis on the diagnostic accuracy of HCV antibody from DBS samples compared to venous samples in those persons identified for HCV testing. We looked at diagnostic accuracy outcomes as well as agreement of DBS against venous blood samples.

In order to better evaluate the sensitivity and specificity of DBS for testing for HCV antibody, the following PICO question was developed for HCV Ab:

> Among persons identified for hepatitis C testing, what is the diagnostic accuracy and impact of detecting HCV Ab from DBS samples versus venous sample?

Population: Samples for serology (HCV Ab) for HCV

Intervention: Using DBS samples

Comparisons: Using plasma or serum from venous samples

Outcomes: Diagnostic accuracy (sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, TN, TP, FN and FP) and agreement (kappa, intra-class coefficients)

3. Method

PRISMA guidelines were followed and QUADAS-2 was used to estimate quality of studies.

i.

ii. Search strategy and selection criteria

Types of studies

Observational (including diagnostic accuracy studies) and interventional studies were included. We chose studies including comparisons of the index test HCV antibody in DBS against the reference test HCV antibody using serum and reported correlations, regression coefficients, specificity, sensitivity or predictive values. Only English language reports were included.

Participants

No date, geographical or population demographic exclusions were made. Patients of all age groups were included.

Target conditions

For use in screening, for diagnosing HCV.

Index test

Testing for HCV antibody in DBS.

Reference standard

Testing for HCV antibody in serum using any commercially available or in-house tests.

Outcome measures

Sensitivity refers to the proportion of samples with true HCV infection diagnosed with HCV antibody test using DBS confirmed with a positive HCV antibody in serum.

Specificity refers to the proportion of samples with negative HCV antibody using DBS and no evidence of HCV antibody confirmed with a HCV antibody in serum.

Any measures of agreement (kappa, intra-class coefficients) will also be included.

iii. Search methods

We searched English language manuscripts from PubMed, MEDLINE, Web of Science, EMBASE, Global Health and LILACS databases using a sensitive search strategy. The search was conducted during August–September 2015.

Title, abstract and full-text review was done using predefined eligibility criteria. The reference lists for articles selected for inclusion were also reviewed for additional manuscripts to review. Additional data and clarifications were sought by contacting study authors.

iv. Data extraction

All the studies were subject to the same data extraction procedure by one reviewer (BL) and form based on the following parameters: author, publication and study dates, country, type of specimen used for DBS, specimen used as gold standard (plasma or serum), test used, storage conditions and effect of storage conditions and assay type.

v. Statistical data analysis

Statistical analysis of the data was performed using STATA 13. For analysis of sensitivity and specificity, a bivariate analysis using maximum likelihood estimate and 95% confidence interval was used. Likelihood ratios were calculated directly from the pooled sensitivity and specificity. We used forest plots to visually assess heterogeneity. If not all diagnostic values could be extracted from the study, univariate and bivariate analysis was compared. Stratified analysis was performed by type of assay and by storage conditions.

vi. Risk of bias and quality assessment

The QUADAS-2 tool was used to assess risk of bias. A GRADE assessment was performed by two reviewers in parallel to assess the quality of included studies.

4. Results

i. Search results and summary of included studies

Our search yielded 485 abstracts for screening after deduplication (manually and by reference software). One hundred fifteen full texts were screened for potential inclusion and 18 studies were chosen to be included for the qualitative review.[4,12,13,17–31] Fourteen of those contributed to the quantitative analysis. Of those not contributing, one did provide a ROC curve but no denominators for sensitivity and specificity,[28] two did not provide any data for calculation of sensitivity or specificity[30,32] and one was testing avidity in comparison to venous blood samples and not overall diagnostic accuracy.[22] Of the 14 studies providing enough data to calculate sensitivity, one did not have any negative references so that no specificity could be calculated.[29] (Fig. 1 and Table 1)

Studies mainly stemmed from Europe, North America and Australia,[33,34] two studies could be included from South America (Brazil)[33,34] and three from South-East and Central Asia (India,[23] Mongolia[35] and Malaysia[28]). Studies were published from 1997 to 2014 and most used 50µL to 100µL of whole blood on filter paper to test for HCV antibody. Only one study included children;[31] however, age ranges or gender for adult patients were rarely reported.

ii. Diagnostic performance

Diagnostic accuracy

Of those studies included in the quantitative analysis, reported sensitivity of HCV-antibody in DBS ranged from 70% to 100% and specificity ranged from 95.1% to 100% (see Table 1).

A pooled bivariate analysis of sensitivity and specificity revealed an overall sensitivity of 98% (CI95% 94–99) and an overall specificity of 99% (CI95% 97–100). From the pooled sensitivity and specificity, the positive likelihood ratio was 171 and the negative likelihood ratio was 0.02 (see Tables 3, 4 and 5). E(logitSE) was 3.6 and E(logitSp) was 5.1 with a covariance between estimates of E(logitSe) and E(logitSp) (tau^2) of 0.11, showing moderate heterogeneity in the bivariate analysis of studies.

Agreement

Three of 18 included studies provided agreement measures with kappa ranging from 0.87 to 0.94 between DBS and venous blood samples.[33, 34, 36]

iii. Effect of test and cut-off used

Fifteen different assays were used in HCV antibody detection. Cut-offs varied widely, and as no standardized cut-offs existed many studies devised their own cut-off via receiver operator characteristics. Nine of the included studies did report some threshold or cut-off used for DBS.[12,13,20,21,25–28,34] No attempt was made to stratify by type of test or cut-off used as too many strata would have rendered results difficult to interpret.

iv. Effect of storage conditions and type of test

Four studies evaluated different storage conditions. In one study, three of three previously negative samples exceeded threshold values after 3 days at room temperature.[27] Similarly, Tuaillon et al. showed that after 6 days of room temperature storage, threshold values were exceeded and previously negative samples would be seen as positive.[37] In another study, stability was shown until 60 days at room temperature, but variation in quantitative values was less after storage at –20 °C.[34] This was confirmed in another study that also tested different storage conditions and found lowest variation of results after storage at –20 °C.[33]

No study had left study samples at room temperature for longer than 24 hours. Therefore, in another pooled analysis, we stratified studies according to whether samples had been left at room temperature for longer than 4 hours or not. This did not change the high heterogeneity found in our meta-analysis (see Appendix).

v. Assessment of study quality and risk of bias

Concerning risk of bias, several studies did not report adequately on major issues; as such, rating risk of bias was difficult. Four of the included studies used case-control designs and only two reported consecutive sampling. However, the rest did provide some report on sampling. Only two studies blinded laboratory personnel to either reference or index test while performing the other one, however all studies performed index and reference tests consistently and reported on the protocol used. Overall, we graded the quality of studies to be moderate (Tables 2 and 6).

b. Discussion

This systematic review and meta-analysis shows that there is evidence of moderate quality on the use of DBS for HCV antibody testing. The pooled analysis of the data available suggests high diagnostic accuracy of DBS samples for detection of HCV antibodies with good precision. The descriptive review also shows that studies looking at agreement found good agreement between DBS HCV antibody testing and testing on venous blood samples.

i. Impact of storage and other factors

No study stored DBS samples at room temperature for longer than 24 hours. Additionally, those studies looking at variation of results after putting samples in different storage conditions found that samples could become false positive with longer exposure at ambient temperatures.[13,27]

In stratification on storage conditions of our pooled results, pooled sensitivity and specificity were only slightly different for samples stored longer at room temperature. However, as no study reported on storing samples for longer than a day, results are not generalizable to conditions often found in low-resource locations.

ii. Key limitations

This review has a number of limitations. We did not look at studies in languages other than English and no unpublished data from laboratories was included. While some studies had only a low risk of bias, overall the quality of studies was moderate at best.

Another important limit of this review is its inability to suggest certain commercial tests over others to use for DBS testing of HCV antibody or to suggest a cut-off that should be used for DBS testing. As tests used were varied, no stratified analysis was done for the type of test. Additionally, to suggest a cut-off, individual-level patient data would have to have been available.

iii. Future work

We would therefore suggest that subsequent diagnostic accuracy studies concentrate on showing applicability of DBS under field transport and storage conditions and report on different cut-offs used with their tests. We would also urge manufacturers to validate their tests for the use of DBS, apply for regulatory approval for this sample type and include instructions for this in their manuals.

5. Conclusion

In conclusion, while diagnostic accuracy of DBS for HCV antibody testing is good in those studies included in this review, uncertainty about the storage conditions needed and the cut-offs to use seriously limit its wider application in low-resource settings.

a. Figures and tables

Fig. 1. PRISMA flowchart

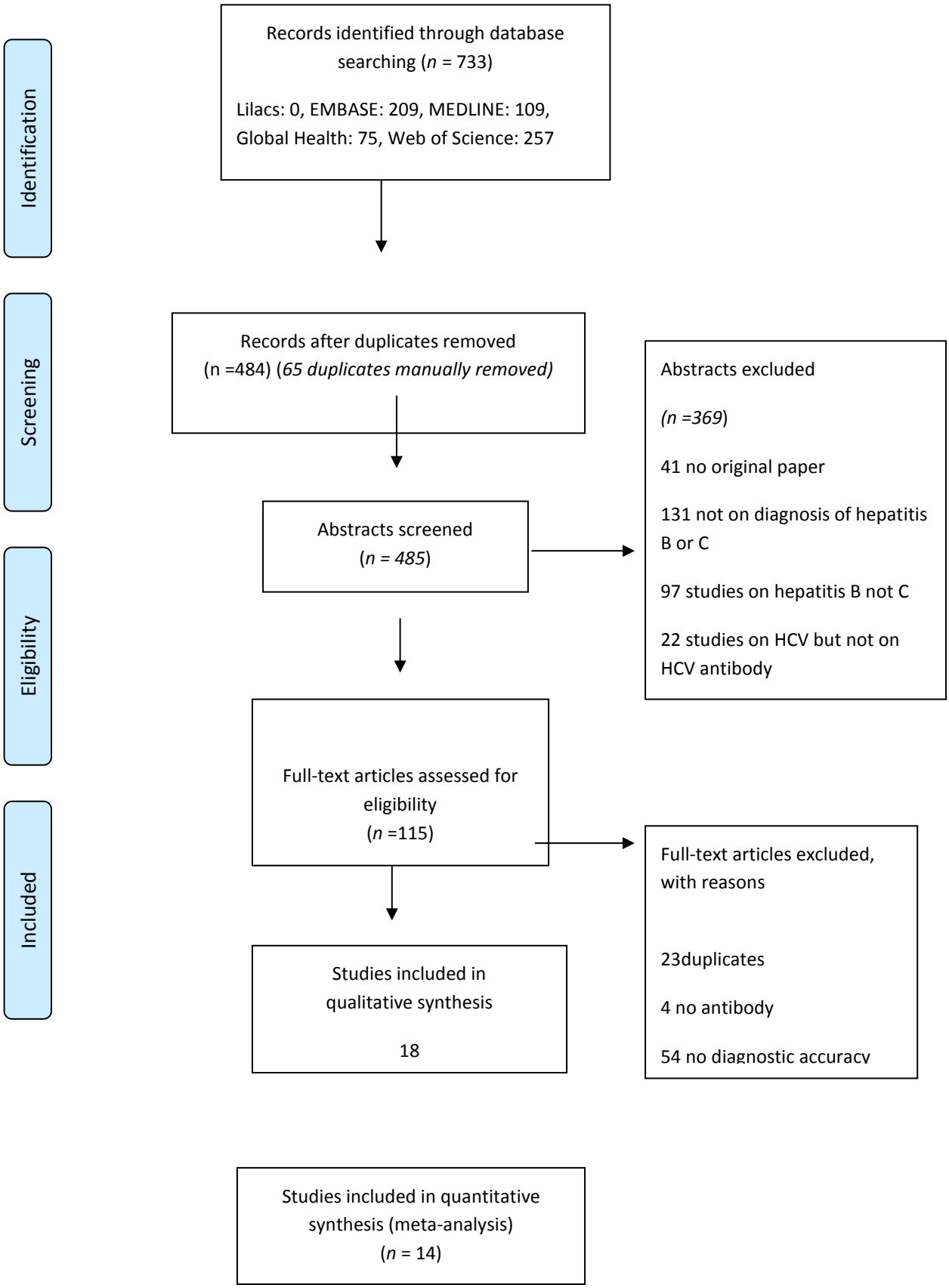


Table 1. Study characteristics

Author	Title	Journal	Year	Country	Study pop, sample size	Storage conditions	DBS collection method	Plasma antibody test	DBS antibody test	Suggested cut-off	Specificity	Sensitivity	Correlation/agreement	Effect of storage conditions
Brandao	Simultaneous detection of hepatitis C virus antigen and antibodies in dried blood spots	*Journal of Clinical Virology*	2013	Brazil	386 persons, 40 anti-HCV positive, 346 blood donors HCV non-reactive	DBS samples air dried at room temperature for 4 hours, stored at –20°C	75µL whole blood onto Whatman filter paper / alternatively 3–5 drops of capillary blood by finger-prick	MonolisaTM HCV AgAb ULTRA, Bio-Rad (Marnes-la-Coquette, France), and Murex HCV AgAb, Abbott (Kyalami, Republic of South Africa)	MonolisaTM HCV AgAb ULTRA, Bio-Rad (Marnes-la-Coquette, France), and Murex HCV AgAb, Abbott (Kyalami, Republic of South Africa)	ROC cut-off: 0.287 nm for Monolisa assay ROC cut-off for Murex assay 0.238 nm	99.7 (98.4–99.9) 95.9 (93.3–97.8)	97.5 (86.8–99.9) 97.5 (86.8–99.9)	PPV and NPV calculated Kappa=0.99 (with ROC cut-off),	Stability up to 60 days of storage at room temperature, but less variation at –20°C
Croom	Commercial enzyme immunoassay adapted for the detection of antibodies to hepatitis C virus in dried blood spots	*Journal of Clinical Virology*	2006	Australia	103 samples from high- risk groups, negative samples from 94 individuals tested at Haematology Lab	Air dried at room temperature, storage at –20°C, plasma at –20°C, time of storage 1 week to 11 months	80 µL of each whole blood sample spotted onto Schleicher and Schuell cards (Grade 903)	Monolisa EIA confirmation test: Murex anti HCV (version 4.0), EIA	Monolisa EIA confirmation test: Murex anti HCV (version 4.0), EIA	NR	100% (96–100) 108/108	100% (94–100) 75/75	NR	NR
Chevaliez	Dried blood spots (DBS), a promising tool for large-scale hepatitis C screening, diagnosis and treatment monitoring	Conference abstracts	2014	France	529 patients, 183 HCV seronegative, 346 seropositive	NR	NR	EIA HCV assay	EIA HCV assay	0.2	98.9 (96.1–99.7)	99.1 (97.4–99.7)	R=0.56	NR
Dokubo	Comparison of hepatitis C virus RNA and antibody detection in dried blood spots and plasma specimen	*Journal of Clinical Virology*	2014	US	148 participants in a prospective study of HCV	DBS airdried for 2 hour, then sent to another institute, then stored at –70°C	Fingerstick on Whatman 903 cards 0.5 ml blood	Standard HCV TMA (Novartis®) ELISA v3.0(Ortho®).	Standard HCV TMA (Novartis®) ELISA v3.0(Ortho®).		100% (71/71)	70% (54/77)	Kappa 0.69	NR

Larrat	Performance of an antigen–antibody combined assay for hepatitis C virus testing without venipuncture	*Journal of Clinical Virology*	2012	France	113 HCV-positive cases consecutively recruited	DSB dried 24 hours at room temperature	Finger-prick blood on Whatman card	Monolisa® HCV-Ag-Ab-ULTRA, Bio-Rad	Oraquick HCV CEIA Biorad	0.1 0.2 cEIA	100 (95.8–100) 88/88 100 (95.8–100) 88/88	97.4(92.5–99.1) 110/113 98.2 (93.8–99.5) 111/113	ROC AUC OMT cEIABiorad: 0.99 ROC AUC FSB cEIABiorad : 0.918	At 3 days room temperature 3/3 HCV negative samples NR
Lee	Evaluation of the dried blood spot (DBS) collection method as a tool for detection of HIV Ag/Ab, HBsAg, anti-HBs and anti-HCV in a Malaysian tertiary referral hospital	*Ann Acad Med Singapore*	2011	Malaysia	600 samples overall, not quite clear how many used anti-HCV	Left to dry overnight at room temperature, then stored –20°C	3 mL blood sample by venous puncture	Abbott	Abbott	ROC cut-off 0.10 RLU	100%	97.3%	ROC curve AUC: 0.99 R=0.631	NR
Lukacs	Simultaneous determination of HIV antibodies, hepatitis C antibodies and hepatitis B antigens in dried blood spots – a feasibility study using a multi-analyte immunoassay	*Clinical Chemistry and Laboratory Medicine*	2005	Germany	7 samples from known HCV patients				Luminex			100% 7/7		
McCarron	Hepatitis C antibody detection in dried blood spots	*J Viral Hepat*	1999	UK						0.99 1.99	87.5% 100%	100% 97.2%		

Marques	Dried blood spot samples: optimization of commercial EIAs for hepatitis C antibody detection and stability under different storage conditions	*Journal of Medical Virology*	2012	Brazil	21 and 24 HCV reactive patients, 234 individual and 132 HCV negative	Serum stored at –20°C	75 µL whole blood on Whatman paper	Two methods: HCV Ab Radim, Pomezzia, Italy and ETI-AB-HCVK-4 DiaSorin, Vercelli, Italy	Two methods: HCV Ab Radim, Pomezzia, Italy and ETI-AB-HCVK-4 DiaSorin, Vercelli, Italy	Radimcut-off: manufacturers cut-off ROC curve for DiaSorin EIA	99.5% (98 – 99.9) 98.9% (96.80–99.55)	97.5% (86.84–99.94) 88.9% (75.95–96.29)		2–8 °C, 20–25 °C, and –20°C were evaluated, –20 °C resulted in lowest variation Methods of cut-off determination: the receiver operating characteristic curve (AUROC)
Nandagopal	Evaluation of dried blood spot as an alternative specimen for the diagnosis of anti-HCV in resource-limited setting	*Indian Journal of Medical Microbiology*	2014	India	Murex	60 samples	50 µL of whole blood 903 Whatman card	NR	NR	NR	100 (29/29)	100 (31/31)	Pearson correlation coefficient 0.98	NR
O Brien	Detection of hepatitis C antibody with at-home collection kits using an innovative laboratory algorithm	*Infectious Diseases in Clinical Practice*	2001	US	1286 subjects enrolled in multicentre study	Air dry for 30 min, sent in FedEx envelope	Self-collected with at-home kit	NR	Hepatitis C check, Home Access Corp. self use DBS home kit	NR Several inconclusive and indeterminate results not included in diagnostic accuracy calculations	100% 686/686	99.5% 402/404	NR	NR
Parker	A method for the detection and confirmation of antibodies to hepatitis C virus in dried blood spots	*Journal for Virological Methods*	1997	UK	80 anti HCV positive samples, 52 negative 569 DBS sample fields from South African neonates	Air dry at room temperature before storage at 4°C	Dried blood field samples	In-house IgG ELISA, immunoblot RIBA 3.0	In-house IgG ELISA, immunoblot RIBA 3.0	T/N 5.0 T/N10.0	541/569 95.1%	78/80 98% 69/80 86.2%	NR	

Ross	Detection of infections with hepatitis B virus, hepatitis C virus, and human immunodeficiency virus by analyses of dried blood spots--performance characteristics of the ARCHITECT system and two commercial assays for nucleic acid amplification	*Virology*	2013	Germany	339 samples	Dried overnight at room temperature	100 µL of whole blood applied to Whatman 903 filter paper	ARCHITECT system (Abbott Diagnostics, Delkenheim, Germany)	ARCHITECT system (Abbott Diagnostics, Delkenheim, Germany)	NR	100% (97.7–100) 160/160	97.8% (96–100) 175/179	NR	NR
Sheperd	A hepatitis C avidity test for determining recent and past infections in both plasma and dried blood spots	*Journal of Clinical Virology*	2013	UK	19 recently infected 300 chronic carrier 82 resolved infection	DBS stored at 4°C until use	50 µL on 903 Whatman Protein saver cards	ORTHO HCV 3.0 ELISA Test System with Enhanced SAVekit (Ortho Clinical Diagnostics) was used to detect anti-HCV in DBS	NR	Avidity cut-off AI<30	98.3% Arc	100%	Comparison to known carriers, not to venous blood samples	NR
Tejada-Strop	Disparate detection outcomes for anti-HCV IgG and HCV RNA in dried blood spots	*Journal of Virological Methods*	2015	US	33 adult patients with chronic Hep C	-20°C until 5 years later	75 µL of whole blood on 12 mm DBS	Two immunoassays, the VITROS anti-HCV IgG chemi-luminescence assay (CIA) and the HCV 3.0 enzyme immunoassay (EIA), both from Ortho Clinical Diagnostics (Rochester, NY)	Two immunoassays, the VITROS anti-HCV IgG chemi-luminescence assay (CIA) and the HCV 3.0 enzyme immunoassay (EIA), both from Ortho Clinical Diagnostics (Rochester, NY)	3.26 CIA 1.5 EIA	Not calculated	CIA 48/52 92% EIA 90% 47/52 For stored samples CIA: 100% (33/33) EIA: 32/33 97%	NR	NR

Tuaillon,	Dried blood spot for hepatitis C virus serology and molecular testing	*Hepatology*	2010	France	100 anti HCV positive serum samples and 100 anti HCV negative samples	18 hours dried at room temperature, stored at –20°C for 1–8 weeks	50 µL of whole blood on Whatman 12 mm paper discs	Ortho HCV 3.0 ELISA, immunoblot assay INNO-LIA HCV Score as confirmatory test	Ortho HCV 3.0 ELISA, immunoblot assay INNO-LIA HCV Score as confirmatory test	Threshold value 0.380	98% (97–100)	99% (97–99)	NR	Stability of anti HCV and HCV RNA investigated by varying room temperature exposure 2–12 days until freezing, after 6 days at room temperature ODs > than cut-off values
Waterboer,	Dried blood spot samples for sero-epidemiology of infections with human papilloma viruses, *Helicobacter pylori*, hepatitis C virus and JC virus	*Journal of Clinical Virology*	2011	Mongolia	1022 sexually active women from cross sectional study (response rate 69%)	Room temperature up to 8 hours, then –20°C up to 1 month (serum + DBS)	Whole blood applied to 5 spots on DBS filter paper cards (Whatman 903)	The HCV (strain H77, subtype 1a) core and NS3 proteins	The HCV (strain H77, subtype 1a) core and NS3 proteins	Sera 1492 (Core) 371 (NS3) DBS 967 (Core) 310 (NS3) c	Not calculable from the data	Not calculable from the data	98% agreement (kappa 0.94) for core 96.1% agreement (kappa 0.90) for NS3	NR

Table 2. Risk of bias

	Patient selection	**Bias**	**Index test**	**Bias**	**Reference standard**	**Bias**	**Flow**	**Bias**
	Was a case–control design avoided? Consecutive or random sample of patients? Inappropriate exclusions?		Blinded to reference standard Could the conduct or interpretation of the index test have introduced bias?		Blinded to index? Could the reference standard have introduced bias?		Is there an appropriate interval between the index test and reference standard? Do all patients receive the same reference standard? Are all patients recruited into the study included in the analysis?	
Brandao	No case–control design, consecutive sample, no exclusions	LR	NR	UR	NR	UR	NR	UR
Croom	No case–control, sampling from different cohorts	LR	NR	UR	NR	UR	All patients included, same reference standard	LR
Chevaliez	NR	UR	NR	UR	NR	UR	NR	UR
Dokubo	No case–control, sampling from a prospective cohort	LR	NR	UR	NR	UR	Sampling reported, same reference standard	LR
Larrat	Consecutive recruitment	LR	Blinded	LR	Blinded	LR	Sampling reported, same reference standard	LR
Lee	Consecutive recruitment	LR	NR	UR	NR	UR	Sampling reported, same reference standard	LR
Lukacs	NR	UR	NR	UR	NR	UR	Sampling reported, same reference standard	LR
McCarron	Case–control	HR	NR	UR	NR	UR	NR	UR
Marques	No case–control design	LR	NR	UR	NR	UR	Sampling reported, same reference standard	LR
Nandagopal	NR	UR	NR	UR	NR	UR	NR	UR
O Brien	No case–control design	LR	Blinded	LR	Blinded	LR	Sampling partly reported, same reference standard	LR
Parker	Case–control design	HR	NR	UR	NR	UR	Sampling partly reported, same reference standard	LR
Ross	No case control design	LR	NR	UR	NR	UR	NR	UR
Sheperd	No case–control design	LR	NR	UR	NR	UR	NR	UR
Tejada-Strop	Case–control	HR	NR	UR	NR	UR	NR	UR
Tuaillon,	Case–control	HR	NR	UR	NR	UR	Sampling reported, same reference standard	LR
Waterboer,	No case–control	LR	NR	UR	NR	UR	NR	UR

Table 1. Sensitivities and specificities of included studies

Study	Sensitivity			Specificity		
	Estimate	CI95% lower bound	CI95% upper bound	Estimate	CI95% lower bound	CI95% upper bound
Brandao/2013/MonolisaTMAgAb	0.98	0.87	1.00	1.00	0.98	1.00
Brandao/2013/Murex	0.98	0.87	1.00	0.96	0.93	0.98
Croom/2006/MonolisaTMAgAb	1.00	0.95	1.00	1.00	0.97	1.00
Chevaliez/2014/EIA HCV	0.99	0.97	1.00	0.98	0.95	1.00
Dokubo/2013/HCV TMA Novartis	0.70	0.59	0.80	1.00	0.85	1.00
Larrat1/2012/Oraquick	0.97	0.92	0.99	1.00	0.96	1.00
Larrat2/2012/cEIABiorad	0.98	0.94	1.00	1.00	0.96	1.00
Lee/2011/Abott				–	–	–
Lukacs/2005/Luminex	1.00	0.59	1.00	–	–	–
McCarron/1999/				–	–	–
Marques1/2012/RadimPomezzia	0.98	0.87	1.00	0.99	0.98	1.00
Marques2/2012/ETI-AB-HCVK-4-Diasorin	0.89	0.76	0.96	0.96	0.94	0.98
Nandagopal/2014/Murex	1.00	0.89	1.00	1.00	0.88	1.00
OBrien/2001/Hepatitis C check	1.00	0.98	1.00	1.00	0.99	1.00
Parker/1997/in-house	0.98	0.91	1.00	0.95	0.93	0.97
Parker2/1997/in-house	0.86	0.77	0.93	0.95	0.93	0.97
Ross/2013/Architect	0.98	0.94	0.99	1.00	0.98	1.00

Table 4. Forest plot of sensitivities and specificities

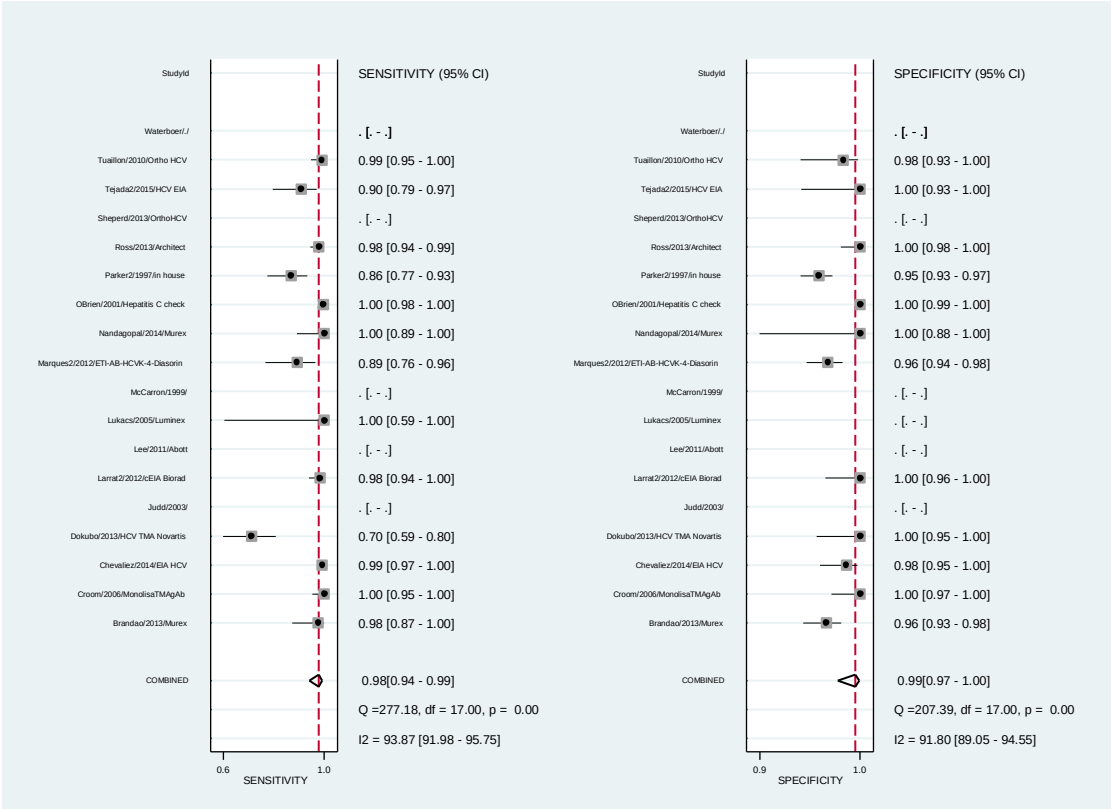


Table 5. Forest plot of likelihood ratios for included studies

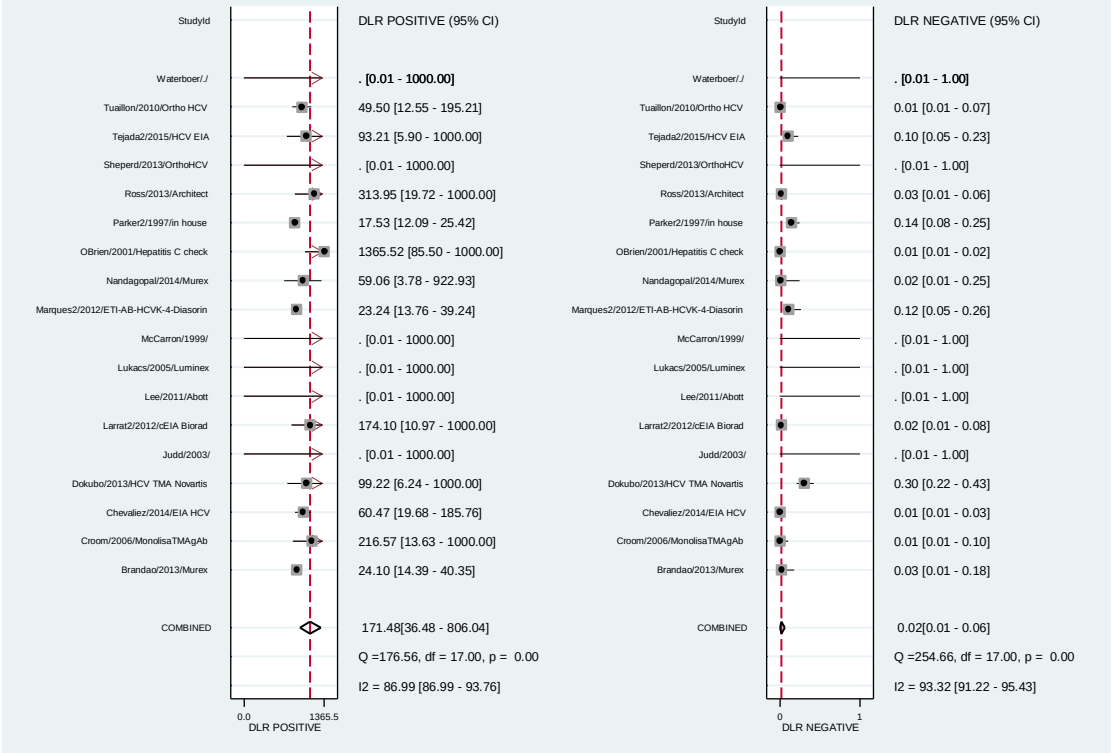


Table 6. GRADE table

Number of studies	Type of study	Directness	Precision	Consistency	Risk of bias	Overall quality
Sensitivity 98% (95% CI 94.0%–99.%)						
14 studies (1549 HCV positive among 4304 samples)	Diagnostic accuracy	No significant indirectness	No significant imprecision	Significant inconsistency	Moderate risk of bias (patient enrollment only partly consecutive or random; several case–control studies)	Moderate
Specificity 99% (95% CI 97–100%)						
13 studies (2756 HCV positive among 4304 samples)	Diagnostic accuracy	No significant indirectness	No significant imprecision	Significant inconsistency	Moderate risk of bias (patient enrollment only partly consecutive or random; several case-control studies)	Moderate

Appendix

i. Sources of heterogeneity

In a univariate analysis of sensitivity and specificity stratified by storage conditions sensitivity and specificity are similar among those reporting to have stored samples for less than 4 h compared to those reporting up to 24 h storage.

Table A1. Sensitivities of included studies stratified on storage conditions

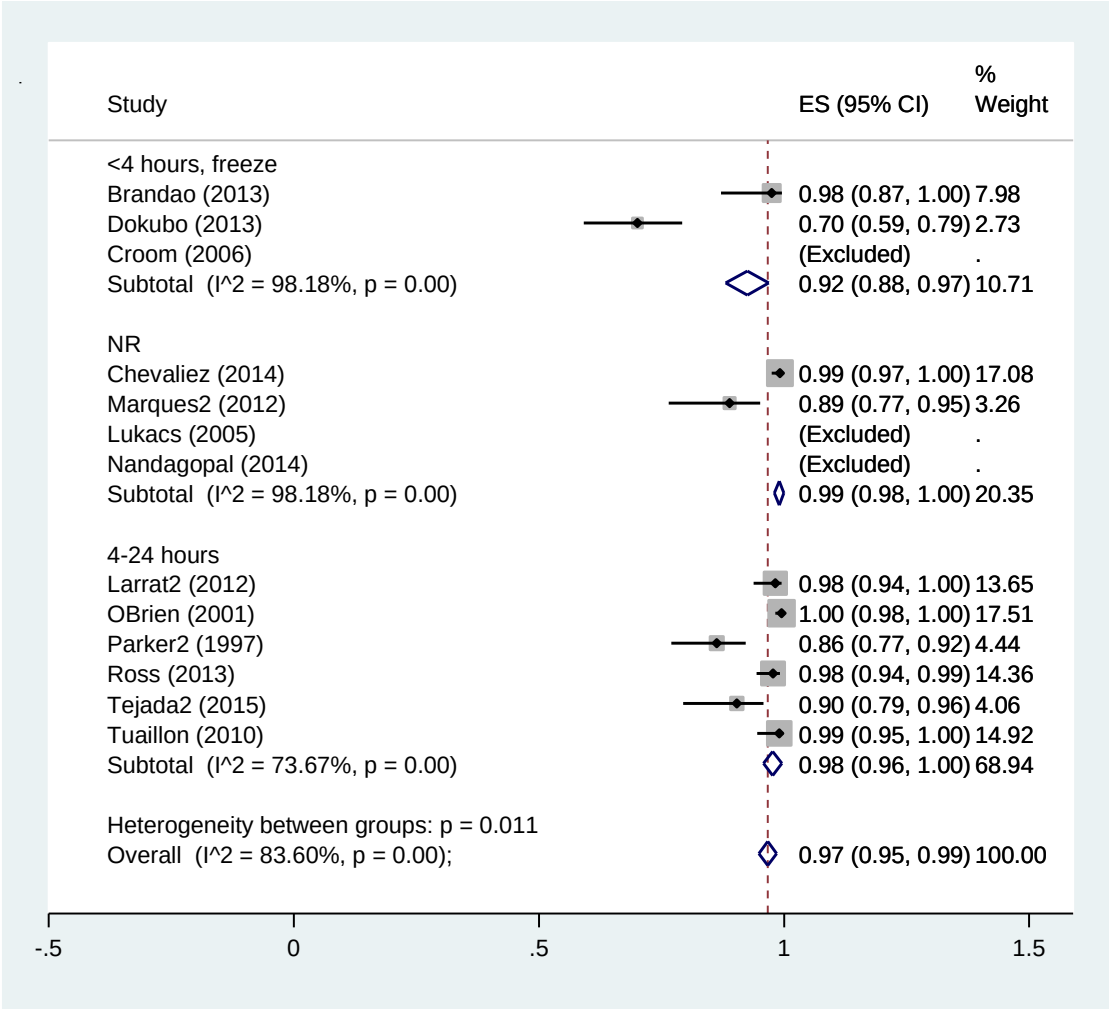
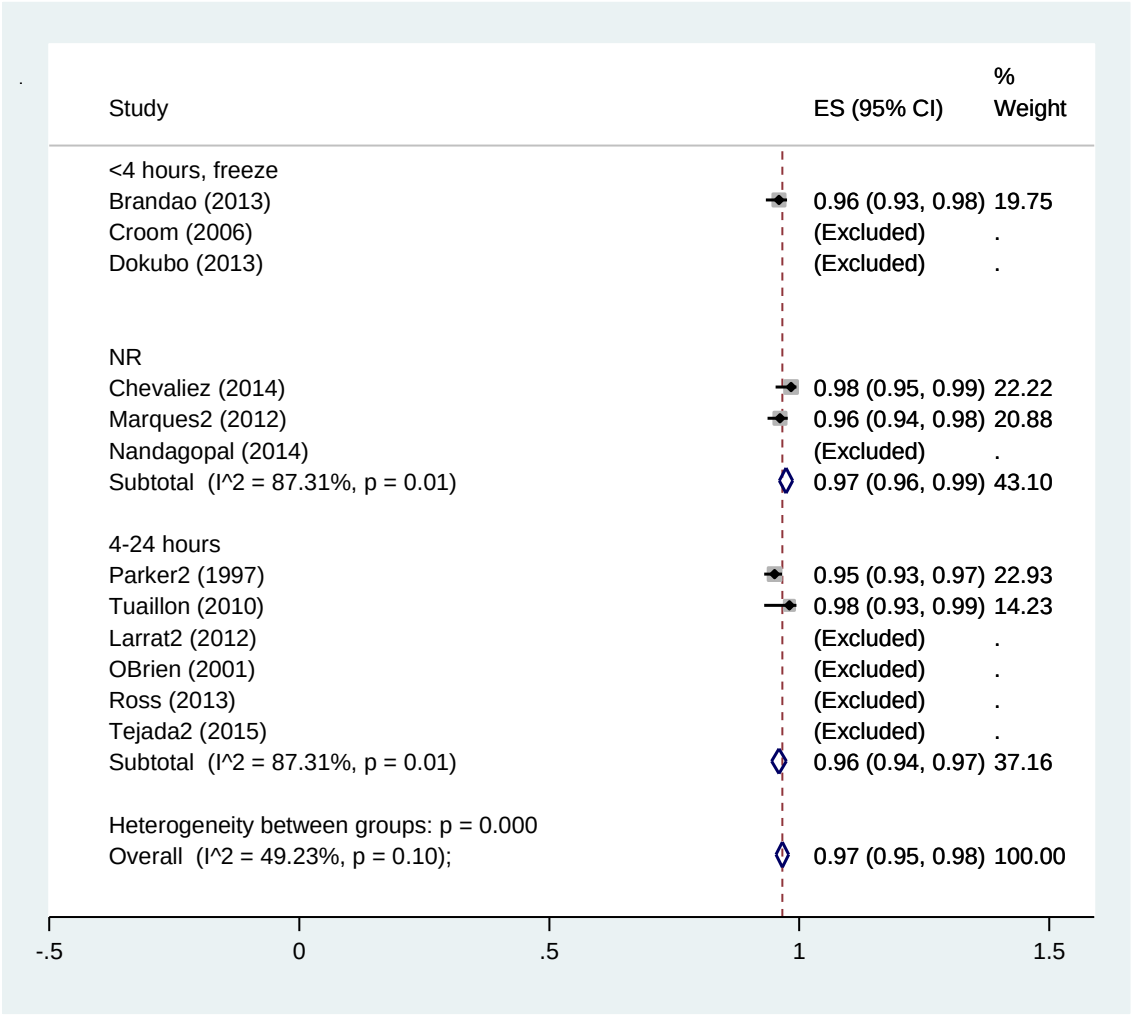


Table A2. Specificities of included studies stratified on storage conditions



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Annex 5.9.3

PICO 7 - Dried blood spots

Dried blood spots as sample collection method for HBV DNA

A systematic review and meta-analysis

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1. Executive summary

Introduction: Dried blood spots are a convenient diagnostic for viral diseases due to transport and logistical advantages over venous blood sampling. The diagnostic accuracy for the detection of hepatitis B (HBV DNA) on DBS samples is not known.

Methods: We conducted a systematic review and meta-analysis on the diagnostic accuracy of HBV DNA from DBS samples compared to venous samples in those persons identified for HBV DNA testing. MEDLINE, EMBASE, Global Health, Web of Science and Cochrane library were searched with a sensitive search strategy and data were extracted following a predefined extraction scheme. We described ranges of diagnostic accuracy outcomes as well as correlation and regression coefficients of DBS against venous blood samples reported by included studies. For pooled analysis of sensitivity and specificity, a bivariate analysis using maximum likelihood estimate and 95% confidence intervals were used and heterogeneity of results was assessed. PRISMA guideline was followed and the QUADAS tool was used to assess for risk of bias.

Results: Ten studies of 485 abstracts were included in the qualitative review, 8 of those contributed to the quantitative analysis. Overall quality of studies was low. A pooled bivariate analysis revealed an overall sensitivity of 96% (CI 95% 91–98) and an overall specificity of 100% (CI 95% 55–100). Positive likelihood ratio was 307 and the negative likelihood ratio was 0.04. Heterogeneity was moderate with an I2 of 18% (95% CI 0–100) and a tau^2 of 0.018.

One study reporting on thresholds reported a sensitivity of 98% for a cut-off of 2000 IU/mL, another reported a limit of detection of a commercial assay for DBS of 914 IU/ml. No study reported on storage conditions >24 hours at room temperature for DBS, but several studies varying storage conditions for individual samples did not find high variation of results.

Discussion: This systematic review and meta-analysis show that data are scarce and of suboptimal quality on the use of DBS for HBV DNA testing. The pooled meta-analysis of data available suggests that sensitivity compared to serum is good while specificity estimate is adequate with high imprecision. Individual study reports seem to suggest that sensitivity of HBV DNA detection above 2000 IU/mL is good. Bigger and better-performed diagnostic accuracy studies reporting diagnostic accuracy at different thresholds are needed.

2. Introduction

i. Epidemiology

A large number of people are infected with hepatitis B (HBV) and nearly a third of those will develop liver cancer and cirrhosis. Prevalence of HBV is highest in sub-Saharan Africa, East Asia, in the Middle East and India.[1] Few patients are aware of their infection until complications are present.

ii. Approach to HBV DNA testing

While for a diagnosis of HBV, serology is often sufficient for follow up and for decisions on treatment, quantitative testing of HBV DNA is important.[2] Most HBV DNA testing currently occurs on platforms in reference laboratories. Several guidelines recommend using a threshold of 2000 IU/mL for decisions on treatment in patients with chronic hepatitis B.[2]

iii. Use of DBS

Dried blood spots (DBS) can facilitate sample transport and simplify logistics, as has been shown for HIV.[3] They do not require venous blood to be taken, storage and transportation are less difficult and biohazard is reduced.[4] Increasingly DBS has therefore been used in the management of viral diseases, including HIV and hepatitis.[5] However, currently DBS protocols for hepatitis are not standardized for many applications and commercial tests and DBS is not yet regulatorily approved as a sample type.[6]

Several studies have shown potential of DBS to increase uptake of hepatitis testing among several vulnerable risk groups,[7-9] while others have not been able to confirm this trend.[10] Even if uptake is not affected, however, procedural advantages make DBS a good choice for diagnosis and follow up of patients with HBV in low-resource regions.[5] Which is why DBS for serology has often been used in epidemiological studies, sometimes without validating it against serum.[11,12] For HBV DNA testing, first efforts to test on DBS stem from more than 20 years ago,[13] but only recently other studies followed.[14,15] While several systematic reviews have been published on HBV diagnostics and on the use of DBS, no systematic review on HBV DNA and DBS exists to our knowledge. Recent reviews on DBS in HCV RNA[16] and in tropical diseases[17] did not include the diagnostic accuracy of HBV DNA in DBS.

Recently, WHO published the first guidelines for the prevention, care, and treatment of individuals with chronic HBV infection.[18] These guidelines focused on assessment for treatment eligibility, initiation of first-line therapies, switching, and monitoring and did not include screening recommendations. WHO is now preparing guidelines for testing for chronic hepatitis B and C infection in low- and middle-income settings. A topic for consideration in these guidelines is the potential use of DBS for serological and molecular testing for HBV and HCV to facilitate access to and uptake of testing. As preparation for this guideline on hepatitis B and C diagnosis, we present this systematic review on the diagnostic accuracy of HBV DNA DBS in comparison to HBV DNA in serum.

In order to better evaluate the sensitivity and specificity of DBS for testing for HBV DNA the following PICO question was developed for HBV DNA.

Among persons identified for HBV DNA testing, what is the diagnostic accuracy and impact of detecting HBV DNA from DBS samples versus venous sample?

Population: Samples for HBV DNA detection

Intervention: Using DBS samples

Comparisons: Using plasma or serum from venous samples

Outcomes: Diagnostic accuracy (Sensitivity, Specificity, Positive likelihood ratio, Negative likelihood ratio, TN, TP, FN, and FP), correlation and regression coefficients

3. Methods

PRISMA guidelines were followed and QUADAS-2 was used to estimate quality of studies.

i. Search strategy and selection criteria

Types of studies

Observational (including diagnostic accuracy studies) and interventional studies were included. We chose studies including comparisons of the index test HBV DNA in DBS against the reference test HBV DNA using serum and reported correlations, regression coefficients, specificity, sensitivity or predictive values. Only English language reports were included.

Participants

No date, geographical or population demographic exclusions were used. Patients of all age groups were included.

Target conditions

Hepatitis B diagnosis, patient follow up and treatment monitoring

Index test

Testing for HBV DNA in DBS for diagnosing HBV and for follow up of HBV patients

Reference standard

Testing for HBV DNA in serum using any commercially available or in house test

Outcome measures

Sensitivity refers to the proportion of samples with true HBV infection diagnosed with HBV DNA test using DBS confirmed with a positive HBV DNA in serum.

Specificity refers to the proportion of samples with negative HBV DNA using DBS and no evidence of HBV infection confirmed with a HBV DNA in serum.

Correlation refers to any bivariate quantitative correlation parameter between HBV DNA quantity amplified in DBS compared to serum.

Regression coefficients refer to any linear regression coefficients describing the association between HBV DNA quantity in DBS compared to serum.

ii. Search methods

We searched English language manuscripts from MEDLINE, Web of Science, EMBASE, Global Health and LILACS databases. The search was conducted in August 2015.

Title, abstract and full-text review was done using predefined eligibility criteria. The reference lists for articles selected for inclusion were also reviewed for additional manuscripts to review. Additional data and clarifications were sought by contacting study authors.

iii. Data extraction

All the studies were subject to the same data extraction procedure and form based on the following parameters: author, publication and study dates, country, percentage of children and adults, age range, gender distribution, type of specimen used for DBS, specimen used as gold standard (plasma or serum), test used, storage conditions and effect of storage conditions. Data extraction was performed by one reviewer (BL).

iv. Statistical data analysis

Statistical analysis of the data was performed using STATA 13. For analysis of sensitivity and specificity, a bivariate analysis using maximum likelihood estimate and 95% confidence intervals was used if >4 studies contributed to the analysis. Likelihood ratios were calculated directly from the pooled sensitivity and specificity. We used forest plots to visually assess heterogeneity. If not all diagnostic values could be extracted from the study, univariate and bivariate analysis was compared. Stratified analysis was performed by studies that used the In house or commercially available tests and by storage conditions.

v. Risk of bias and quality assessment

The QUADAS-2 tool was used to assess risk of bias. A GRADE assessment was performed by two reviewers in parallel to assess the quality of included studies.

4. Results

i. Search results and summary of included studies

Our search yielded 485 abstracts for screening after de-duplication (manually and by reference software). Forty-eight full-text papers were screened for potential inclusion and 10 studies were chosen to be included for the qualitative review.[13-15,19-25] Of the 10 studies, one of these studies was a conference abstract[22] that was later published as a paper[15] so this was not included in the quantitative analysis. Another study did not represent the data in analysable form[24] (Fig. 1 and Table 1). So eight studies remained for analysis, out of which only 4 contributed to both sensitivity and specificity estimates.

Of those studies included, three stemmed from Europe (France[22], Germany[15], Spain[23]) while three were from Africa (Congo[26], Egypt[21] and Zambia[25]) two from Asia (India,[13] China[24])

and one from Mexico.[14] No data from children were included, one study only included data on women[13] and one study only included HIV-positive patients.[25] Overall studies underreported on demographic characteristics. All studies used whole blood apart from one study that used plasma[26] for preparation of DBS samples.

ii. Diagnostic performance

Diagnostic accuracy

In the 4 studies that contributed both to sensitivity and specificity, sensitivity of HBV DNA in DBS ranged from 93% to 100% and specificity ranged from 86% to 100% (*see* Table 1). A pooled bivariate analysis revealed an overall sensitivity of 96% (CI95% 91–98) and an overall specificity of 100% (CI95% 55–100). From the pooled sensitivity and specificity, the positive likelihood ratio was 307 and the negative likelihood ratio was 0.04. Heterogeneity was low with a covariance between estimates of E(logitSe) and E(logitSp) (tau^2) of 0.018 (E(logitSE) 3.3 and E(logitSP) 5.8) (Tables 3, 4 and 5).

In a univariate analysis of the 7 studies contributing to sensitivity, pooled sensitivity was 97% (CI95% 94–99). I^2 in this analysis was 54% with a tau^2 of 0.0006 (see Appendix).

Association, correlation and agreement

Five of the 10 included studies reported regression coefficients showing a high association between quantitative results of HBV DNA in DBS and in serum[14,15,20,21,23] (regression coefficients between 0.61 and 0.96).

In terms of correlation one study reported good Pearson correlation (0.93)[14] and one study reported a good Spearman correlation coefficient of 0.84.[27] One study reported an agreement of kappa >0.7 between binary results of HBV DNA on DBS and on serum.

iii. Limit of detection and thresholds of HBV DNA of assays

No guidelines for using HBV DNA assays on DBS exist and so several studies performed testing to understand the limit of detection of this method. Limit of detections of used assays (commercial and in-house) for a serum sample ranged from 10 to 100 IU/mL (see Table 1). On DBS the limit of detection in one study was 914 IU/mL for an often-used commercial assay (COBAS Taqman), with a plasma limit of detection of 20 IU/mL.[15] Furthermore three studies reported that quantitation of HBV DNA in DBS below 3000–4000 IU/mL was difficult.[6,23,28] A recent study from Zambia used diluted samples and reported that 13.8% (CI95% 7.7–23.7) of those detected in plasma were missed in DBS with a cut-off of 200 IU/mL, 1.8% (95% CI: 0.5–6.6) with a cut-off of 2000 IU/mL and 0.2% (95% CI: 0.03–1.7) with a cut-off of 20 000 IU/mL (see Table 1).

iv. Effect of storage conditions, type of test and DBS/DPS

Two studies evaluating different storage conditions ranging from 4°C to 37°C for up to 7 days did not find a decline in diagnostic accuracy.[14,23] For the diagnostic accuracy studies, all studies

stored DBS samples at –20 °C, so we did not attempt a stratified meta-analysis on storage conditions.

We stratified studies according to whether an in-house polymerase chain reaction (PCR) assay or a commercial assay was used in a univariate analyses for sensitivity and specificity. Pooled sensitivities were similar in both groups (95% and 98%) (see Table A3 and A4 in Appendix).

Only one study used plasma instead of whole blood for preparation of dried samples, so no stratified analysis was undertaken to investigate heterogeneity. This study showed similar sensitivity and specificity to the other studies and the pooled estimate.[21]

v. Assessment of study quality and risk of bias

Concerning risk of bias, most studies did not report adequately on major issues, so that rating risk of bias was limited. None of the studies blinded laboratory personnel to either the reference or the index test when performing the other one, or if so, this was not reported. However, as all studies used and reported a clear and consistent protocol for both reference and index test with an output that does not allow for interpretation we did not see a major cause of bias in this. We downgraded 4 of the included studies because they used a case–control design, while the other 5 did not adequately report on their sampling or the flow of participants. This leads to a general high risk of bias in these diagnostic accuracy studies and an overall low quality of evidence (Table 2 and Table 6).

5. Discussion

This systematic review and meta-analysis shows that data are scarce and of suboptimal quality on the use of DBS for HBV DNA testing. The pooled meta-analysis of data available suggests that sensitivity compared to serum is good while the specificity estimate is adequate with high imprecision, and that different storage conditions do not effect DBS unduly. The descriptive review also shows a good correlation and high association between quantitative values for HBV DNA on DBS and in serum samples.

i. Impact of storage and other factors

Some studies did test samples at varying storage conditions and found no effect on the results of these tests. However, only one study stored dried plasma samples (also the only study using dried plasma samples and not DBS)[28] at room temperature, while in all other studies those samples used in diagnostic accuracy calculations were stored at room temperature no longer than 24 hours and stored frozen afterwards. This means that all pooled results we present can only be considered valid under these conditions, which might limit applicability in field conditions tremendously.

ii. Key limitations

This review has a number of limitations. Overall the number of studies was small. Only the English literature was looked at and no unpublished studies were examined. The few studies included were of limited quality and had small sample sizes.

This review is not able to answer whether the sensitivity and specificity of certain HBV DNA thresholds – for example, 2000 IU/mL, the threshold below which treatment for HBV is not recommended – are high enough, as this could not be calculated due to not having individual sample data. This would be important, however, as guidelines suggest treatment not be started in certain cases below this threshold because it is considered inactive, immune-controlled chronic hepatitis B. Therefore, any field test might not necessarily have to be able to detect HBV DNA below 2000 IU/mL.[2]

In our meta-analysis, sensitivity was high in general, with narrow confidence intervals. However, no conclusions regarding specificity were possible because the pooled meta-analysis of available data yielded very wide confidence intervals. Furthermore, no pooled receiver operator characteristics (ROC) to establish good overall cut-offs for the data could be performed.

iii. Future works

Bigger and better-performed diagnostic accuracy studies that avoid case–control designs, test HBV DBS under real-life storage and transport conditions and report diagnostic accuracy for different time, temperature and clinically relevant LOD thresholds are therefore called for. We would also advocate to perform a meta-analysis of individual-level patient (sample) data of published and unpublished studies on HBV DNA in DBS, as that approach might yield more data and would allow for quantitative analyses.

Prisma flowchart

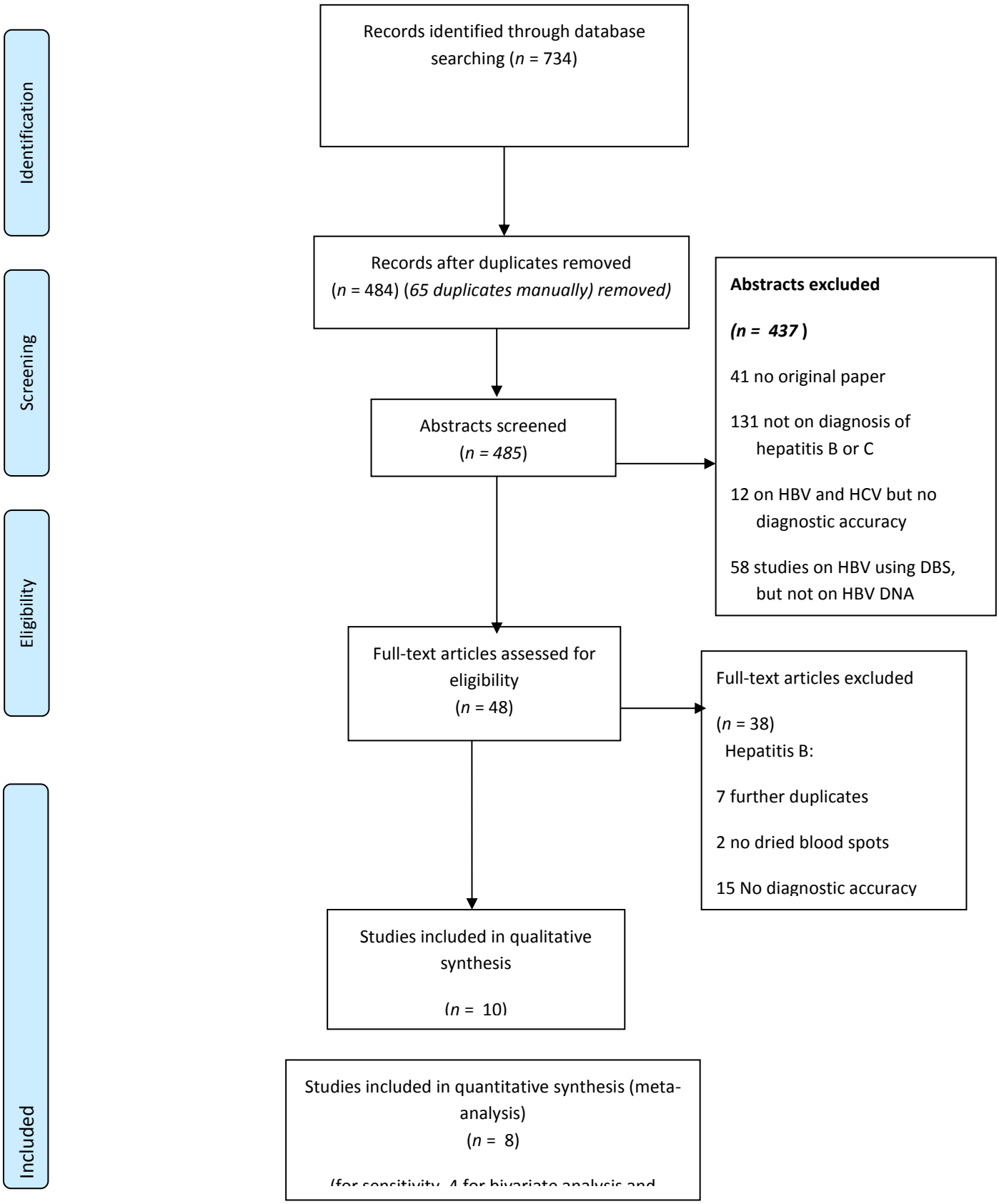

Fig. 2. Prisma Flowchart

Table 2. Characteristics of included studies

Author	Title	Journal	Year	Country	Study pop and sample size	Storage conditions	DBS collection method	Plasma method PCR	DBS method PCR	Limit of detection	Specificity	Sensitivity	Correlation/Association	Effect of storage conditions	Comments
Alidjinou	Detection of hepatitis B virus infection markers in dried plasma spots among patients in Congo-Brazzaville	*Diagnostic Microbiology and Infectious Disease*	2014	Congo-Brazzaville	32 HBV patients	DBS at room temperature, Frozen plasma samples at –80 °C	30 µL of plasma onto filter paper	COBAS Taqman/COBAS AmpliPrep	COBAS Taqman/COBAS AmpliPrep	Detection limit for plasma was 12 IU/mL. In 3 patients, viral load in plasma was 152, 250, and 1727 IU/mL, respectively, whereas HBV DNA could not be quantified in DPS, but was detected.		96% (25/26)	Spearman correlation coefficient r=0.84		
Alhusseini	Hepatitis B virus DNA can be amplified directly from dried blood spot on filter paper	*American Journal of Biochemistry and Biotechnology*	2012	Egypt	50 HBs Ag pos patients, 10 negative controls	Stored at –80 °C	Watman 903, 50 µL from venous blood sample	In house	In house	No cut-off suggested	100%	100%	Good correlation (r=0.88) between DBS and plasma viral load		
Gupta	Direct detection of hepatitis B virus from dried blood spots by polymerase chain reaction amplification	*Journal of Clinical Microbiology*	1992	India	Submitted for routine serological testing of HBs pos mothers 60 mothers with chronic HBV infection, 5 laboratory personell	–20 °C for filter paper	In house (end-point)	In house (end-point)	Whatman paper	Limit of detection 10E4 virus particles in each 5-,u blood spot,	86% (13/15)	96% (43/45)			No diagnostic accuracy calculation but can be calculated from data
Halfon	Dried blood spot for Hepatitis B virus serology and molecular testing	Conference abstract	2012	France	100 Hbs Ag neg, 100 Hbs Ag pos with HBV DNA	–20°C	3 blood whole blood on paper card	HBV Cobas Taqman	HBV Cobas Taqman	1400 IU/mL 2000 IU/mL	100 100	98 (95–100) 91 (85–97)			Conference abstract so no more data, same study as Mohamed below
Jardi	Usefulness of dried blood samples for quantification and molecular characterization of HBV-DNA	*Hepatology*	2004	Spain	82 patients with chronic HBV infection (23 HBeAg pos, 39 HBeAg neg, 20 HBeAg inactive, 15 HBe neg under	Room temperature for up to two hours, then –20 °C	20 µL of capillary blood on 5mm paper disks (Scheicher)	In house	In house	LOD 100 cop/mL among eight samples with serum HBV DNA between 103 and 104 copies/mL, seven			Regression coefficient HBV DNA concentration in DBS versus serum samples $r(^2)$ = 0.96 (P<.001).	Stability of DBS samples assessed by leaving samples for several days in differed	

					lamivudine therapy					tested positive using DBS samples, and among four samples with detectable serum HBV-DNA levels _103 copies/mL, none were positive using DBS samples.				conditions, no effect on HBV DNA levels	
Lira R	Use of dried blood samples for monitoring hepatitis B virus infection	*Virology Journal*	2009	Mexico	47 HBV Monoinfected patient	Plasma samples at –70 °C, filter paper at –20 °C	50 µL per card (Schleicher + Schull)	QIAamp® Ultrasens® Virus kit (QIAGEN GMBH, Germany),				100%	The Pearson correlation 0.93 (p = 0.01)	No adverse effect by sample storage s from ten patients were stored at 4 °C, 25 °C, and 37 °C for 7 days	
Mohamed S	Dried blood spot sampling for hepatitis B virus serology and molecular testing	PLOS One	2013	France	50 HBV-positive patients, 10 HBV-negative patients	Dried for 18 h in room temperature	15 µL on 12 mm discs (Whatman)	Cobas AmpliPrep/Cobas Taqman HBV test,	Cobas AmpliPrep/Cobas Taqman HBV test,	Limit of detection of HBV DNA 20 IU/mL plasma, limit of detection DBS 914 IU/mL		50/50 100%	Correlation good between DBS and plasma (r^2=0.86, P<0.001),		
Ross	Detection of infections with hepatitis B virus, hepatitis C virus, and human immunodeficiency virus by analyses of dried blood spots – performance characteristics of the ARCHITECT system and two commercial assays for nucleic acid	Virology	2013	Germany	299 samples		100 µL applied to filter paper (Whatman, Schleicher+Schüll)	Artus HBV LC PCR (Qiagen, Hilden, Germany)	Artus HBV LC PCR (Qiagen, Hilden, Germany)	Limit of detection 100 IU/mL in plasma, 7 samples with HBV concentrations of 409–3643 IU/mL missed	100 (96–100)	93 (92.9–93.1)			
Vinikoor	Hepatitis B viral load in dried blood spots: a validation study in Zambia	*Clinical Journal of Virology*	2015	Zambia	68 HBs pos patients,	Dried for 12 hours at room temperature	50 µL applied to filter paper	COBAS		The probability of a undetectable DBS result at a plasma viral load of 200 IU/mL was 13.8% (95% CI:	91.2 62/68				

										7.7–23.7) but this dropped to 1.8% (95% CI: 0.5–6.6) when a 2000 IU/mL threshold was used and 0.2% (95% CI: 0.03–1.7) at 20,000 IU/ml.					
Zhang	Detection of HBV-DNA in dried bloodstains on filter paper by nested polymerase chain reaction	*Laboratory Medicine*	2010	China	Hospital patients;60 blood samples	DBS samples at –20 °C, whole blood samples at –80 °C	10–20 µL on Whatman	In house (end-point)	In house (end-point)	All samples of 5 copies of HBV DNA per ml detected			Kappa >0.7 for agreement between nested PCR and ELISA, 61% positive with whole blood sample, but only 51% with filter paper		No diagnostic accuracy or agreement calculations for whole blood against filter paper

Table 3. Risk of bias of included studies

Author	Patient selection	Bias	Index test		Reference standard		Flow and timing	
	- Was a case–control design avoided - Consecutive or random sample of patients - Inappropriate exclusions		- Blinded to reference standard - Could the conduct or interpretation of the index test have introduced bias?		Blinded to index? Could the reference standard have introduced bias?		**Patient flow?**	
Alidjinou	NR, but no case–control design	UR	Not blinded, interpretation unbiased	LR	Not blinded, interpretation unbiased	LR	NR	UR
Alhusseini	Case–control design, sampling NR	HR	Not blinded, interpretation unbiased	LR	Not blinded, interpretation unbiased	LR	NR	UR
Gupta	Case–control design, sampling NR	HR	Not blinded, interpretation unbiased	LR	Not blinded, interpretation unbiased	LR	Partly reported	LR
Halfon	NR	UR	Not blinded, NR	UR	Not blinded, NR	UR	NR	UR
Jardi R	Selection only of cases	HR	Not blinded, interpretation unbiased	LR	Not blinded, interpretation unbiased	LR	Partly reported	LR
Lira R	Selection of only cases	HR	Not blinded, interpretation unbiased	LR	Not blinded, interpretation unbiased	LR	NR	UR
Mohamed S	Case–control design	HR	Not blinded, interpretation unbiased	LR	Not blinded, interpretation unbiased	LR	NR	UR
Ross	No case–control design, sampling NR	HR	Not blinded, interpretation unbiased	LR	Not blinded, interpretation unbiased	LR	Flow reported	LR
Vinikoor	No case–control design, only cases	HR	Not blinded, interpretation unbiased	LR	Not blinded, interpretation unbiased	LR	Flow reported	LR
Zhang	No case–control design, sampling NR	HR	Not blinded, interpretation unbiased	LR	Not blinded, interpretation unbiased	LR	Partly reported	LR

HR: high risk of bias; LR: low risk of bias; UR: unclear risk of bias; NR: not reported

Table 4. Forest plot of sensitivities and specificities of included studies.

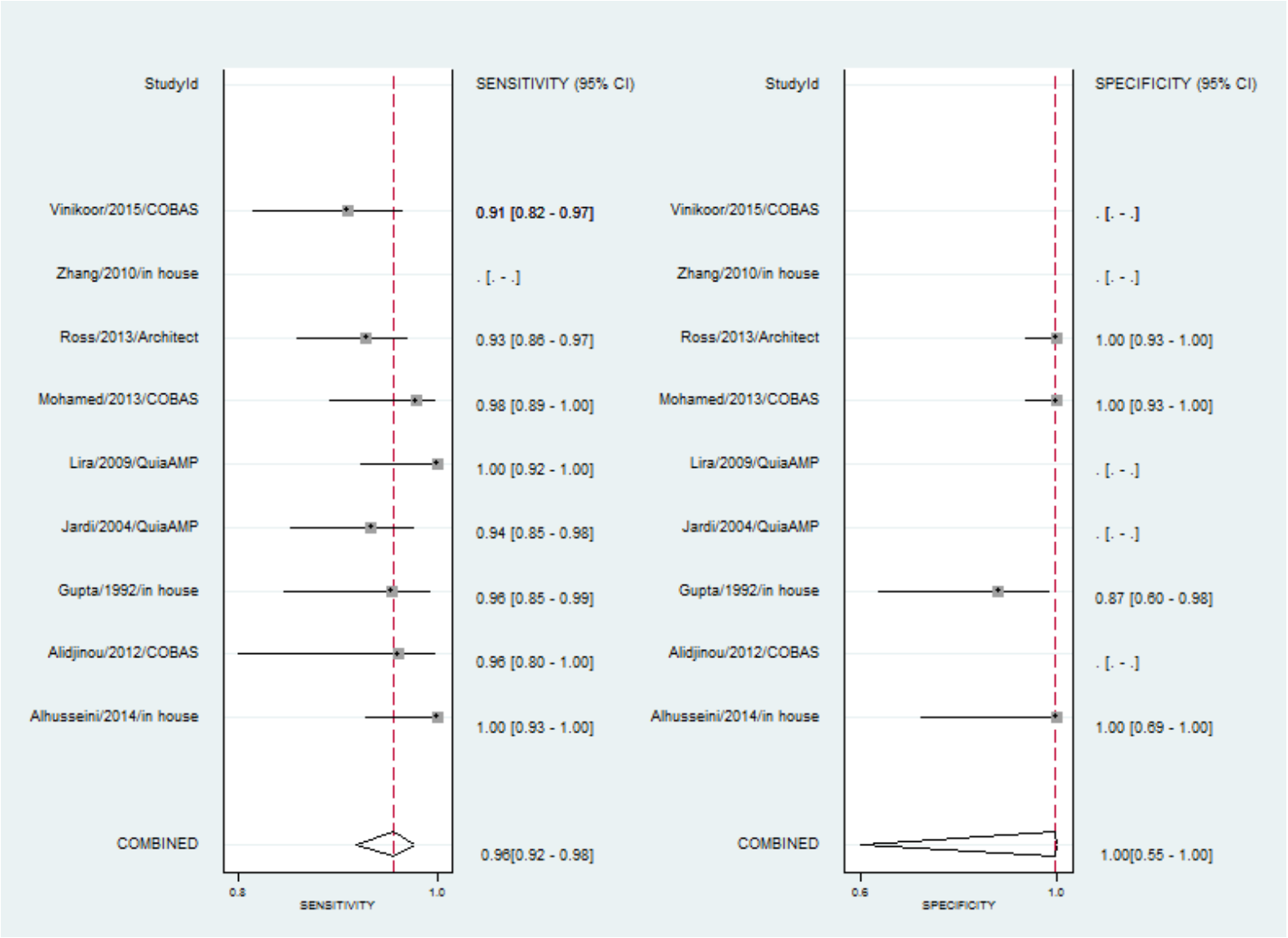

Table 5. Sensitivities and specificities of included studies

Study	Sensitivity	Lower 95% confidence interval	Upper 95% confidence interval	Specificity	Lower 95% confidence interval	Upper 95% confidence interval
Alhusseini/2014/in house	1.00	0.93	1.00	1.00	0.69	1.00
Alidjinou/2012/COBAS	0.96	0.80	1.00		–	–
Gupta/1992/in house	0.96	0.85	0.99	0.87	0.60	0.98
Jardi/2004/QuiaAMP	0.94	0.85	0.98		–	–
Lira/2009/QuiaAMP	1.00	0.92	1.00		–	–
Mohamed/2013/COBAS	0.98	0.89	1.00	1.00	0.93	1.00
Ross/2013/Architect	0.93	0.86	0.97	1.00	0.93	1.00
Vinikoor/2015/COBAS	0.91	0.82	0.96			
Zhang/2010/in house					–	–
Combined	**0.96**	**0.92**	**0.98**	**1.00**	**0.39**	**1.00**
	Heterogeneity (Chi-square): Q = 9.68, df = 7.00, *P* = 0.21 Inconsistency (I-square): I^2 = 27.69, 95% CI = [0.00–85.52]			Heterogeneity (Chi-square): Q = 16.89, df =7.00, *P* = 0.02 Inconsistency (I-square): I2 = 58.56, 95% CI = [26.14 – 90.98]		

Table 6. Forest plot of likelihood ratios

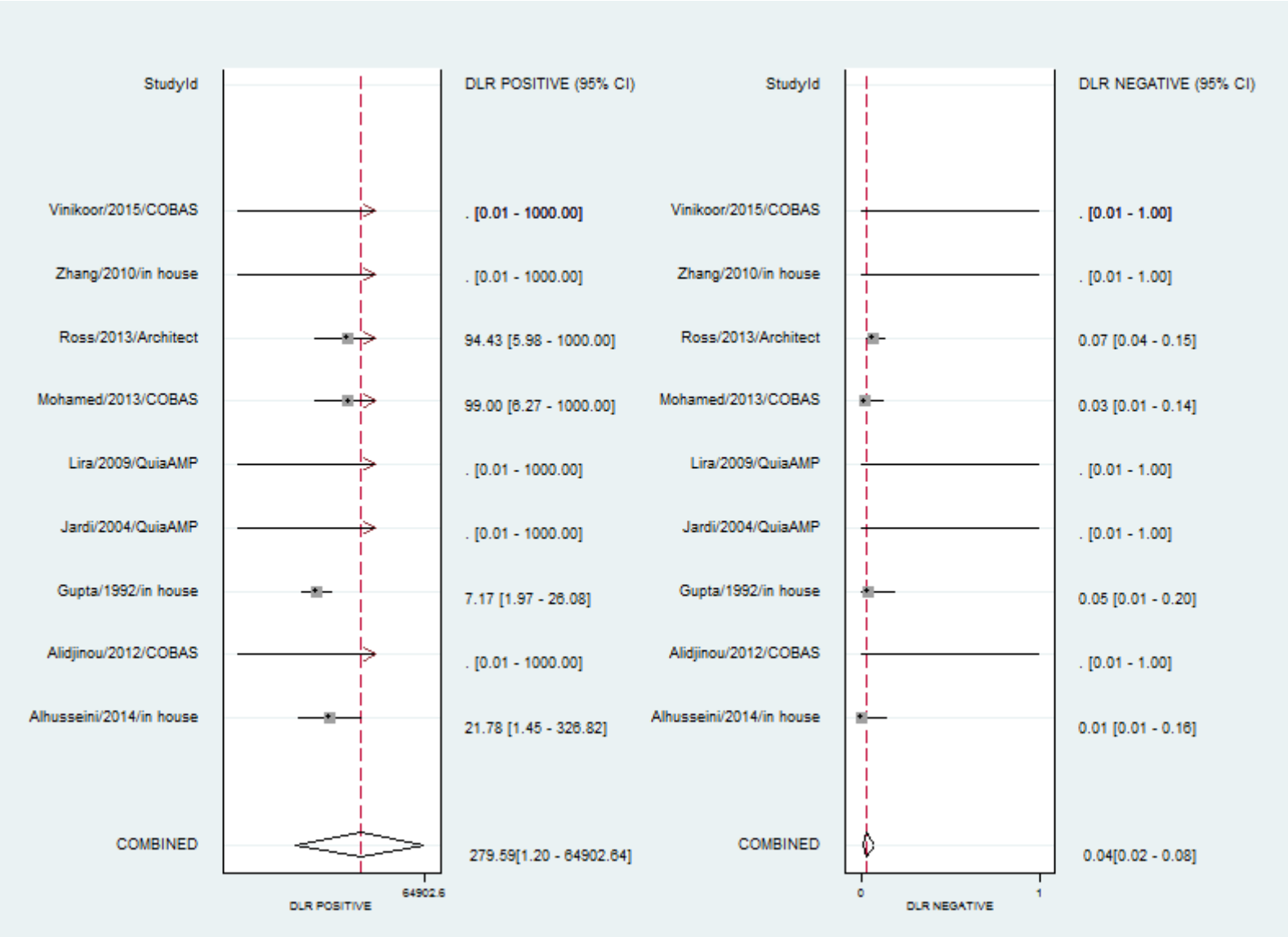

Table 7. GRADE table

Number of studies	**Type of study**	**Directness**	**Precision**	**Consistency**	**Risk of bias**	**Overall quality**
Sensitivity 96% (95% CI 92.0%–98.0%)						
8 studies (463 HBV positive among 588 samples)	Diagnostic accuracy	No significant indirectness	No significant imprecision	Significant inconsistency	High risk of bias (patient enrolment not consecutive or random in all studies; several case–control studies)	Low
Specificity 100% (95% CI 54–100%)						
4 studies (125 HBV DNA neg among 588 samples)	Diagnostic accuracy	No significant indirectness	Significant imprecision with small sample size	Significant inconsistency	High risk of bias (patient enrolment not consecutive or random in all studies; several case–control studies)	Low

Appendix

iv. Bivariate vs univariate analysis

Comparing the analysis of bivariate sensitivity and specificity estimates and confidence intervals were similar. Heterogeneity was slightly lower in the univariate analysis.

Univariate sensitivity

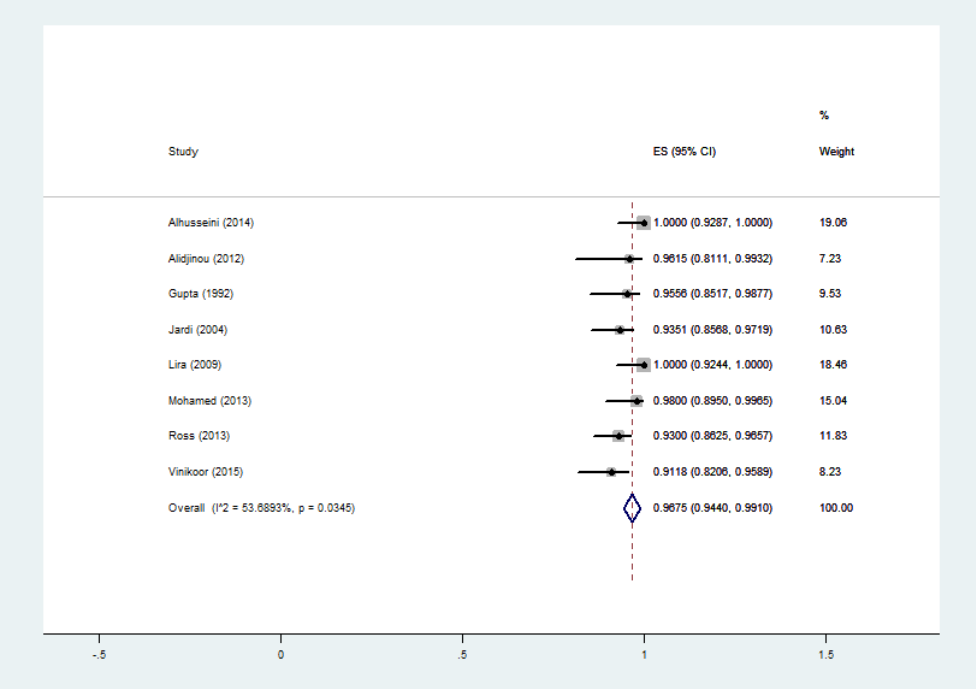

Table A3. Sensitivity of included studies in univariate analysis

Univariate specificity

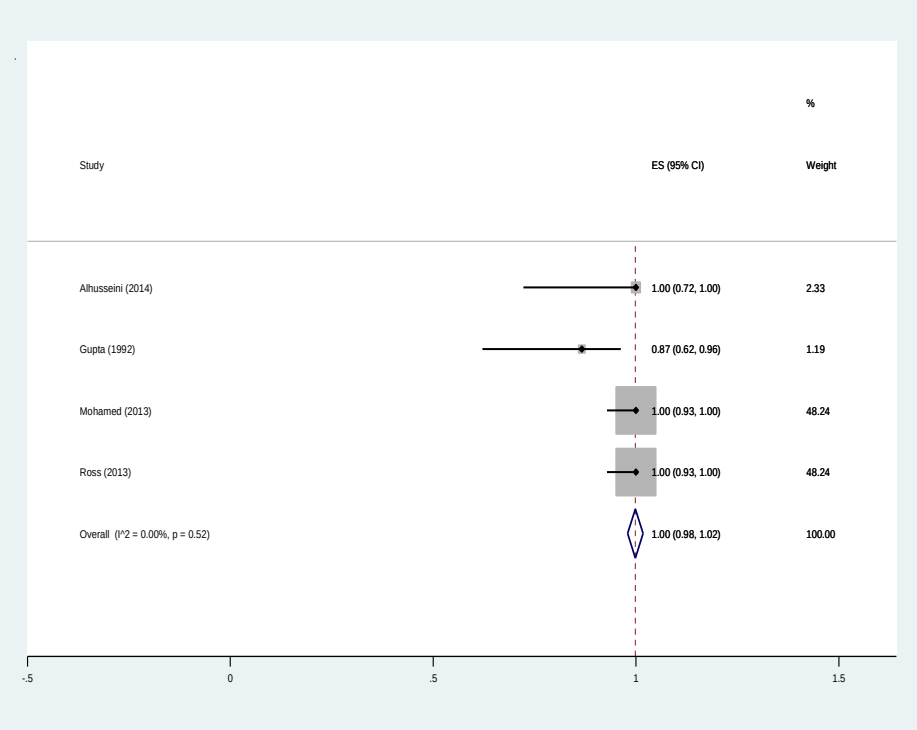

Table A4. Specificity of included studies in univariate analysis

v. Sources of heterogeneity – univariate analysis

Looking at heterogeneity in the different assays used, sensitivity and specificity was not different in those with commercial or in-house assays.

Different assays

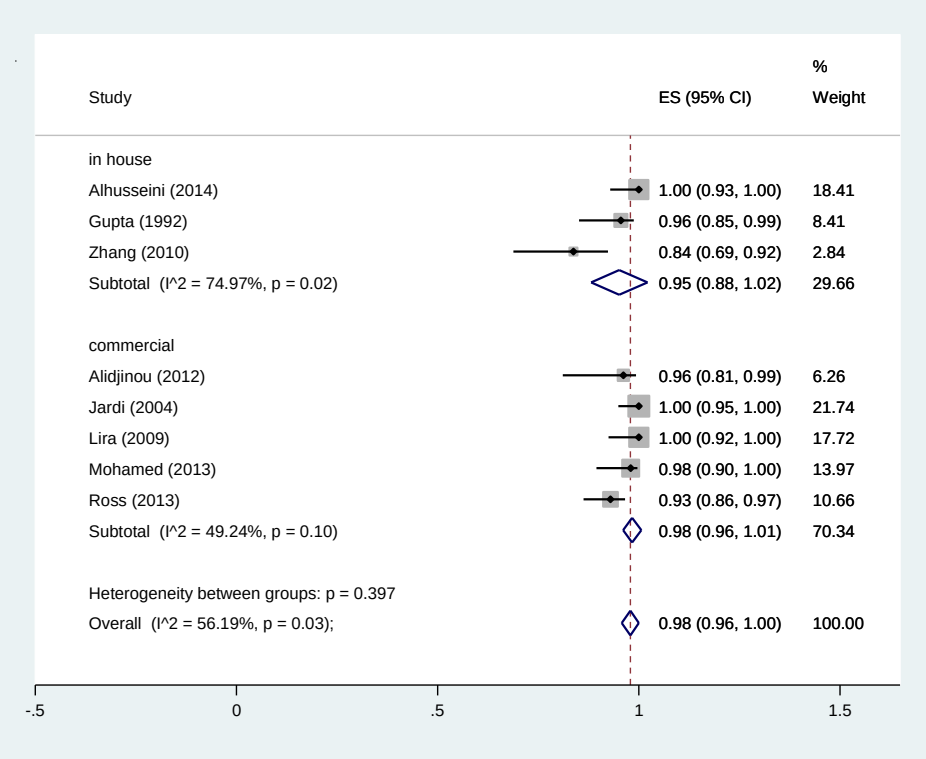

Table A5. Sensitivity of included studies stratified on type of assay

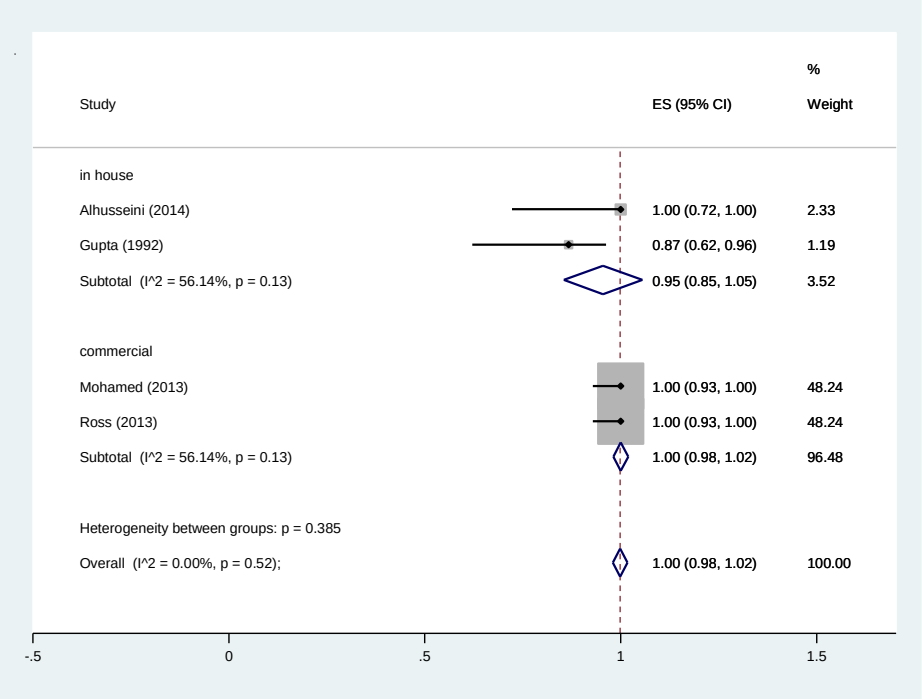

Table A6. Specificity of included assay stratified on type of assay

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Annex 5.9.4

PICO 7 - Dried blood spots

Dried blood spots as sample collection method for hepatitis C virological testing: a systematic review and meta-analysis

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August 2015

Abstract

b. Introduction

The entry of new all-oral direct acting antiviral therapy for hepatitis C provides an opportunity to scale up HCV care in low- and middle-income countries. In HIV, use of dried blood spots (DBS) has facilitated the diagnosis and management of HIV in resource-poor settings. DBS may be used in a similar way to facilitate diagnosis and management of HCV. Here, we present a systematic review of the literature of DBS for HCV RNA detection to address the WHO PICOT 7 question. This is an update of the Greenman et al. 2014 paper addressing this question.

c. Methods

Following an *a priori* protocol, PubMed, MEDLINE and Web of Science databases were searched by two reviewers duplicating each other's efforts. Data was extracted with the primary outcome of HCV viral load DBS test accuracy using the gold standard of a venous sample. For analysis of sensitivity and specificity, a bivariate analysis using maximum likelihood estimate and 95% confidence intervals was used. Likelihood ratios were calculated directly from the pooled sensitivity and specificity. QUADAS-2 was used to assess bias and a GRADE evaluation was performed to evaluate quality of included studies.

d. Results

The previous review found six papers eligible for inclusion and the update included three papers, one of which is in press, making for a total of nine papers eligible for inclusion. Eight studied DBS and one dried serum. The pooled sensitivity and specificity were 96.0% (upper-lower bounds 93.4–97.6) and 97.7% (upper-lower bounds 94.7–99.0), respectively. Heterogeneity was identified and a stratified analysis on capillary versus venous blood samples was performed which revealed a pooled sensitivity of 92.7% (lower-upper bounds 87.5–95.8%) and 97.4% (lower-upper bounds 95.9–98.3%) for capillary and venous samples, respectively, and pooled specificity of 97.4% (91.6–99.2%) and 98.3% (93.9–99.5%) for capillary and venous samples, respectively. Although there were insufficient data on storage conditions to perform a quantitative analysis, several papers stored DBS at ambient temperature and for prolonged periods of time. While these storage conditions did not affect accuracy, two studies found deterioration of HCV RNA in DBS samples stored at room temperature, while two others failed to detect such deterioration.

e. Discussion

These results support the potential use of DBS for HCV RNA detection. Further information is needed on the use of DBS for quantitative HCV RNA viral load when stored in DBS outside the cold chain.

1. Introduction

Globally, there are approximately 130–150 million people living with hepatitis C virus (HCV) and the majority of these live in low- and middle-income countries (LMICs).[1,2] In part because of the complexity and cost of the current algorithm for diagnosis and treatment using pegylated interferon, the majority of LMICs do not support HCV programming. As a result, without accessible and effective treatment, nearly half a million people die of HCV annually.[2,3] However, the more effective and tolerable oral direct-acting antivirals (DAAs) offer the opportunity to significantly simplify both the treatment and diagnostic algorithm, enabling the implementation, decentralization and scale-up of HCV care in LMICs. In order to further simplify diagnosis and monitoring of HCV, dried blood spots (DBS) may be considered.

DBS have been used to aid in the diagnosis of a wide variety of pathogens, including assessment of antibody to viral or bacterial infections such as HIV, hepatitis B, HPV and measles virus[4,5] and qualitative and quantitative viral load detection in HIV.[6] In particular, use of DBS in detecting and measuring HIV RNA has aided in decentralization of HIV services in low-resource areas and expanded the ability to perform early infant diagnosis for children at risk of vertical transmission.[7]

DBS for detection and monitoring of HCV viral load has a number of advantages. Especially salient for resource-poor settings is the possibility of using DBS to store and transport samples to a central laboratory without having to use refrigeration or dry ice, which is necessary for serum/plasma samples.[8–10] Furthermore, plasma/serum should be processed within 6 h of venous blood draw, necessitating transportation to the laboratory within that time – an impossibility in most resource-limited settings, especially those outside urban areas. DBS can also be prepared using capillary blood, which obviates the need, seen in venipuncture, for centrifugation to separate blood cells from serum/plasma.[12] In injecting drug users, venipuncture can be complicated by difficulty in finding an accessible vein and thus, capillary blood from finger-prick samples may be easier to obtain.[11] DBS holds advantages over oral fluid sampling, which has also been used to detect HCV, as it has been shown that HCV RNA in saliva is independent of plasma viral load,[13] and patients with low serum HCV RNA viral loads are less likely to have detectable HCV in saliva.[14] Other advantages include lower cost, minimal storage facility and transportation requirements, decreased donor discomfort and decreased risk to health-care

workers.[10] If DBS could be used for HCV RNA detection and monitoring, it would facilitate the simplification and decentralization of HCV diagnosis and monitoring.[8,11]

Today a significant amount of literature supports the use of DBS as an alternative to serum/plasma obtained via venepuncture for the detection of HCV antibody,[16–18] yet the use DBS in the detection and monitoring of HCV RNA and genotyping HCV, especially following storage at room temperature, has not been systematically reviewed.[15] To address the question of whether DBS can be used in the diagnosis of HCV in resource-limited settings, we undertook a systematic review of use of DBS for HCV RNA detection and genotyping, examining a range of storage conditions.

This review was published in the *Journal of Viral Hepatitis* in 2014.[19] The previous review included nine papers – eight studied DBS and one studied dried serum**.** Two studies measured concordance between genotype and subtype determined by DBS and whole plasma and both found 100% concordance. Four studies measured end-point detection limits of HCV RNA positive samples by DBS and found sensitivity of 100% down to 250 IU/mL, 331 IU/mL, 2500 IU/mL and 24 160 IU/mL. Two studies found deterioration of HCV RNA in DBS samples stored at room temperature (10-fold reduction in HCV RNA at 4 weeks using dried serum spots and 3-fold reduction in RNA at 6 days with DBS), while two others failed to detect such deterioration.

In 2014, WHO published the first guidelines for the prevention, care and treatment of individuals with HCV infection. These guidelines focused on assessment for treatment eligibility, initiation of first-line therapies and monitoring. WHO is now undertaking guidelines for testing for chronic hepatitis B and C infection in low- and middle-income settings. A topic for consideration in these guidelines is the potential use of DBS for serological and molecular testing for HBV and HCV to facilitate access to and uptake of testing.

In order to inform these WHO guidelines, the following PICOT was developed: Among persons identified for HCV testing, what is the diagnostic accuracy and impact of detecting HCV NAT from DBS samples versus venous samples?

Population: Samples for HCV NAT

Intervention: Using DBS samples

Comparisons: Using plasma or serum from venous samples

Outcomes: Diagnostic accuracy (sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, TN, TP, FN and FP).

2. Methods

The protocol used for this update is the same as that used in the Greenman et al. paper. This protocol is copied here from the *Journal of Viral Hepatitis* manuscript.

a. Search strategy

Using a sensitive search strategy as part of a predefined protocol (Appendix 1), we searched MEDLINE, CAB abstracts and Web of Science (ISI Citation Index) published up to August 2013 for studies meeting our pre-specified inclusion criteria. No date or geographical exclusions were applied; only English language publications were included. Following an initial screening of abstracts by two separate reviewers, full-text copies of potential eligible articles were reviewed independently by two reviewers. A title search of references was performed on articles meeting the inclusion criteria to determine potential articles for inclusion not identified during the initial database search. After all articles meeting the inclusion criteria were identified, data was abstracted by two reviewers according to prespecified categories.

b. Inclusion/exclusion criteria

We sought studies that reported on the use of dried DBS as a tool for monitoring and genotyping HCV RNA that included at least one of the following: the sensitivity and/or specificity of DBS in HCV RNA viral load quantification; the accuracy of DBS for genotyping HCV RNA; and the rate of degradation of HCV RNA during transport and storage at room temperature. Nine of the 73 articles met these criteria.

c. Data analysis

For studies measuring HCV RNA presence and quantification, studies were analysed for viral load end-point detection, sensitivity, specificity, PPV and NPV of DBS compared to HCV RNA in whole plasma. For the question of HCV RNA deterioration at room temperature in DBS, reviewers extracted reports of the rate of deterioration and the definition of "room temperature". The results of this analysis are displayed in Table 1. Studies determining HCV genotype were analysed for measures of genotype concordance between whole plasma and DBS, proportion of HCV RNA-positive DBS samples successfully genotyped and genotypes observed.

The updated review searched Medline and PubMed with the same search terms as from the original SR, but with the limit of 2013–present.

d. Statistical data analysis – update

Statistical analysis of the data was performed using OpenMeta[Analyst]. For analysis of sensitivity and specificity, a bivariate analysis using maximum likelihood estimate and 95% confidence intervals was used. Likelihood ratios were calculated directly from the pooled sensitivity and specificity. We used forest plots to visually assess heterogeneity.

e. Risk of bias and quality assessment

The QUADAS-2 tool was used to assess risk of bias (Table 1). A GRADE assessment was performed to assess quality of included studies (Table 2).

3. Results

In the initial review search, 184 titles were identified. Seventeen were selected for full-text review. Overall, nine met eligibility criteria for the published review (Fig. 1) of which six reported on the outcomes for this PICOT, sensitivity and specificity of HCV NAT DBS versus venous samples.[20–25] In the review update, 14 articles were identified of which 13 underwent title and abstract review (there was one article duplication in the search). Four articles were selected for full-text review. The remainder were not selected as they did not address HCV (*n*=4), did not address HCV viral load (*n*=4) or did not include outcome data (*n*=1). Of the four articles selected for full-text review, two were excluded as they did not deal with HCV viral load (VL). Two were retained.[26,27] One further article that has been accepted for publication was identified by an expert in the field and was included in the update.[28] Two of these articles[26, 28] provided sufficient data to include in the meta-analysis (Fig. 2).

a. Study characteristics

The selected studies were published between 1998 and 2015; all but two were published between 2007 and 2015. Two of the studies were located in the UK, two in Italy, two in France, two in the United States and one each in Lebanon, Brazil, Guinea-Bissau and Japan. Six studies reported outcomes in terms of end-point sensitivity of HCV RNA detection using DBS when compared to plasma.[20–25] Four studies measured the stability of HCV RNA in DBS at room temperature (Table 1).[20–21, 23–24]

b. Sample characteristics

The patient characteristics varied across studies. Five studies drew from patients attending liver clinics[21, 25, 27, 28] or in HCV treatment studies.[23] One study drew from a population of injecting drug users (IDUs), one from HIV-infected patients[22] and one from a general population.[24] One study's[23] participants were on PEG-RBV treatment.

Finally, the preparation of DBS varied among the samples. Eight of the nine studies used DBS[21–28] and one used dried plasma spot.[20] Three studies used capillary blood[23, 25, 26] and five used venous blood for preparation of DBS.[21, 22, 24, 27, 28] The studies used a variety of RNA extraction and elution methods, and nucleic acid amplification assays.

c. Diagnostic accuracy values

Overall, the pooled sensitivity was 96.0% (upper–lower bounds 93.4–97.6) and specificity was 97.7% (upper–lower bounds 94.7–99.0) (Figs 3 and 4). The negative and positive likelihood ratios were calculated to be 0.041 and 41.74, respectively. There was minor heterogeneity identified on the forest plots for sensitivity, with one study in particular contributing to the heterogeneity.[26] This study used capillary blood; however, other studies with higher sensitivity (e.g. 25), also used capillary blood. Thus, a stratified analysis was performed on capillary versus venous samples.

i.

d. Impact of sample type

A stratified analysis looking at sample type (capillary versus venous) revealed a pooled sensitivity of 92.7% (lower–upper bounds 87.5–95.8%) and 97.4% (lower–upper bounds 95.9–98.3%) for capillary and venous samples, respectively (Fig. 5). The overall pooled specificity was 97.4% (91.6–99.2%) and 98.3% (93.9–99.5%) for capillary and venous samples, respectively (Fig. 6).

e. Impact of storage conditions

There was insufficient information to perform a meta-analysis on different storage conditions. However, from information provided in the included manuscripts, storage at room temperature or over time up to 1 year does not appear to affect sensitivity, though several studies did identify a reduction in viral yield.[20, 25]

f. Bias and quality assessment

The QUADAS-2 revealed some risk of bias due to patient selection that was not consecutive or randomized in some studies or due to a case–control design. Otherwise, the included studies showed low risk of bias (Table 2). The GRADE table showed a moderate quality of data (Table 3).

4. Discussion

a. Overall conclusions

This systematic review and meta-analysis includes evidence of moderate quality that supports acceptable accuracy of DBS for testing HCV NAT as compared to use of plasma samples, and suggests that DBS may be used for diagnosis of HCV using HCV NAT DBS where there is limited access to venepuncture or inadequate technology to prepare and transport plasma samples. Although studies are limited, DBS is likely stable and maintains good accuracy in conditions with higher temperatures, although viral degradation may occur when storing for prolonged durations, which may affect quantitative assessment of viral load. As there are relatively little data on accuracy of DBS in real-life conditions (including high humidity), operational research will be useful to determine accuracy in such conditions.

In a stratified analysis that examined studies that used capillary blood versus those that used venous blood for DBS, capillary blood sample use resulted in somewhat lower pooled sensitivity than venous blood sample, although bounds were wide and overlapping. One study that used capillary blood examined the discordant results and found that the signal to cut-off ratio for discordant results was lower than for concordant results, suggesting that a lower cut-off may help to improve accuracy for capillary blood samples.

b. Key limitations

This review also has a number of limitations. Overall, the number of studies was small. In particular, there is a dearth of studies that systematically examines the effects of storage conditions on the accuracy of DBS; and of those that did assess this, several did not specify the exact conditions, e.g. exactly what is room temperature. This is particularly important as the majority of these studies came from high-income countries and room temperature in these areas will be very different than temperature in sub-Saharan Africa. Thus, should DBS be adopted for screening for HCV NAT, it will be important to pursue further operational research using field specimens prepared and stored in real-life conditions. Ideally, to fit operational needs, these

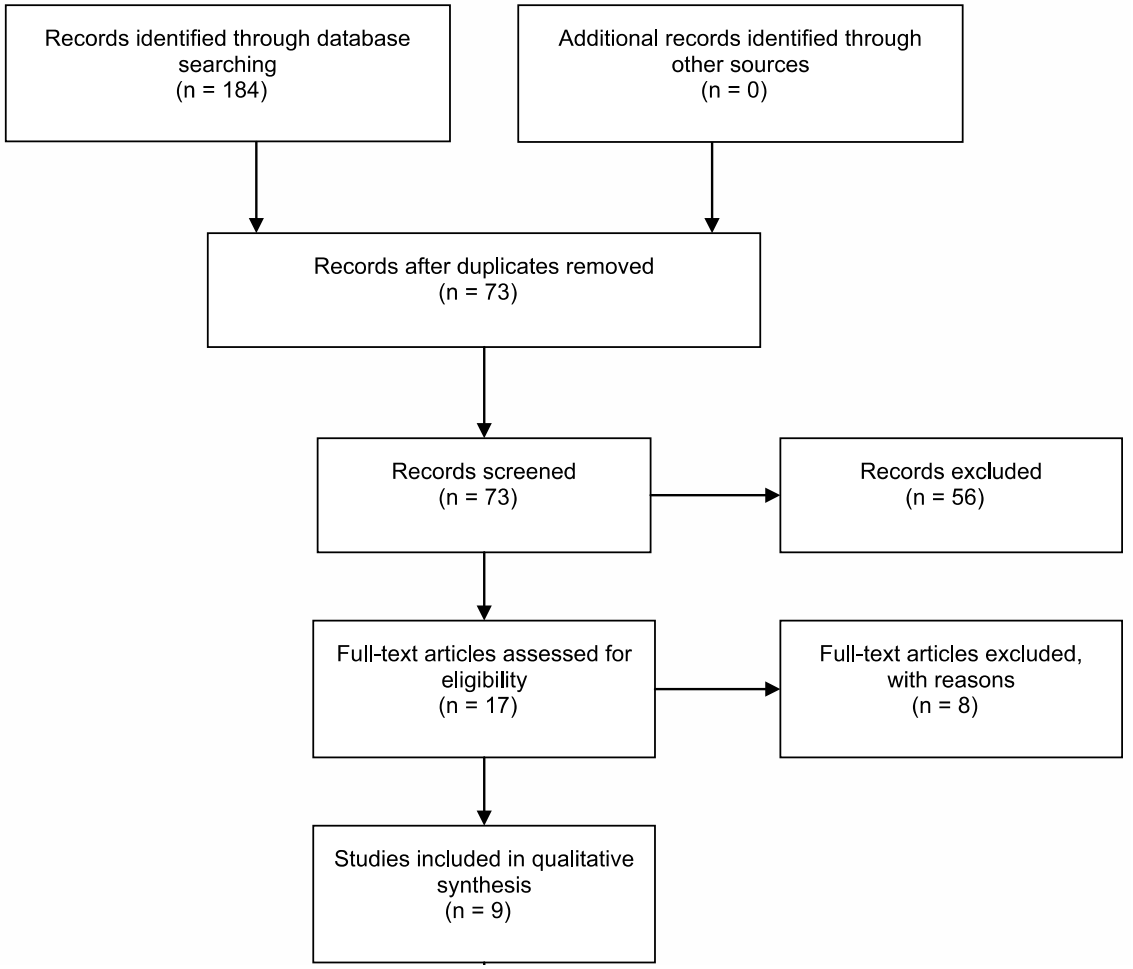
studies should use capillary whole blood to prepare the DBS, using a commonly available type of filter paper while comparing several commercial test kits and conditions of storage.

Future work

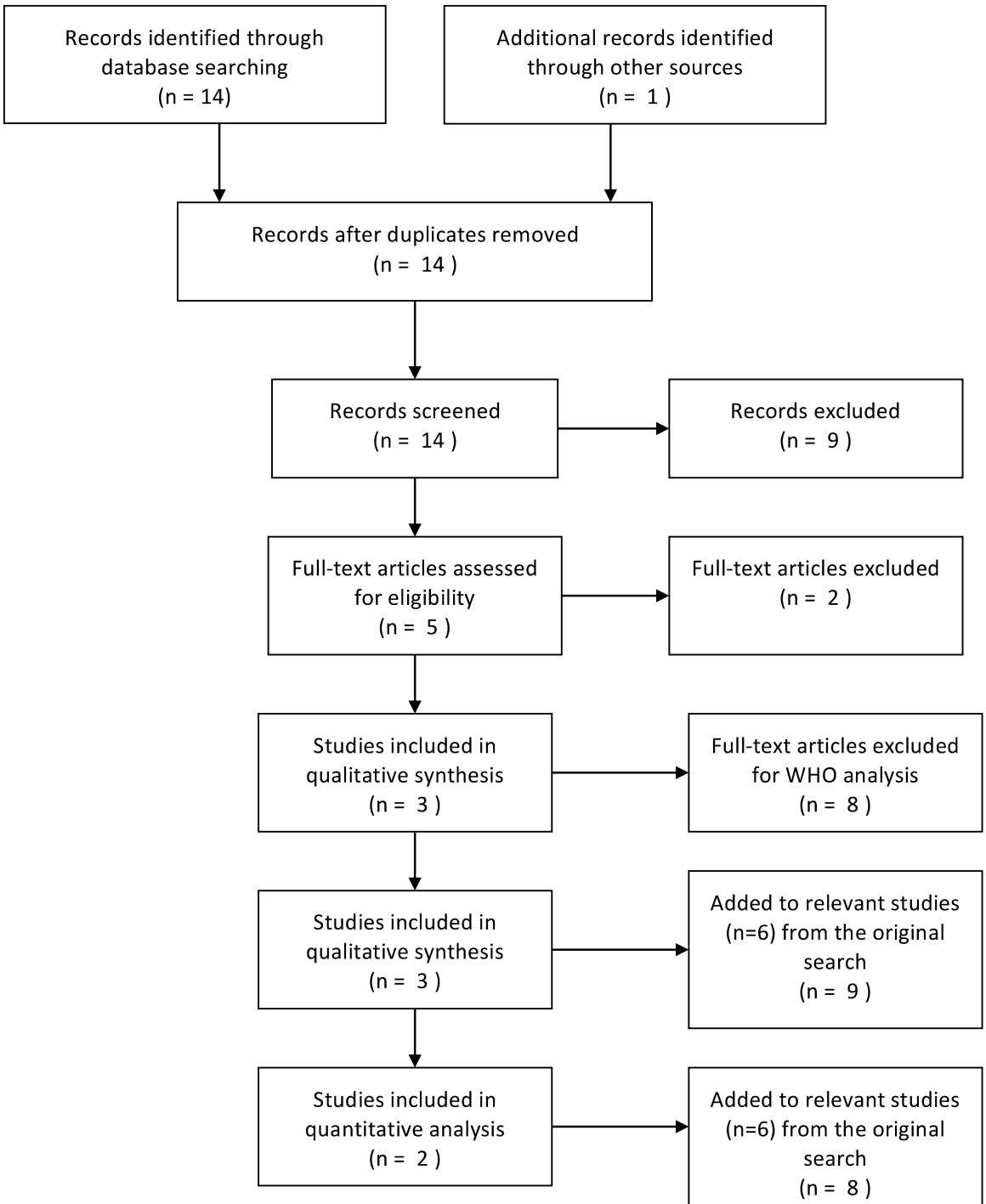
Expanded use of HCV NAT testing will be critical to improving the diagnosis and treatment of HCV globally. The use DBS for improved preparation, storage and transport of samples for decentralized testing may help to expand the reach of this important diagnostic test.

Tables and figures

c. Fig. 1. Flow diagram



d. Fig. 2. Additional search flow diagram



), Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems

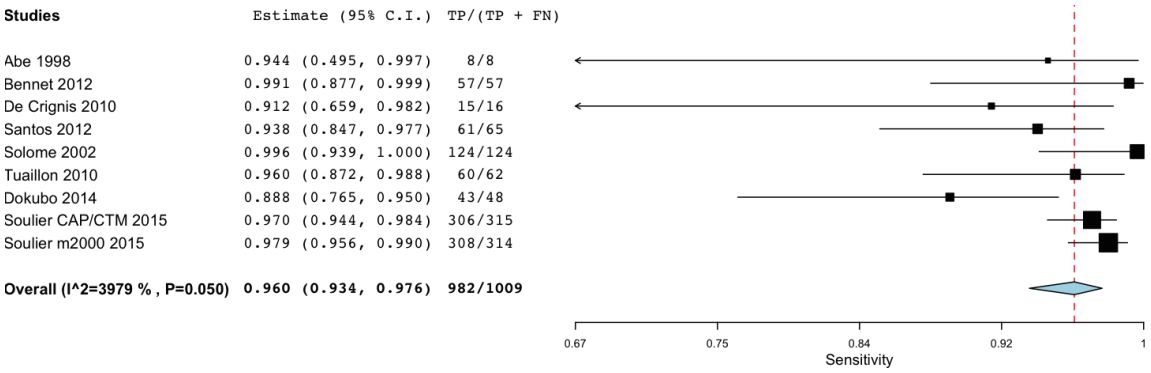
e. Table 1: Study-level characteristics

Study	Design	Study site and population	DBS collection method	Storage conditions	Assay	Sensitivity/specificity	Effect of storage conditions	Cut-off value
Abe (1998)	Case–control	*n* =12 Japan	Plasma on Whatman filter paper	Room temperature and tested at 1, 2, 3, 4 weeks	ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer)	Sensitivity: 100% Specificity: 100%	10-fold reduction in virus yield in 4 weeks (in 6 of 8 samples, no samples lost HCV RNA positivity)	
Bennett (2012)	Cross-sectional	*n*=80 HCV antibody positive patients attending liver clinic, United Kingdom	Venous blood on Whatman 903 cards	Room temperature, 4 °C, –20 °C, –80 °C	ABI 9700 and ABI 7500	Sensitivity: 100% (57/57) Specificity: 95.7% (22/23)	No significant variation in cycle threshold over 1 year (2 DBS from 1 patient)	Suggested: 150 –250 IU/mL
De Cringis (2010)	Case–control	*n*=25 13 HIV–HCV coinfected 4 HIV monoinfected 3 HCV 5 healthy blood donors Italy	50 uL venous blood on Whatman No. 3 card	Stored at –80 °C	HIV-1/HCV multiplex SYBR Green real-time RT-PCR assay	Sensitivity: 93.8% (15/16) Specificity: 100% (9/9)		
Dukobo (2014)	Cross sectional	*n*=132 Adult (18–30 years) active IDU in San Francisco	Capillary blood on Whatman 903 cards		dHCV TMA (Norvatis)	Sensitivity: 89.6% (95% CI 77.8–95.5) Specificity: 100% (95% CI 95.6–100)		

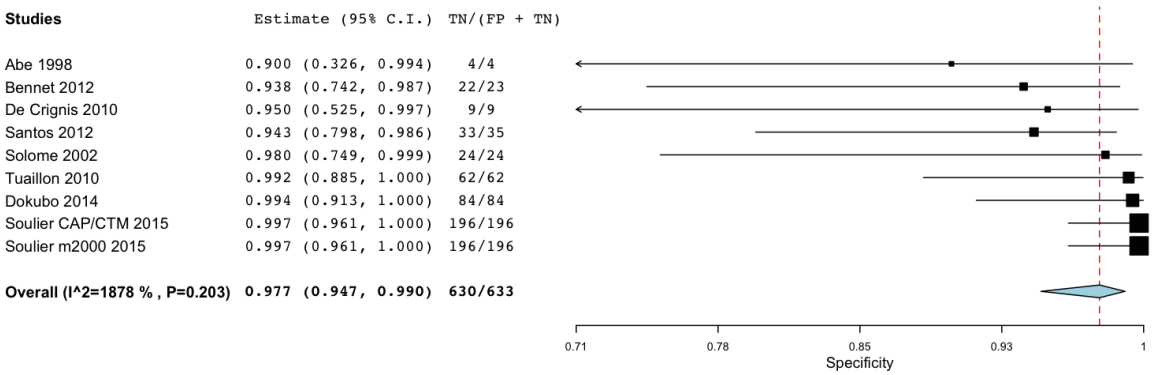
Tejada-Strop (2015)	Case–control	*n*=33 Adult patients with chronic hepatitis C at the Liver Clinic of the University of California, San Francisco	Venous samples on Whatman 903 paper (GE Healthcare)	Control: DBS freshly prepared Long-term storage: 5 years at –20C	Superscipt III Platinum 1 Step qRT-PCR kit	Control Sensitivity: 88% Specificity: 100% Long-term storage Sensitivity: 33% Specificity: 100%		
Soulier (2015)	Case–control	*n*=511 315 adults with HCV-Ab-positive chronic hepatitis C 26 adults with HCV-Ab-positive resolved infection 170 adults HCV-Ab-negative France	Venous samples on Whatman 903 paper (GE Healthcare)	–80 °C with desiccant package	CobasAmpliprep/CobasTaq Man HCV version 2 (CAP/CTM v2.0, Roche Molecular Systems) and m2000 platform (Abbott Molecular)	CAP/CTM v2 Sensitivity: 97.1% (94.7%–98.5%) Specificity: 100% (97.8%-100%) M200 Sensitivity: 98.1% (95.9%–99.1%) Specificity: 100% (97.8%–100%)		
Santos	Cross-sectional	*n* = 100 HCV chronically infected patients in a PEG-RBV study at 4 (*n* = 100) and 24 (*n* = 68) weeks post-treatment initiation Brazil	Capillary blood on SS903 collection cards (Schleicher and Schuell, Keene, NH, USA)	Room temperature	pCR-II-TOPO plasmid (Invitrogen)	Sensitivity: 98.0% (99/101) Specificity: 94.0% (63/67)		
Solmone (2002)	Cross-sectional	*n* = 55 Residual samples from patients undergoing routine haematological	50 uL EDTA venous blood on SS903 card	Subset of 16 paired samples stored at room temperature and tested every 2–4 weeks over	In-house RT-PCR	Sensitivity: 100% (124/124) Specificity: 100% (24/24)	No loss of positivity after 11 months (16 paired samples)	

		controls (39 HCV antibody (Ab)–positive and 16 HCV Ab-negative patients) Italy		11 months				
Tuallion (2009)	Case–control	*n* = 200 100 anti-HCV-positive and 100 anti-HCV-negative France	3 drops (50 lL) capillary blood on Whatman 903 card	Stored at –20 °C for 1–8 weeks until use	CobasAmpliprep Total Nucleic Acid Isolation 100 kit (Roche); CobasTaqMan HCV test and real-time PCR COBAS TaqMan 48 instrument plus COBAS Ampliprep analyzer (Roche); One Step RT-PCR kit from Qiagen (Qiagen)		3-fold decrease in RNA in 6 days (No. of samples not reported)	

f. Fig. 3. Forest plot of sensitivity

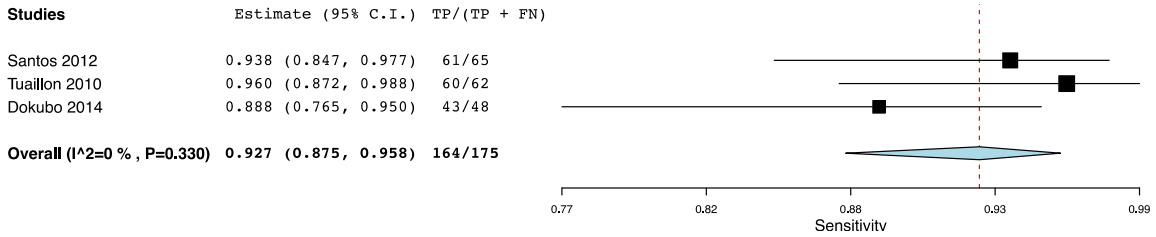


g. Fig. 4. Forest plot of specificity

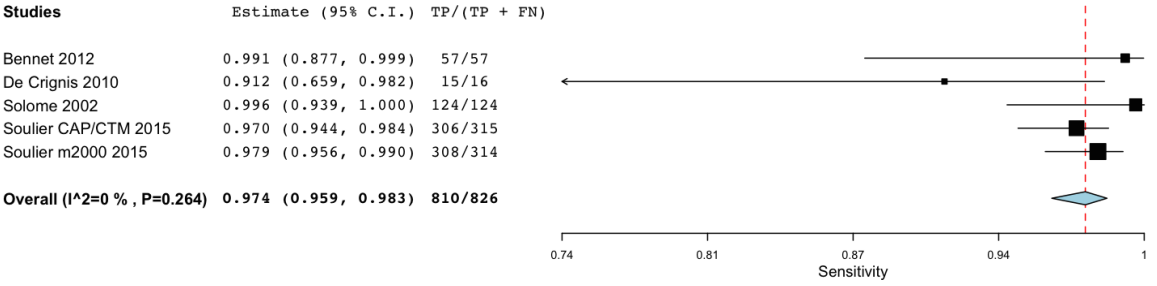


h. Fig. 5. Forest plot of sensitivity of capillary and venous samples

Capillary samples

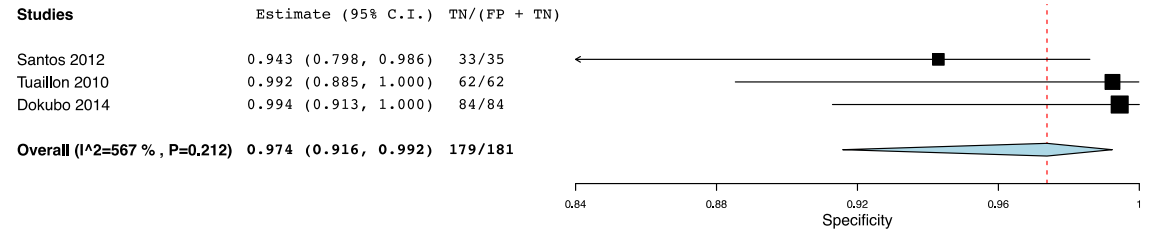


Venous samples

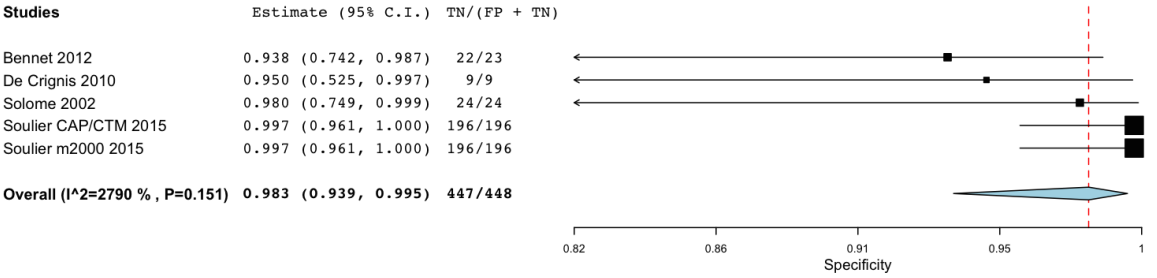


i. Fig. 6. Forest plot of specificity of capillary and venous samples

Capillary samples



Venous samples



j. Table 2: QUADAS-2

Author	Patient selection	Index test	Reference standard	Flow and timing
Abe	HR	LR	LR	LR
Bennet	LR	LR	LR	LR
De Crignis	HR	LR	LR	LR
Dokubo	LR	LR	LR	LR
Santos	HR	HR	LR	LR
Solome	HR	LR	LR	LR
Soulier	HR	LR	LR	LR
Tejada-Strop	HR	HR	LR	LR
Tuaillon	HR	LR	LR	LR

HR: high risk; LR: low risk

k. Table 3. GRADE table

Number of studies	Type of study	Directness	Precision	Consistency	Risk of bias	Overall quality
Sensitivity of DBS for HCV VL: 96.0% (upper-lower bounds 93.4–97.6)						
9 studies 1335 samples	Cross-sectional, case–control or cohort	No significant indirectness	No significant imprecision	No significant inconsistency	Significant risk of bias (non-randomized or consecutive patient recruitment or case-control design)	Moderate
Specificity of DBS for HCV VL: 97.7% (upper-lower bounds 94.7-99.0)						
9 studies 1335 samples	Cross-sectional, case–control or cohort	No significant indirectness	No significant imprecision	No significant inconsistency	Significant risk of bias (non-randomized or consecutive patient recruitment or case-control design)	Moderate

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Appendix 1: Terms used in database searches

MEDLINE-International Database for Medical Literature:

Dried blood spot [Ab] AND Hepatitis C [Ab]
Dried plasma spot [Ab] AND Hepatitis C [Ab]
Dried blood spot [Ab] AND HCV RNA [Ab]
Dried plasma spot [Ab] AND HCV RNA [Ab]
Dried blood spot [Ab] AND HCV [Ab] AND genotype [Ab]
Dried plasma spot [Ab] AND HCV [Ab] AND genotype [Ab]
Dried blood spot [Ab] AND HCV RNA [Ab] AND storage [Ab]
Dried plasma spot [Ab] AND HCV RNA [Ab] AND storage [Ab]

Web of Science (ISI Citation Index)

Dried blood spot [topic] AND Hepatitis C [topic]
Dried plasma spot [topic] AND Hepatitis C [topic]
Dried blood spot [topic] AND HCV RNA [topic]
Dried plasma spot [topic] AND HCV RNA [topic]
Dried blood spot [topic] AND HCV [topic] AND genotype [topic]
Dried plasma spot [topic] AND HCV [topic] AND genotype [topic]
Dried blood spot [topic] AND HCV RNA [topic] AND storage [Ab]
Dried plasma spot [topic] AND HCV RNA [topic] AND storage [Ab]

CAB abstracts

Dried blood spot [all fields] AND Hepatitis C [all fields]
Dried plasma spot [all fields] AND Hepatitis C [all fields]
Dried blood spot [all fields] AND HCV RNA [all fields]
Dried plasma spot [all fields] AND HCV RNA [all fields]

Annex 5.10

PICO 8 - Diagnostic accuracy of HBsAg/HBeAg test versus NAT to confirm successful treatment response: a meta-analysis and review of the literature

London School of Hygiene and Tropical Medicine team
*Olivia Varsaneux, *Ali Amini, Weiming Tang, Wen Chen, Debi Boeras, Jane Falconer, Helen Kelly, Joseph Tucker, Rosanna Peeling (Team lead)
London School of Hygiene and Tropical Medicine team
*Co-leaders of this Review

September 2015

1. Executive summary

Background: Advances in hepatitis B virus detection technology create new opportunities for enhancing screening, referral and treatment. The purpose of this review was to determine the diagnostic accuracy of HBsAg or HBeAg test versus nucleic acid testing (NAT) to confirm successful treatment response among patients receiving treatment for HBV.

Method: A literature search was conducted focused on hepatitis B, diagnostic tests and diagnostic accuracy. Studies were included if they evaluated an assay to determine the sensitivity and specificity of a HBsAg or HBeAg test compared to a quantitative HBV RNA reference among humans. Two reviewers performed a quality assessment of the studies and extracted data for estimating test accuracy.

Results: It was found that, despite HBV NAT being considered the gold standard in confirming response to treatment, both HBsAg and HBeAg were useful in monitoring patients receiving treatment as in many resource-limiting settings NAT is not readily available. Studies showed that the kinetics of HBsAg and HBV DNA followed similar profiles during treatment with pegylated interferon (PEG-IFN) and follow up in patients who developed sustained virological response (SVR). Further studies determined that this correlation was present for all four genotypes. It was also reported that HBsAg quantification can allow for detection of active cases of chronic HBV from true inactive carriers, therefore reducing the need to rigorously monitor HBV DNA levels. Studies showed that HBeAg was capable of differentiating late responders from non-responders to HBV DNA after 24 weeks of treatment.

Conclusions: There is limited evidence for the sole use of HBsAg or HBeAg compared to HBV DNA for monitoring treatment response. More studies are needed to determine which tests for HBV antigen detection may be useful as a marker of treatment response for which therapeutic agent.

2.Background

An estimated 240 million individuals worldwide[1] are chronically infected with hepatitis B virus (HBV) and there are an estimated 4 million acute HBV infections each year. Of those with chronic hepatitis B infection, 20–30% will develop cirrhosis[2] or hepatocellular carcinoma,[3] leading to approximately 650 000 deaths each year.[4] However, most individuals with chronic HBV infection are not aware of their serostatus, contributing to delayed diagnosis and complications from advanced disease.[5] HBV testing is critically important in order to refer infected individuals to HBV treatment and care, to refer uninfected individuals to vaccination and to mobilize prevention and control efforts.

The introduction of NAT is an integral step in the control of the disease as it allows for rapid diagnosis and early treatment of HBV. The virus can be transmitted by blood from asymptomatic donors with acute HBV infection before the development of HBsAg or an anti-HBc response. Therefore, NATs can used to detect HBV DNA in a donor's blood before antigen or

antibody response are detected.[6] Though NAT testing has been proven to be more sensitive in detecting viral infections, serological testing is better suited for the detection of active infections.[7]

Treatment with tenofovir or entecavir is effective for HBV. Their efficacy can be measured by a sustained reduction in viral load, but the quantitative HBsAg response may remain high. The data for measuring HBsAg quantitatively largely works for interferon-based agents. Locarini reviewed the literature on quantitative HBsAg in hepatology and highlighted some of the challenges of quantitative HBsAg testing in using other therapeutic agents.

In March 2015, the World Health Organization (WHO) published the first guidelines for the prevention, care and treatment of individuals with chronic HBV infection.[5] These guidelines focused on assessment for treatment eligibility, initiation of first-line therapies, switching and monitoring. These initial guidelines did not include screening recommendations. Given the large burden of HBV in low- and middle-income settings where there are limited or no existing HBV testing guidelines, there is a substantial need for HBV testing guidelines.

Advances in HBV detection technology create new opportunities for enhancing screening, referral and treatment. Previous systematic reviews on hepatitis B infection have focused on immunological responses,[7] surveillance of cirrhosis[8] and treatment.[9] Existing systematic reviews[10–13] on hepatitis B testing focused on point-of-care (POC) tests and included tests with unclear reference standards. No systematic reviews have examined the diagnostic accuracy of using HBsAg/HBeAg compared to HBV DNA detection to monitor treatment response.

PICO 8	Among patients receiving treatment for HBV, what is the diagnostic accuracy of HBsAg/HBeAg test versus NAT to confirm successful treatment response?
P	Patients receiving treatment for HBV
I	HBsAg/HBeAg testing
C	NAT for HBV DNA detection
O	Diagnostic accuracy True negatives (TNs) – who are screen negative and have cleared the HBV infection. False negatives (FN) – who are screen negative but have HBV infection. These will be misclassified and treatment will be stopped resulting in disease progression leading to liver-related morbidity (fibrosis, cirrhosis, end-stage liver disease, hepatocellular carcinoma), progression of liver disease and mortality. True positives (TP) – who are screen positive and truly have HBV infection. This will increase the number of treated cases and cure rate. False positives (FP) – who are screen positive, but do not have HBV infection. (These will continue treatment inappropriately, and and will have unnecessary referral). Costs – cost of testing strategy, including lab reagents and running costs, cost of further evaluation of a false positive. Cost–effectiveness

	Acceptability to health-care workers and patients Other outcomes (missed cases of liver disease because of false-negative results, unnecessary referral, investigations and/or treatment in false positives).

3. Objectives

The purpose of this review was to identify evidence on the sensitivity and specificity of HBsAg/HBeAg compared to HBV DNA detection for HBV treatment monitoring and to summarize the key test characteristics associated with detection of HBsAg/HBeAg.

4. Methodology

We followed standard guidelines and methods for systematic review and meta-analyses of diagnostic tests.[14,15] We prepared a protocol for the literature search, article selection, data extraction and assessment of methodological quality.

Selection criteria

i. Types of studies

We included observational studies and randomized controlled trials (RCTs) that provide original data from patient specimens, including cross-sectional and case–control studies, and studied HBsAg/HBeAg testing compared to a reference standard of HBV DNA detection.

ii. Participants

Little information on participants was provided in the selection of papers included in the systematic review; therefore, we set a wide inclusion criterion. We included patients of all age groups from all settings and countries as well as all types of specimens.

iii. Index tests

Studies that utilized commercially available HBsAg/HBeAg and HBV DNA assays were eligible for inclusion. The following four are the index tests included:

- Architect HBsAg assays, Abbott
- COBAS AMPLICOR TM HBV Test v2.0 assay. Roche Diagnostics Systems
- IMx HBeAg assay, Abbott
- Iprobe, Abbott

iv. Reference standard

The reference standards acceptable for a definitive diagnosis included tests for detection of HBV by the following HBV DNA detection techniques—polymerase chain reaction (PCR), branched-chain DNA (bDNA), or transcription-mediated amplification (TMA) and DNA hybridization assays.

Outcome measures

Sensitivity refers to the proportion of samples with true HBV infection diagnosed with positive HBsAg/HBeAg test confirmed with a positive HBV DNA detection method.

Specificity refers to the proportion of samples with negative HBsAg/HBeAg test confirmed with a negative HBV DNA detection method.

Search methods

A database search of LILACS, MEDLINE, EMBASE, PubMed, Scopus, Web of Science, Cochrane and WHO Global Index Medicus was performed through April 2015. No language restriction was applied. The references of published articles found in the above databases were searched for additional pertinent materials.

Study selection proceeded in three stages. First, titles/abstracts were screened by a single reviewer according to standard inclusion and exclusion criteria. Second, full manuscripts were obtained and assessed against inclusion criteria. Papers were accepted or rejected and reasons for rejection were specified. Third, two independent reviewers assessed each manuscript and differences were resolved by a third independent reviewer.

Data extraction

Information on the following variables were extracted by a reviewer if the study met the exclusion and inclusion criteria—first author, total sample size, country (and city) of sampling, sample type (oral fluid, finger-prick, venous blood, etc.), point-of-care (Y/N), eligibility criteria, reference standard, manufacturer, raw cell numbers (true positives, false negatives, false positives, true negatives), sources of funding and reported conflicts of interest. We define point of care as being able to give a result within 60 min and having the results guide clinical management at the same encounter.

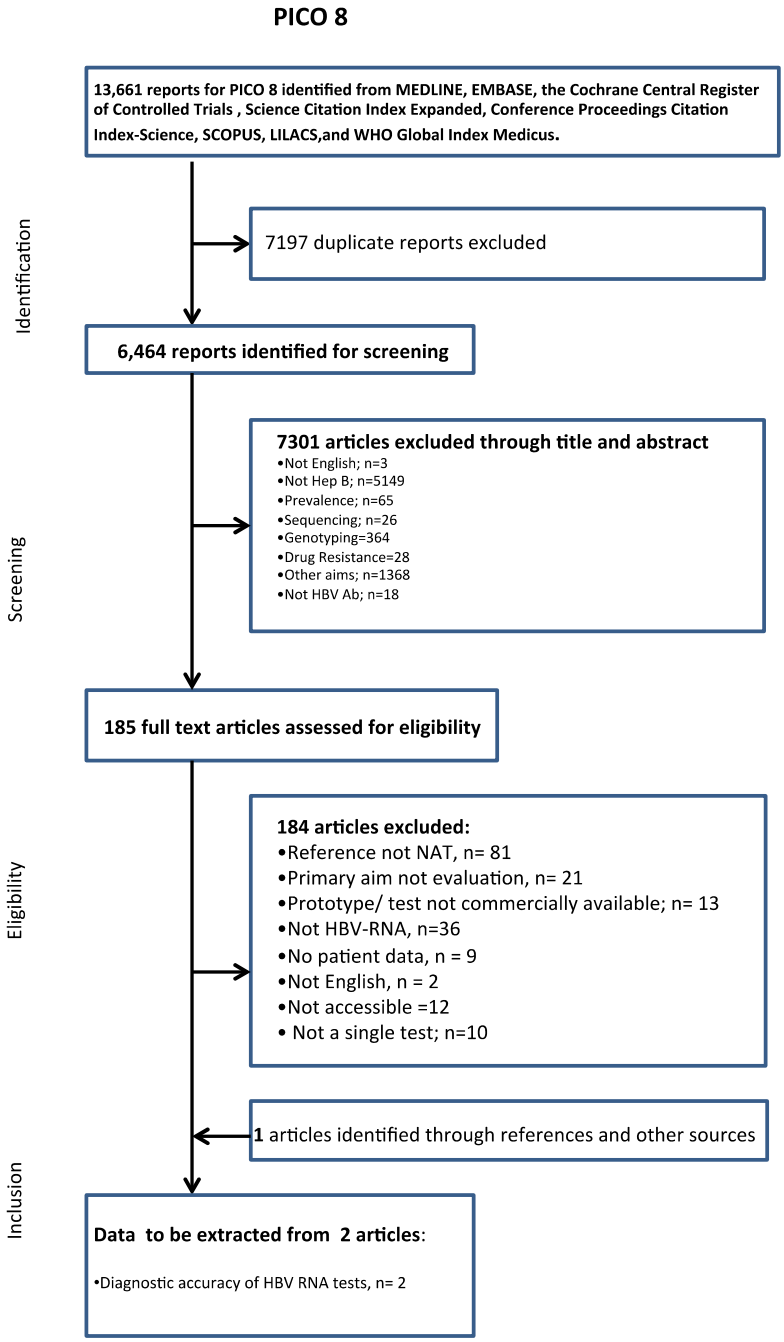
Assessment of methodological quality

Study quality was evaluated using the QUADAS-2 tool,[16] the STARD checklist[17] and the GRADE method.[18] QUADAS includes domains to evaluate bias in the following categories—risk of bias (patient selection, index test, reference standard, flow and timing); applicability concerns (patient selection, index test, reference standard). The GRADE method evaluates the strength of evidence by assessing the risk and probability of bias, imprecision and inconsistency as well as dose–respondent gradient and residual confounding.[18]

5. Results

PRISMA flowchart

Fig. 1. PRISMA flow diagram outlining study selection examining diagnostic accuracy of HBV antibody tests compared to HBV DNA in confirming successful treatment response



Characteristics of included studies

Only two of the studies analysed met the PICO criteria and data were extracted from both these studies. These studies took place in Sweden and the United States of America. The patient population for the Larsson 2013 study was derived from a clinical setting; there was no information on the patient population for the Perrillo 1993 study. The assays evaluated for this

systematic review were Architect HBsAg assays, COBAS AMPLICOR TM HBV Test v2.0 assay, IMx HBeAg assay and Iprobe. Of these two studies, only one reported sensitivity/specificity of HBsAg and one reported sensitivity/specificity of HBeAg.

The lack of information on these diagnostic accuracy measures is a large limitation in the quality of the studies. Other issues with the quality of these studies were insufficient information on the populations studied, randomization and sample collection.

Table 1. Description of study design, study population and setting of all studies (*n*=2)

	First author	**Sample type & size**	**Country**	**Treatment**	**Study population**	**Eligibility criteria**	**Index diagnostic test**	**Reference test**	**Sensitivity**	**Specificity**
1	Larrson, 2013	Liver tissue and blood sample *N*= 160	Sweden	INF	Infectious Disease clinic *N* =160	Patients with chronic HBV	Architect HBsAg assays, Abbott	COBAS AMPLICORTM HBV Test v2.0 assay. Roche	34%	89%
3	Perrillo, 1993	Plasma *N*= 34	United States of America	INF	?	29 patients on treatment and 5 neg. controls	IMx HBeAg assay, Abbott	Iprobe, Abbott	95%	44%

Larsson et al. (2013) monitored HBsAg levels (Architect assays, Abbott) and HBV DNA quantitation (COBAS AMPLICOR^{TM} HBV monitor, Roche) in 160 patients treated for chronic HBV infection at the Infectious Disease Clinic at Sahlgrenska University Hospital between 1993 and 1995. Sensitivity of HBsAg compared to HBV DNA was 34% and the specificity was 89%. A correlation between HBsAg and HBV DNA in serum samples ($R^2 = 0.39$; $P< 0.0001$) was also noted, in that a 90% reduction of HBV DNA corresponded to a 48% decline in HBsAg. The authors also measured HBeAg levels and found that HBeAg-positive patients had a 300 times higher HBV DNA/HBsAg ratio compared to those who were HBeAg-negative. These results indicate that HBsAg quantification could be complementary to HBV DNA quantification for treatment monitoring and confirming successful treatment response.

Perrillo et al. (1993) evaluated whether the HBeAg assay (Abbott IMX) was capable of providing comparable information to HBV DNA assays (Iprobe, Abbott) during and after IFN therapy in 29 consecutive, IFN-treated patients and five untreated controls. The authors found that decremental and incremental changes in HBeAg concentration during and after therapy mirrored those observed with HBV DNA with a significant correlation (R = 0.768 P>0.0001). Only 56% of HBV DNA-negative patients tested positive for HBeAg but 95% of HBV DNA-positive samples were also positive for HBeAg. Though this information allows us to understand that HBeAg concentrations can provide similar clinically relevant information compared to HBV DNA

assays, it is difficult to state the accuracy of these tests against each other as they are traditionally used to measure different indicators.

Narrative summary of each systematic review's findings

Monitoring response to treatment is an essential mechanism in the control of HBV and requires both the sustained disappearance of HBV DNA and the clearance of HBsAg/ HBeAg from the blood. The systematic review showed that monitoring of HBeAg concentration can provide clinically relevant information, though only two of the 6464 studies identified for screening (Larrson et al. 2013 and Perrillo et al. 1993) were included in the systematic review as they were the sole articles that met both the inclusion and exclusion criteria for PICO 8 (showed sensitivity and specificity of assays).

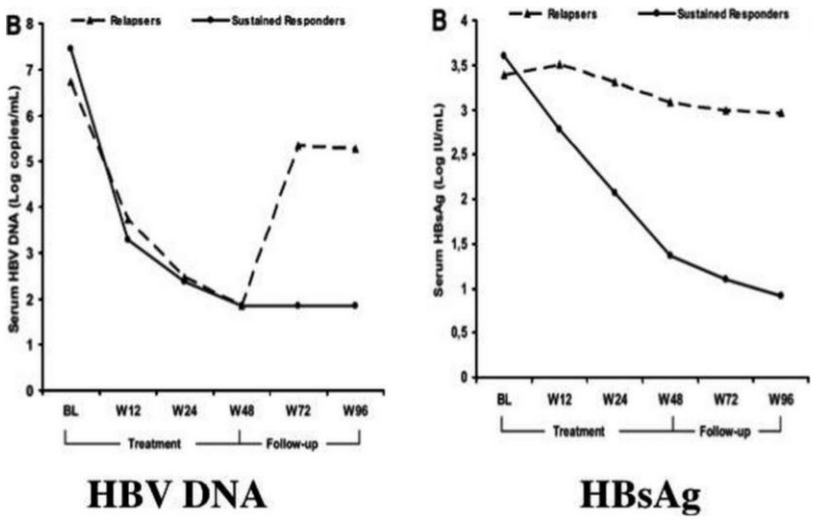
i. Diagnostic accuracy of nucleic acid testing

HBV DNA is essential when determining the presence of the virus as it is quantitatively expressed and allows for prompt detection of HBV. With the advent of reverse transcriptase-polymerase chain reaction (RT-PCR), it quickly became regarded as the gold standard or confirming response to therapy due to its accuracy and cost–effectiveness. However, in many resource-limited settings, such assays are not widely available, therefore it is important to determine if HBsAg or HBeAg can be used for monitoring response to treatment.[19]

ii. Diagnostic accuracy of HBsAg test compared to HBV DNA

Although they did not include specific accuracy values, three supplemental studies provided useful information on the quantitation of HBsAg for treatment monitoring in chronic HBV patients. Martinot-Peignoux et al. (2015) reported that the kinetics of HBsAg and HBV DNA followed similar profiles during treatment with PEG-IFN and follow up in patients who developed SVR (solid line) (*see* Fig. 2).[18]

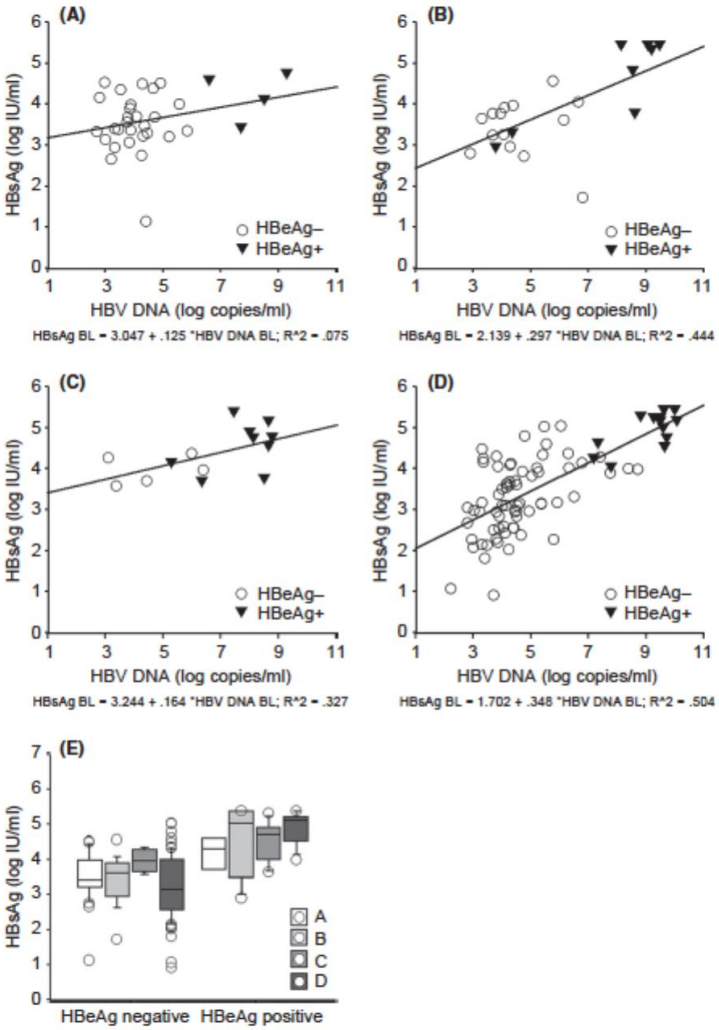
Fig. 2. Serum HBV DNA and HBsAg kinetics during treatment with PEG-IFN and follow up in patients who developed SVR (solid line) [20]



This was confirmed by Ganji et al. (2011) who that showed HBsAg had strong correlation with HBV DNA (r =0.69; *P*<0.01) for both genotypes investigated.[20] Larsson et al. 2014 further proved that that there was a correlation between HBsAg and HBV DNA for all four genotypes (Fig. 3).[19] This highlights the potential for HBsAg to be a useful serological marker to predict response to treatment.

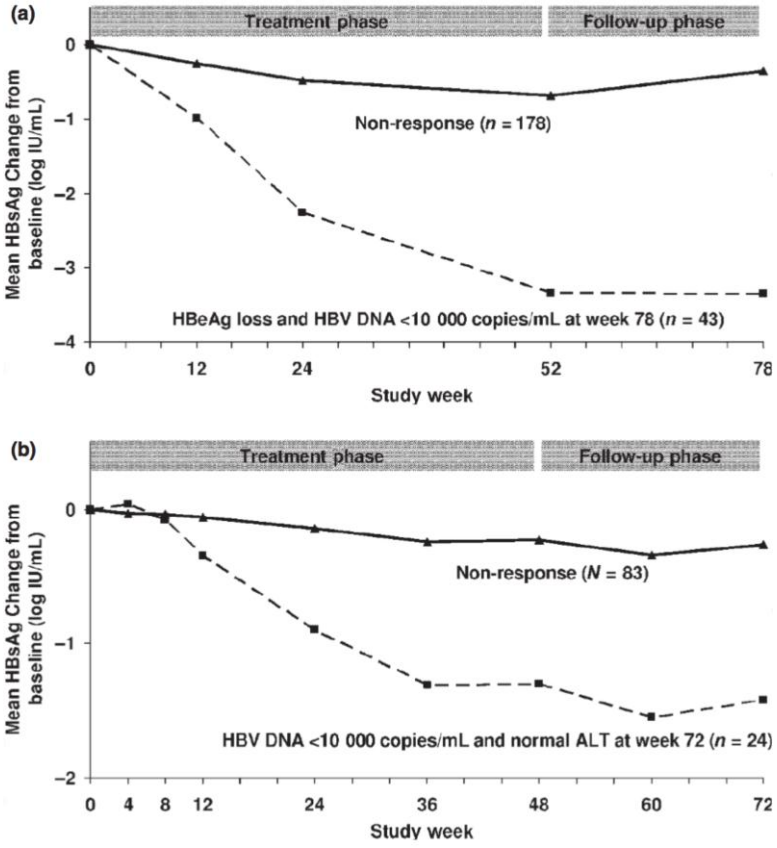
Fig. 3 (A–D). Correlation between HBsAg and HBV DNA in genotypes A–D

Fig. 3 (E). Box plot of HBsAg levels in HBeAg-positive and -negative patients by genotype (no significant differences)[19]



Another important use for HBsAg assays is in monitoring treatment response for HBeAg-negative chronic patients with low HBV DNA levels. Sonneveld et al. (2011) reported that when monitoring PEG-IFN treatment in patients with chronic hepatitis B, HBsAg reduction is most pronounced in patients who achieve a response to therapy at 6 months post treatment.[19] This suggests that HBsAg quantification can allow for detection of active cases of chronic HBV from true inactive carriers, thereby reducing the need to rigorously monitor HBV DNA levels.

Fig. 4. Hepatitis B surface antigen decline during PEG-IFN treatment of hepatitis B e antigen (HBeAg)-positive and HBeAg-negative patients[26]



iii. 4.5.3 Diagnostic accuracy of HBeAg test compared to HBV DNA

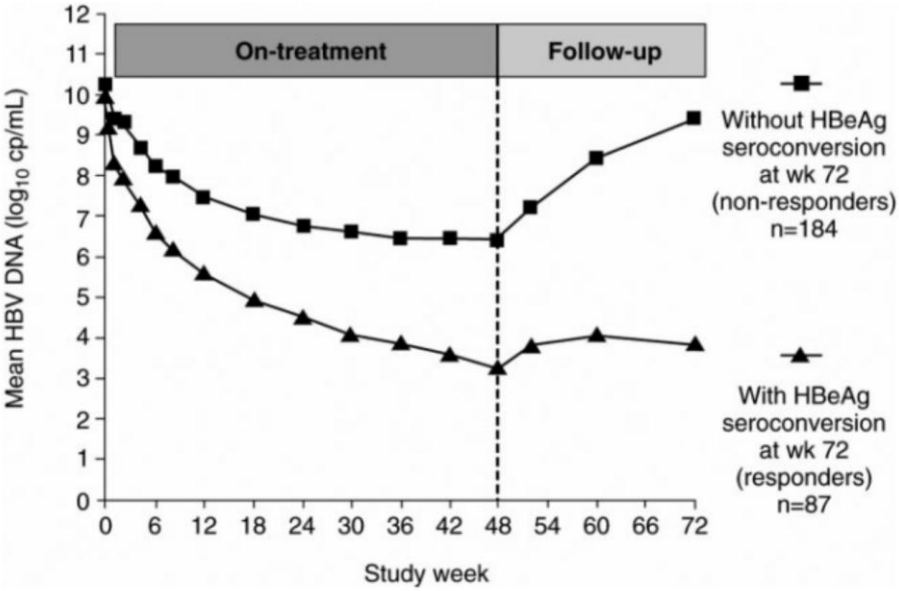
Monitoring HBeAg has been shown to be important due to its association with the disappearance of replicative viral intermediates and its persistence in the blood once HBV DNA has cleared. [19] Using PEG-IFN alfa-2a, Fried et al. (2008) showed that HBeAg levels proved to be a stronger indicator of non-response compared to HBV DNA after 24 weeks of treatment. Lower levels of HBV DNA were seen to closely predict seroconversion (Table 2).

Table 2. Serum HBV DNA at weeks 12 and 24 of treatment: relationship to HBeAg seroconversion

HBV DNA (log_{10} copies/mL)	Patients with HBeAg Seroconversion at Week 72	
	Week 12	Week 24
<3	64%	69%
3–5	49%	41%
5–7	29%	19%
≥7	21%	14%

It was also shown that those who reached HBeAg seroconversion had a consistent decline in their levels of HBeAg and remained at the lowest levels while under the follow-up period. This was in contrast to those who failed to achieve seroconversion after treatment was discontinued, as a rebound was observed allowing for better determining of seroconversion and a higher negative predictive value (Fig. 5). This highlights the importance of HBeAg in differentiating late responders from non-responders and is an important aspect of treatment.[22]

Fig. 5. HBV DNA levels: responders versus non-responders at 24 weeks post treatment – HBeAg seroconversion [23]



However, monitoring treatment response using HBeAg can be complicated as the response may vary with the therapy used. Non-interferon agents rarely cause HBeAg loss or might cause only a transient HBeAg loss while on therapy. Interferon agents are toxic and might convert ~35% of

HBeAg positives to negatives but only in a subset of people with high alanine aminotransferase (ALT).[23]

Monitoring HBV treatment response remains a challenge. Guidelines for chronic HBV management and treatment state that the ideal end-point of treatment should be dictated by a lack of detectable HBsAg. The use of HBsAg/HBeAg as a marker to detect sustained virological response is essential, because on-treatment decrease in HBV DNA shows similar patterns for both sustained responders and relapsers (Fig. 3).[20] Due to the infrequency of obtaining this point with the current anti-HBV agents, the primary goal of antiviral therapy is defined as viral remission, PCR non-detectability (<300 copies/mL [57 IU/mL]).[19,21]

For the time being, NAT can be used as the reference standard to confirm this response to therapy. More studies are needed to determine which tests for HBV antigen detection may be useful as effective markers of treatment response for therapeutic agents.

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Annex 5.11

PICO 10 - Interventions to optimize the chronic viral hepatitis care continuum: a systematic review and meta-analysis of interventions to improve hepatitis B and C screening, linkage to care, treatment uptake, treatment adherence, and viral suppression

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Abstract

Background: Recent advances in hepatitis B virus (HBV) and hepatitis C virus (HCV) therapeutics have ushered in a new era of effectively addressing chronic viral hepatitis. To optimize the real-world effectiveness of these medicines requires engaging and retaining individuals from screening through care and ultimately viral suppression. We carried out a systematic review of operational interventions to enhance chronic viral hepatitis screening, linkage to care, treatment uptake, treatment adherence, and ultimately viral suppression.

Methods: The review was registered in PROSPERO (42014015094) and carried out according to PRISMA guidelines. We searched PubMed, EMBASE, the WHO library, Clinicaltrials.gov, International Clinical Trials Registry Platform, Psychinfo, and Cinahl. We included randomized controlled trials (RCT) or controlled non-randomized studies (NRS) targeting one or more steps along the chronic viral hepatitis (HBV, HCV) continuum of care. We used the Cochrane risk of bias tool and GRADE methodology to assess the quality of included studies. Pool data from studies of similar interventions were included in the meta-analyses for each specific step of the care continuum.

Results: We identified 7581 citations and included 54 studies. All studies except one were from high-income countries. Studies reported outcomes for chronic viral hepatitis screening, linkage to care, treatment uptake, treatment adherence, or viral suppression. No studies evaluated interventions to improve HBV treatment uptake or treatment adherence. Six randomized controlled trials (RCTs) showed that lay health worker HBV test promotion interventions increased HBV testing rates (RR = 2.68 [1.82–3.93], moderate quality evidence). Two NRS and one RCT found clinician reminders to prompt HCV screening during clinical visits increased HCV testing rates (RR = 3.70 [1.81–7.57], very low-quality evidence). Three RCTs demonstrated that interventions facilitating referral and scheduling to specialist sites increased patient attendance at HCV specialist visits (RR = 1.57 [1.03–2.41], moderate-quality evidence). Coordinated care between mental health and treatment specialists along with psychological therapy and counselling for patients with mental health and/or substance use comorbidities increased HCV treatment initiation (OR = 3.03 [1.24–7.37]), improved treatment completion (RR = 1.22 [1.05–1.41]), and increased sustained virological response (SVR) (RR = 1.21 [1.07–1.38]) compared to usual care (very low-quality evidence). Nurse-led therapeutic educational interventions improved treatment completion (RR = 1.14 [1.05–1.23], low-quality evidence) and increased SVR (OR = 1.93 [1.44–2.59], low-quality evidence).

Conclusion: A range of relatively simple, inexpensive operational interventions can significantly improve engagement and retention along the chronic viral hepatitis care continuum. In addition, integrated approaches to hepatitis screening, care and treatment for specific vulnerable populations are effective. In the era of highly effective antiviral therapies, further implementation science research specifically carried out in low and middle-income settings, is needed to optimize engagement and retention for people living with HBV and HCV in the chronic viral hepatitis continuum of care.

Systematic review and meta-analysis manuscript

1. Background

Globally, 250 million people are chronically infected with hepatitis B virus (HBV),[1] and 80–140 million are chronically infected with hepatitis C virus (HCV)[2,3] resulting in 1.45 million annual deaths – the seventh leading cause of mortality worldwide. Chronic HBV and HCV responsible for over 90% of these deaths.[4] Recent advances therapeutic in HBV and HCV therapeutics are now providing the impetus for substantial changes in the clinical management of chronic viral hepatitis.

Optimizing the high efficacy of these new medicines will require engagement and retention across the care continuum, ranging from initial screening to viral suppression (HBV) or cure (HCV) (Fig. 1). Similar to the HIV continuum of care, each step of the chronic viral hepatitis continuum of care is contingent on the previous steps. This importance of the entire HBV care continuum is underlined in Australia where although 57% of the estimated population living with HBV are diagnosed, only 8% receive viral load testing and only 5% are on treatment.[5] Operational interventions may enhance engagement and retention at each step along the continuum of care: screening, linkage to care, treatment uptake, treatment adherence, and viral suppression.

Population-level data on the viral hepatitis treatment care continuum for viral hepatitis is limited, though even in high-income countries only a small fraction of the estimated population living with HBV or HCV are ultimately treated and achieve viral suppression.[5,6] Indeed, large proportions of people living with viral hepatitis B and C are unaware of their infection, especially those from vulnerable groups and those living in low- and middle-income countries.[7,8–10]

To investigate the potential of operational strategies to facilitate engagement and retention in the care continuum, we conducted a systematic review to identify interventions in adults living with chronic HBV or HCV infection. We further quantified the effect size of these interventions and highlighted gaps in knowledge on progression through the care continuum.

2. Methods

We searched PubMed, EMBASE, WHO library, International Clinical Trials Registry Platform, Psychinfo, and Cinahl for full-text or abstract entries published before 31 December 2014. Accepted scientific conference abstracts and clinical trials registered on Clinicaltrials.gov were also screened. References of articles selected for inclusion were searched for additional citations. Only peer-reviewed English language randomized controlled trials (RCT) or controlled non-randomized studies (NRS) were included. Where study details were unclear, we contacted authors directly.

Details on our search strategy can be found in Supplementary Materials. Briefly, we included studies on operational interventions at any point in the chronic viral hepatitis continuum for people living with diagnosed or undiagnosed chronic viral hepatitis. Only studies where the primary or secondary outcomes were engagement, retention, or progression along the care continuum were included. Exclusion criteria included study designs lacking a comparator or control, dissertations, studies enrolling only pediatric populations, and publications failing to report the outcome data necessary for extraction.

Titles, abstracts, and full-texts were sequentially screened for inclusion by two authors independently, with a third author consulted where there was disagreement. Reasons for excluding abstracts and full-texts were recorded. The PRISMA flowchart for included studies is shown in Fig. 2. Data extraction was also performed independently by two reviewers. Differences in the data extracted by the two reviewers was first attempted to be reconciled through discussion. A third reviewer was consulted if disagreement remained. The following variables were extracted: authors, journal of publication, publication year, study design, studied population, inclusion criteria, exclusion criteria, participant characteristics, sample size, study context, intervention description, control description, duration of intervention, results, and conclusions. Data was extracted and analyzed according to an intention-to-treat approach, even if individual authors reported results or conclusions based on per-protocol analyses.

Following data extraction, risk of bias was assessed for each included RCT and NRS using the Cochrane Collaboration's risk of bias tool. Risk of bias was ranked along six domains: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias. Detailed risk of bias tables for each stage of the chronic viral hepatitis continuum of care are included in Supplementary Materials.

All included publications were assessed for comparability on the basis of intervention type, control condition, and outcome. Studies determined to be similar for intervention, control, and outcome were included in meta-analyses to determine pooled effect size. Pooled risk or odds ratios with confidence intervals and forest plots were generated using a random-effects model in Review Manager 5.3. The degree of heterogeneity between studies in a comparison was assessed by calculating I^2.

When studies only reported odds ratios with confidence intervals, data was pooled using the generic inverse variance method. If a portion of the studies included in a comparison reported

outcomes that had been adjusted using matching or statistical modeling (e.g. regression modelling), a sub-analysis was generated using only adjusted results. Funnel plots were used to screen for reporting bias.

The strength of evidence was assessed according to the methodology described by the GRADE working group, and a GRADE table was generated for each meta-analysis and sub-analysis. Where specific interventions were directed at specific populations (e.g. mental health or substance use patients living with hepatitis C) we did not downgrade for indirectness as any recommendation from this analysis would be relevant to that specific population. For imprecision, the pooled sample size for each meta-analysis was compared against the optimal information size (OIS), which was calculated using an alpha of 0.05 and power of 80%.

Comparisons determined to be most relevant to current viral hepatitis treatment guidelines were included in the results section. All other comparisons can be found in Supplementary Materials.

3. Results

A total of 11 806 titles were identified through database searches, and 19 additional titles were identified through searching article references and contacting authors. After duplicates were removed, 7581 titles were screened according to standard inclusion and exclusion criteria. 469 abstracts were selected for further screening, 353 of which were excluded. Based on the results of abstract screening, 116 articles were selected for full-text review. Ultimately, 54 studies were selected for inclusion in this systematic review,[11–64] including 45 full-text publications,[11,13,14,16–31,33–39,41,45–47,49–55,57–64] 5 abstracts,[12,15,32,40,48] and 4 clinical trials[42–44,56] (Table 1). Of the 54 total included studies, 31 were included a meta-analysis that calculated a pooled effect size (Fig. 2).

Of the total 54 included studies, 37 reported an outcome along the HCV continuum of care, 15 reported an outcome along the HBV continuum of care, and 2 studies reported outcomes involving both HBV and HCV. Interventions to improve retention along the HBV continuum of care were limited to screening and linkage to care, while interventions to improve retention along the HCV continuum of care addressed all five steps. The most commonly reported outcomes among included studies were HCV treatment adherence (including treatment completion) and HCV viral suppression, with 21 and 20 studies, respectively, followed by HBV and HCV screening, with 15 and 11 studies, respectively. HBV and HCV linkage to care and treatment uptake were comparatively less well studied (Table 1). None of the interventions to improve HBV or HCV screening specifically targeted symptomatic or asymptomatic individuals.

All included studies were conducted in high-income countries, except a single study conducted in Turkey.[31] Of the included studies, 44.4% (24/54) were RCTs, with seven of those being cluster RCTs, and the remaining 55.5% (30/54) of included studies were NRS. Sample sizes ranged from 21 to 36,987 (Table 1).

Thirteen meta-analyses were performed where studies were determined to be similar in terms of intervention, control and outcome so that reported data could be pooled. The number of

studies included in a meta-analysis ranged from two to six. The limited number of studies in each comparison prevented us from performing stratified analyses for risk of bias, intervention intensity, and other relevant factors. Funnel plots did not detect reporting bias for any of the 13 meta-analyses.

Nearly half (7/15) of the interventions to improve HBV screening were lay health worker (LHW) HBV test promotion interventions.[16,29,38,53–55,64] Six of the seven LHW-led interventions were one-time activities that delivered educational content tailored to a particular community's cultural and social context.[16,29,53–55,64] All six studies targeted Asian communities in the United States or Canada. Results from these six studies were pooled in a meta-analysis. Self-reported HBV testing rates were higher among groups that received a single LHW educational intervention to improve HBV knowledge and promote testing compared to groups that received no or unrelated educational interventions (RR = 2.68, CI 95; 1.82–3.93). All studies found LHW-led interventions had a positive effect on HBV screening; however, these results were moderately heterogeneous (I^2 = 56%). This heterogeneity in effect size may be due to differing study design (three were RCTs randomized by cluster, and three were RCTs randomized by individual) and intervention setting (three were home visits, and three were delivered at a community-based organization).

Unlike interventions to improve HBV screening, which were primarily delivered in community settings, all 11 of the interventions to improve HCV screening either targeted health-care providers or took place at an established health-care facility.[18,19,21,23–25,32,36,41,50,51] At this stage in the HCV continuum of care, the two most common interventions were clinician reminders to prompt HCV screening during clinical visits (three studies)[21,32,36] and pre-test counselling with on-site HCV testing at a health-care facility serving high-risk populations (three studies).[19,41,50] Clinician reminders to prompt HCV screening during clinical visits consistently increased HCV testing rates compared to no clinician reminders (RR = 3.70, CI 95; 1.81–7.57). While all three studies found clinician reminders to have a positive effect on HCV screening rates, there was a large degree of heterogeneity between reported effect sizes (I^2 = 99%). Providers were prompted by reminders to order HCV tests if patients belonged to a high-risk birth cohort (one study),[32] reported risk behaviour (one study),[21] or both (one study).[36] Two studies used physical reminder stickers attached to patient charts,[21,36] while one study incorporated reminders into an electronic medical records system.[32] All three studies examined patients seeing primary care providers in New York City clinics.

Three interventions had a facilitated referral component where staff at a site of established care actively assisted HCV+ patients with a history of substance use in scheduling specialist visits.[19,39,50] Interventions that provided facilitated referral increased patient attendance to HCV specialist visits compared to no facilitated referral in all three studies (RR = 1.57, CI 95; 1.03–2.41). However, there was significant heterogeneity between reported effect sizes (I^2 = 74%). Some of the interventions in this meta-analysis provided patient education and case management at varying degrees of intensity in addition to facilitated referral, which may partially explain why the three interventions reported widely varying effect sizes.

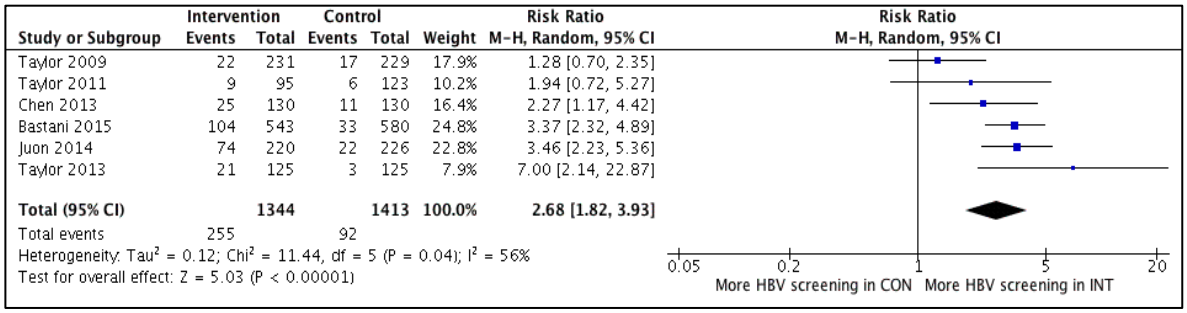
In certain contexts HCV+ patients had been deemed ineligible for HCV treatment because of ongoing mental health and/or substance use comorbidities. Individually tailored mental health counselling and motivational therapy to treat mental health and/or substance use issues increased the number of patients who were referred eligible to treatment compared to usual care (OR = 3.43, CI 95; 1.81–6.49). There was little heterogeneity in this meta-analysis (I^2 = 0%) despite the fact standards for treatment eligibility differed between the two included studies.[22,30]

Along the treatment uptake, treatment adherence, and viral suppression steps of the HCV continuum of care, six interventions provided "integrated" or "multidisciplinary" care.[11,20,30,46,62,63] These interventions involved regular contact between mental health and specialist treatment providers throughout treatment, and also arranged regular psychological therapy and counselling for patients with mental health and/or substance use comorbidities. Some interventions also provided varying degrees of patient education and case management before and during treatment. Coordinated care between mental health and treatment specialists along with psychological therapy and counselling for patients with mental health and/or substance use comorbidities increased HCV treatment initiation (OR = 3.03, CI 95; 1.24–7.37), improved treatment completion (RR = 1.22, CI 95; 1.05–1.41), and increased SVR (RR = 1.21, CI 95; 1.07–1.38) compared to usual care. Studies included in these meta-analyses differed in terms of additional services provided beyond coordinated care, percentages of patients with mental health and/or substance use comorbidities, and patient genotype. However, little heterogeneity in effect size was found for treatment adherence and viral suppression.

An additional six studies investigated the impact of educational activities about HCV infection, treatment, side-effects of therapy, and the importance of treatment adherence for HCV^+ patients beginning or maintained on interferon-based therapy.[14,33,37,42,48,52] Nurse-led therapeutic educational sessions were found to improve treatment completion (RR = 1.14, CI 95; 1.05–1.23) and increase SVR (OR = 1.93, CI 95; 1.44–2.59).

Meta-analyses for interventions to improve HBV screening

Single culturally tailored LHW educational session to improve HBV knowledge and promote testing vs no or unrelated educational session for self-reported HBV screening.

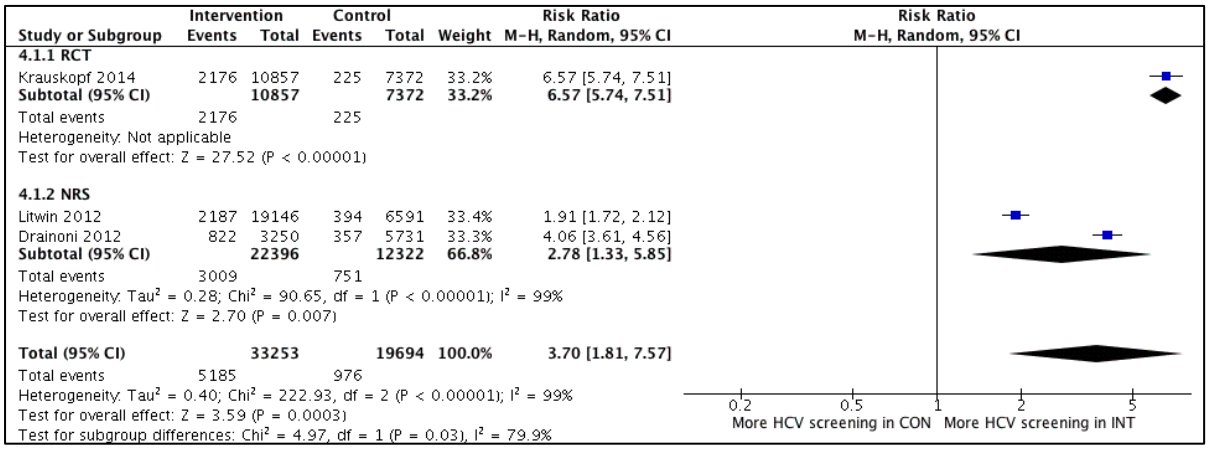


Quality assessment							No. of patients		Effect		Quality
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Single LHW educational session	No or unrelated educational session	Relative (95% CI)	Absolute (95% CI)	
HBV screening											
6	Randomized trials	Serious[1]	Not serious	Not serious[2]	Not serious[3]	None	255/1344 (19.0%)	92/1413 (6.5%)	**RR 2.68** (1.82–3.93)	109 more per 1000 (from 53 more to 191 more)	⨁⨁⨁◯ Moderate
								6.6%		110 more per 1000 (from 54 more to 192 more)	

1. 6/6 studies are at high risk of detection bias because the outcome was self-reported HBV screening 6 months post intervention. 5/6 studies are at high risk of attrition bias because the ratio of participants with missing data to participants with HBV screening outcome was high (>1.0).
2. Although all included studies involved Asian immigrants in North America, this was not judged to be a significant enough difference in populations to downgrade because the intervention strategies are not exclusive to Asian immigrant populations.
3. The confidence interval is not wide. The OIS was calculated to be 222, and the pooled sample size exceeded the OIS. 3/6 included studies were cluster RCTs, none of which performed analyses that accounted for clustering. Consequently, this meta-analysis commits a unit-of-analysis error and produces over-precise results. Additionally, no ICC were reported in the included studies, so statistical methods could not be used to reduce the effective sample size of the cluster RCTs. Despite this limitation, it is unlikely proper adjustment for cluster design would significantly impact the precision of the pooled results.

II. Meta-analyses for interventions to improve HCV screening

Clinician reminder to prompt HCV screening during clinical visits with or without supplementary provider education vs no clinician reminder for HCV screening.

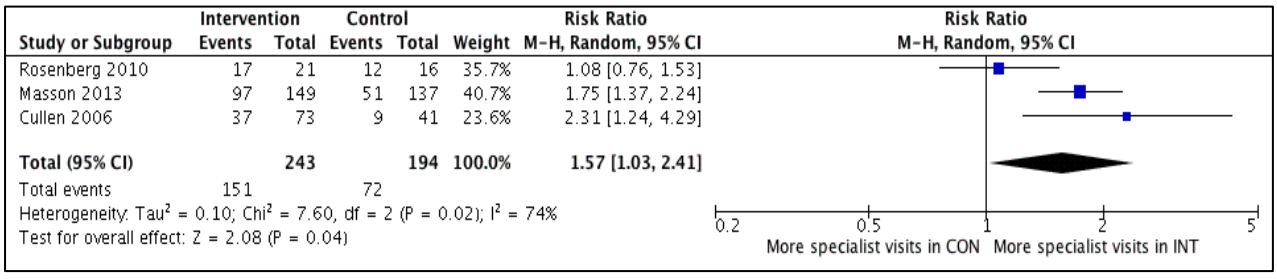


Quality assessment							No. of patients		Effect		Quality
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Clinical testing reminder	No reminder	Relative (95% CI)	Absolute (95% CI)	
HCV screening											
3	Other design[1]	Serious[2]	Serious[3]	Not serious	Serious[4]	None[5]	5185/33253 (15.6%)	976/19694 (5.0%)	RR 3.70 (1.81–7.57)	134 more per 1000 (from 40 more to 326 more)	⊕◯◯◯ very low
								6.0%		161 more per 1000 (from 48 more to 393 more)	

1. This meta-analysis includes 1 cluster RCT and 2 NRS.
2. Drainoni (2012) is at high risk of performance bias and did not employ methods to adjust for confounding potentially introduced by its non-randomized study design. Krauskopf (2014) did not report comparability of randomized clusters and therefore was at high risk of bias.
3. All included studies report a risk ratio >1.0. However, I^2 = 99%. The high degree of heterogeneity may be due to differences between HCV screening algorithms used in each intervention.
4. Although the pooled sample size exceeds the calculated OIS, the confidence interval is wide. Additionally, Krauskopf (2014) was a cluster RCT that did not account for clustering in its analysis. Consequently, this meta-analysis commits a unit-of-analysis error and produces overprecise results. No ICC was reported, so statistical methods could not be used to reduce the effective sample size of the cluster RCT.
5. All included studies report a risk ratio >2.0. However, the pooled results have not been upgraded for large effect because the non-randomized design of 2/3 studies introduces a significant possibility of confounding.

III. Meta-analyses for interventions to improve HCV linkage to care

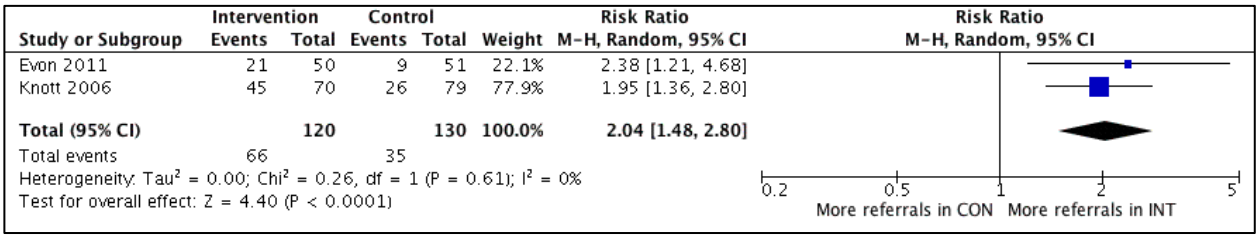
Facilitated referral and scheduling to specialist visit by staff at site of established care with or without supplementary HCV education and post-test counselling vs no facilitated referral for attendance at HCV specialist visit



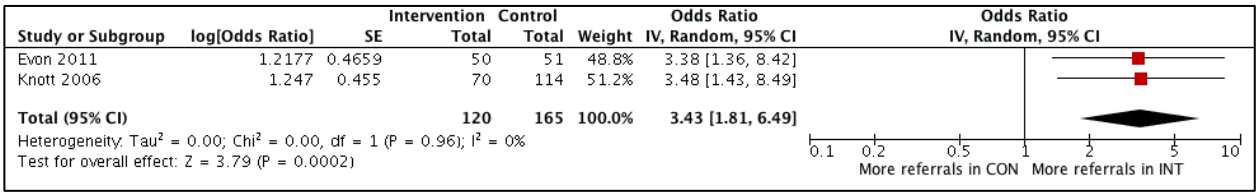
Quality assessment							No. of patients		Effect		Quality
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Facilitated referral to specialist visit at site of established care	No facilitated referral	Relative (95% CI)	Absolute (95% CI)	
Attended HCV specialist visit											
3	Randomized trials	Not serious[1]	Serious[2]	Not serious	Not serious[3]	None	151/243 (62.1%)	72/194 (37.1%)	**RR 1.57** (1.03–2.41)	212 more per 1000 (from 11 more to 523 more)	⨁⨁⨁◯ Moderate
								37.2%		212 more per 1000 (from 11 more to 525 more)	

1. Rosenberg (2010) relied on self-reported HCV status and self-reported attendance to an HCV specialist visit, putting the study at high risk of detection bias. However, because this study had a relatively small sample size it was not judged to put the entire meta-analysis at high risk of bias.
2. I^2 = 85%. This high degree of heterogeneity may be due to differences between the intensity of interventions in the included studies.
3. The confidence interval is not wide. The OIS was calculated to be 124, and the pooled sample size exceeded the OIS.

Individually tailored mental health counselling and motivational therapy for HCV+ patients with mental health and/or substance use comorbidities vs usual care for physician referral to initiate treatment.



Unadjusted results



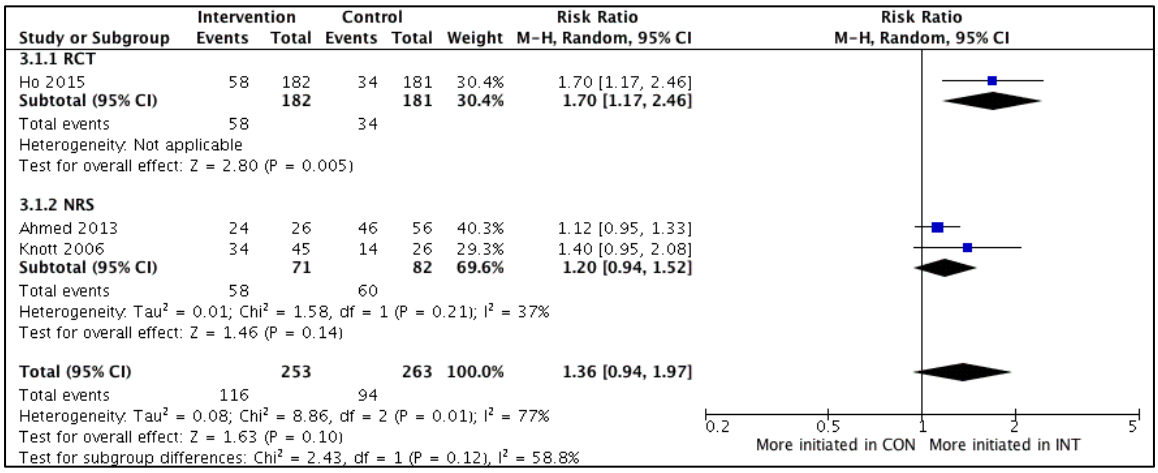
Adjusted results

Quality assessment							No. of patients		Effect		Quality
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Individually tailored mental health counselling and motivational therapy	Usual care	Relative (95% CI)	Absolute (95% CI)	
Physician referral to initiate treatment											
2	Other design[1]	Serious[2]	Not serious	Not serious[3]	Not serious[4]	None	66/120 (55.0%)	35/130 (26.9%) 25.3%	RR 2.04 (1.48–2.80)	280 more per 1000 (from 129 more to 485 more) 263 more per 1000 (from 121 more to 455 more)	⨁◯◯◯ Very low
Adjusted physician referral to initiate treatment											
2	Other design[1]	Serious[5]	Not serious	Not serious[3]	Serious[6]	None	–/120	–/165	OR 3.43 (1.81–6.49)	0 fewer per 1000 (from 0 fewer to 0 fewer)	⨁◯◯◯ Very low

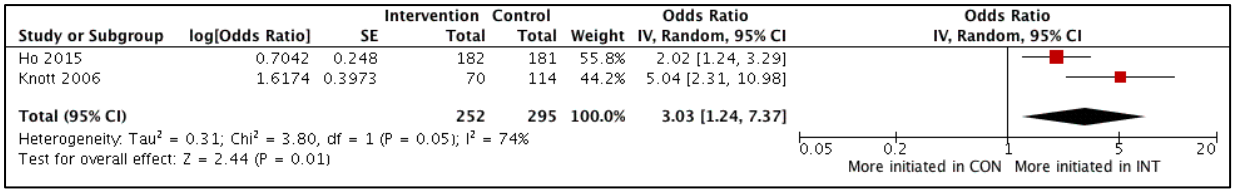
1. Evon (2011) is a RCT, while Knott (2006) is a NRS.
2. Knott (2006) is at high risk of detection bias because the outcome was subjective and determined by the physician overseeing treatment who was not blinded. Unadjusted results from Knott (2006) were used in this meta-analysis that did not employ methods to adjust for confounding potentially introduced by its non-randomized study design.
3. The decision to not downgrade for indirectness assumes guidelines are applied to other contexts where mental health or substance use comorbidities are also contraindications to recommending HCV+ patients for treatment.
4. The confidence interval is not wide. The OIS was calculated to be 94, and the pooled sample size exceeded the OIS.
5. Knott (2006) is at high risk of detection bias because the outcome was subjective and determined by the physician overseeing treatment who was not blinded.
6. The confidence interval for the pooled adjusted outcomes is wide.

IV. Meta-analyses for interventions to improve HCV treatment initiation

Coordinated care between mental health and treatment specialists with psychological therapy and counselling for patients with mental health and/or substance use comorbidities vs usual care for treatment initiation



Unadjusted results



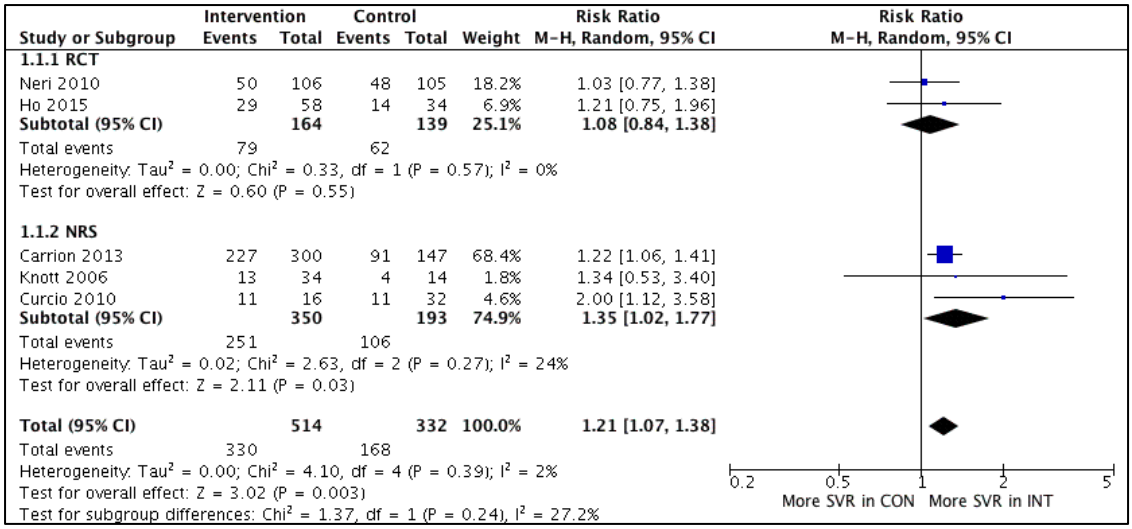
Adjusted results

Quality assessment							No. of patients		Effect		Quality
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Coordinated care with psychological therapy and counselling	Usual care	Relative (95% CI)	Absolute (95% CI)	
Treatment initiation											
3	Other design[1]	Serious[2]	Serious[3]	Not serious	Serious[4]	None	116/253 (45.8%)	94/263 (35.7%)	**RR 1.36** (0.94–1.97)	129 more per 1000 (from 21 fewer to 347 more)	⨁◯◯◯ Very low
								53.9%		194 more per 1000 (from 32 fewer to 522 more)	
Adjusted treatment initiation											
2	Other design[5]	Not serious	Serious[6]	Not serious	Serious[7]	None	–/252	–/295	**OR 3.03** (1.24–7.37)	0 fewer per 1000 (from 0 fewer to 0 fewer)	⨁◯◯◯ very low

1. Ho (2015) is a RCT, while Ahmed (2013) and Knott (2006) are NRS.
2. Unadjusted results from Ahmed (2013) and Knott (2006) were used in this meta-analysis that did not employ methods to adjust for confounding potentially introduced by their non-randomized study design.
3. I^2 = 77%. This high degree of heterogeneity may be due to differences between the populations under investigation. Ahmed (2013) included general HCV+ patients, while Ho (2015) and Knott (2006) only included patients with mental health and/or substance use comorbidities. Interventions also differed between studies. Ho (2015) provided additional case management, and Ahmed (2013) provided participants with therapeutic education and community support programmes in addition to coordinated care and psychological support.
4. The confidence interval is not wide. However, the OIS was calculated to be 741, and the pooled sample size did not meet this threshold.
5. Ho (2015) is a RCT, while Knott (2006) is a NRS.
6. I^2 = 74%. This high degree of heterogeneity may be due to differences between the interventions under investigation. Ho (2015) provided case management in addition to coordinated care and psychological support.
7. The confidence interval for pooled adjusted results is wide.

V. Meta-analyses for interventions to improve HCV-sustained virological response

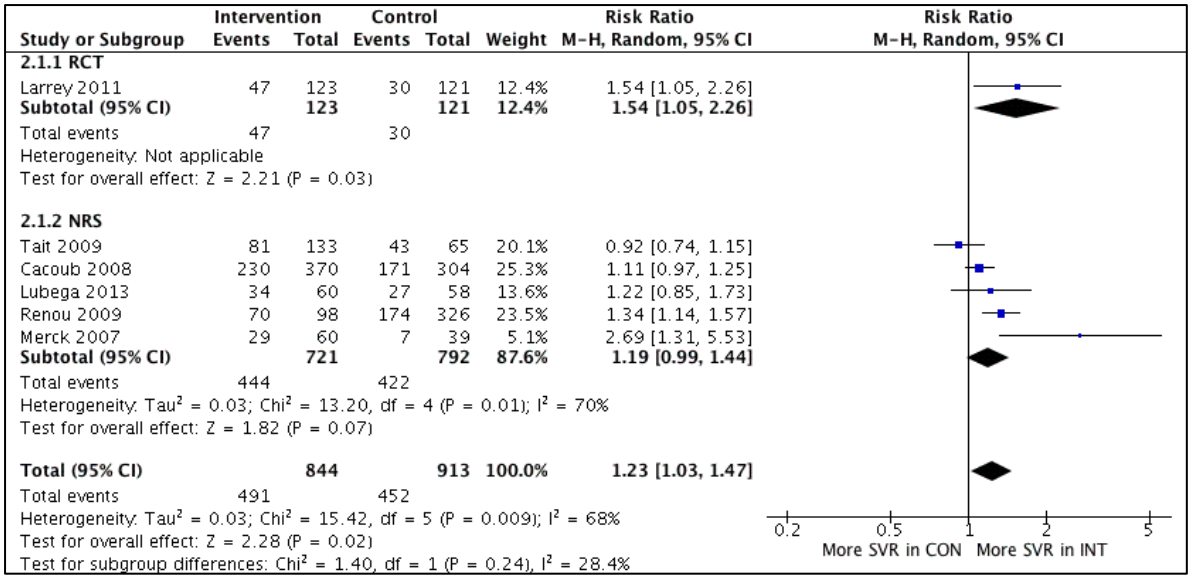
Coordinated care between mental health and treatment specialists with psychological therapy and counselling for patients with mental health and/or substance use comorbidities vs usual care for SVR



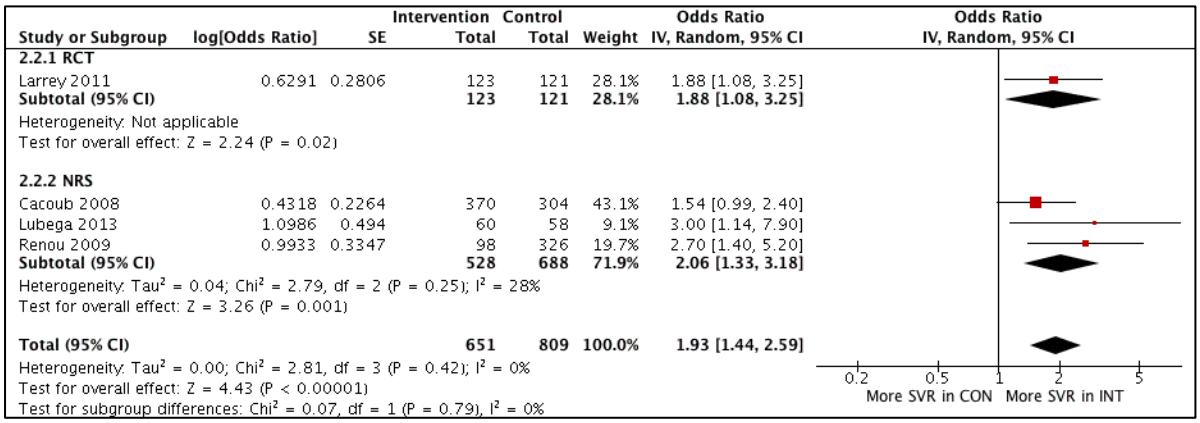
Quality assessment							No. of patients	Effect			Quality
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Coordinated care with psychological therapy and counselling	Usual care	Relative (95% CI)	Absolute (95% CI)	
SVR											
5	Other design	Serious[1]	Not serious	Not serious	Not serious[2]	None	330/514 (64.2%)	168/332 (50.6%)	**RR 1.21** (1.07–1.38)	106 more per 1000 (from 35 more to 192 more)	⨁◯◯◯ Very low
								41.2%		86 more per 1000 (from 29 more to 156 more)	

1. Curcio (2010) is at high risk of performance bias because of differences between the treatment received by the two cohorts besides the intervention under examination. Knott (2006) did not employ methods to adjust for confounding potentially introduced by its non-randomized study design.
2. The confidence interval is not wide. The OIS was calculated to be 434, and the pooled sample size exceeded the OIS.

Nurse-led therapeutic educational sessions with information on HCV infection, treatment, side-effects, and/or adherence vs no therapeutic education for SVR



Unadjusted results



Adjusted results

Quality assessment							No. of patients		Effect		Quality
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Therapeutic education	No specific education	Relative (95% CI)	Absolute (95% CI)	
SVR											
6	Other design[1]	Serious[2]	Serious[3]	Not serious	Not serious[4]	None	491/844 (58.2%)	452/913 (49.5%)	**RR 1.23** (1.03–1.47)	114 more per 1000 (from 15 more to 233 more)	⊕◯◯◯ Very low
								50.0%		115 more per 1000 (from 15 more to 235 more)	
Adjusted SVR											
4	Other design[5]	Not serious[6]	Not serious	Not serious	Not serious	None	–/651	–/809	**OR 1.93** (1.44– 2.59)	2 fewer per 1000 (from 1 fewer to 3 fewer)	⊕⊕◯◯ Low

1. Larrey (2011) is a RCT. Cacoub (2008), Lubega (2013), Renou (2009), Tait (2009), and Merck (2007) are NRS.
2. Tait (2009) and Merck (2007) did not employ methods to adjust for confounding potentially introduced by non-randomized study design. Tait (2009) and Cacoub (2008) are both at high risk of performance bias, and Merck (2007) is at high risk of attrition bias.

3. Tait (2009) reports a risk ratio that is <1.0 while all other included studies report a risk ratio >1.0. Additionally, I^2 = 68%.
4. The confidence interval is not wide. The OIS was calculated to be 1029, and the pooled sample size exceeded the OIS.
5. Larrey (2011) is a RCT. Cacoub (2008), Lubega (2013), and Renou (2009) are NRS.
6. Cacoub (2008) is at high risk of performance bias because there was no standardization of intervention procedures. However, this risk of bias is not sufficient to downgrade the quality of evidence.

4. **Discussion**

This systematic review and meta-analysis demonstrated that operational interventions can improve engagement, retention, or progression through the chronic viral hepatitis care continuum. Specifically, LHW HBV screening promotion activities increased HBV test uptake, while clinician reminders to prompt HCV screening during clinical visits increase HCV testing rates. Coordinated care between hepatitis and mental health specialists along with psychological therapy and counselling for patients with mental health and/or substance use comorbidities can increase HCV treatment initiation, improve treatment completion, and result in higher SVR rates.

Our review covered all 5 major steps along the continuum of care for HBV and HCV. Previous systematic reviews examining progression along the care continuum have either limited their analysis to specific components of the care continuum,[65] or limited analysis to either HBV or HCV.[66] Additionally, these reviews did not focus on studies that had comparison groups. We used GRADE methodology to rigorously evaluate the quality of reported evidence and our data substantially extend the 2012 NICE guidelines – Hepatitis B and C: ways to promote and offer testing to people at increased risk of infection.

Our results demonstrate that culturally appropriate lay health workers educational programs to promote HBV testing are effective. All six studies had consistently favorable results with relatively strong study designs and were graded as moderate quality evidence. Although all these interventions were conducted among Asian immigrant populations in the US, this intervention may be relevant in a range of other settings. Our findings are consistent with the growing body of evidence demonstrating that lay health workers can effectively perform a range of interventions that would otherwise be undertaken by trained medical personnel. The lay health workers in the six studies received training in order to help tailor the educational intervention and this training component was relatively simple and of low cost. Qualitative investigation supports these types of interventions as being feasible and acceptable to both those individuals screened and lay health workers themselves.[67] While the importance of cultural context in developing interventions to improve HBV screening in high-risk populations and the usefulness of community settings in the delivery of healthcare is important, the low-cost nature of this intervention could facilitate its used in resource limited settings. Task shifting to LHW are well documented as strengthening service delivery capacity in a variety of clinical settings in low- and middle-income countries.[68–71]

Our results show that clinician reminders to prompt HBV and HCV screening during clinical visits increased HCV testing rates. While of obvious use in electronic medical records, one included study used a clinical “risk screening” sticker placed on a print charts.[21] Clinician reminders are consistent with the broader shift towards standardizing clinical practice, including provider initiated screening and systems-based approaches to improving clinical outcomes. While this style of intervention does not operate through the lens of addressing patient barriers, implementation is

relatively easy and similar systems have demonstrated effectiveness in multiple disease modalities, such as breast[72] and colorectal cancer screening.[73]

We found that coordinating care between mental health and treatment specialists along with psychological therapy and counselling for patients with mental health and/or substance use comorbidities was effective in promoting HCV treatment initiation, treatment completion, and achieving SVR. Hepatitis C disproportionally affects individuals with comorbid mental health or substance use issues. Traditionally, services for hepatitis, mental health and addiction have been provided by separate clinicians or teams often located in different health facilities. This may contribute to HCV treatment dropout and/or treatment failure.[74] While the interventions addressing multidisciplinary or integrated care in this review were diverse, a likely key contributor to improved outcomes was co-location and coordination of services. Integrating HCV screening and treatment with mental health and addiction services is feasible and acceptable to the targeted clients.[75,76] Our funding also builds on the limited literature regarding integration of HIV and mental health services, which can also improve treatment outcomes.[77]

We identified substantial gaps in current knowledge examining progress along the chronic viral hepatitis continuum of care. Implementation science in viral hepatitis will become increasingly important as access to effective HBV and HCV medicines expands across the world. High quality evidence provides a strong basis for forming guidelines recommendations for program managers, clinicians and others working in the field. Most of our included studies were graded low or very low. The lack of studies with robust design in particular in HCV screening and HBV treatment are a significant gap. Our analysis found no studies investigating HBV treatment uptake, adherence, or viral suppression.

While we did not provide an economic analysis of the value of incorporating operational interventions in the viral hepatitis care continuum, mathematical modelling in HCV suggests that imperfect follow-up during the HCV care continuum greatly reduces the real-world effectiveness of HCV therapy.[78] It follows that interventions impacting on multiple steps along the care continuum are more resource efficient. Future research should focus quantifying the costs and effectiveness of elements or combinations of interventions to optimize treatment outcome.

There are several limitations to our review. First, outcomes that were studied were intermediate outcomes related to diagnosis and treatment, not disease end-points such as morbidity and mortality associated with HBV and HCV. However, it is well known that treatment of HBV or HCV infection reduces liver-related deaths, hepatocellular carcinoma incidence and all-cause mortality.[79,80] Second, almost all studies addressing treatment uptake, adherence, and viral suppression in HCV were carried out with interferon-based therapies. Current DAA-based regimens are simpler to administer, more effective, and better tolerated. In the era of DAAs, with near 100% efficacy, retention and progression along the care continuum is likely to become an important determinant of achieving SVR. Finally, all included studies were carried out in high-income settings, with the exception of one study from Turkey. More implementation science research is needed in low- and middle-income contexts where the majority of people living with chronic viral hepatitis live.

Our systematic review demonstrates that a range of relatively simple, inexpensive operational interventions can significantly improve engagement and retention along the chronic viral hepatitis care continuum. In addition, we identified the importance of integrated approaches to hepatitis screening, care and treatment for specific vulnerable populations. Further implementation

science research, robust in design and specifically carried out in low and middle-income settings, is needed to evaluate current gaps in our knowledge to improve engagement and retention for people living with HBV and HCV in the chronic viral hepatitis continuum of care.

1. Figures and tables

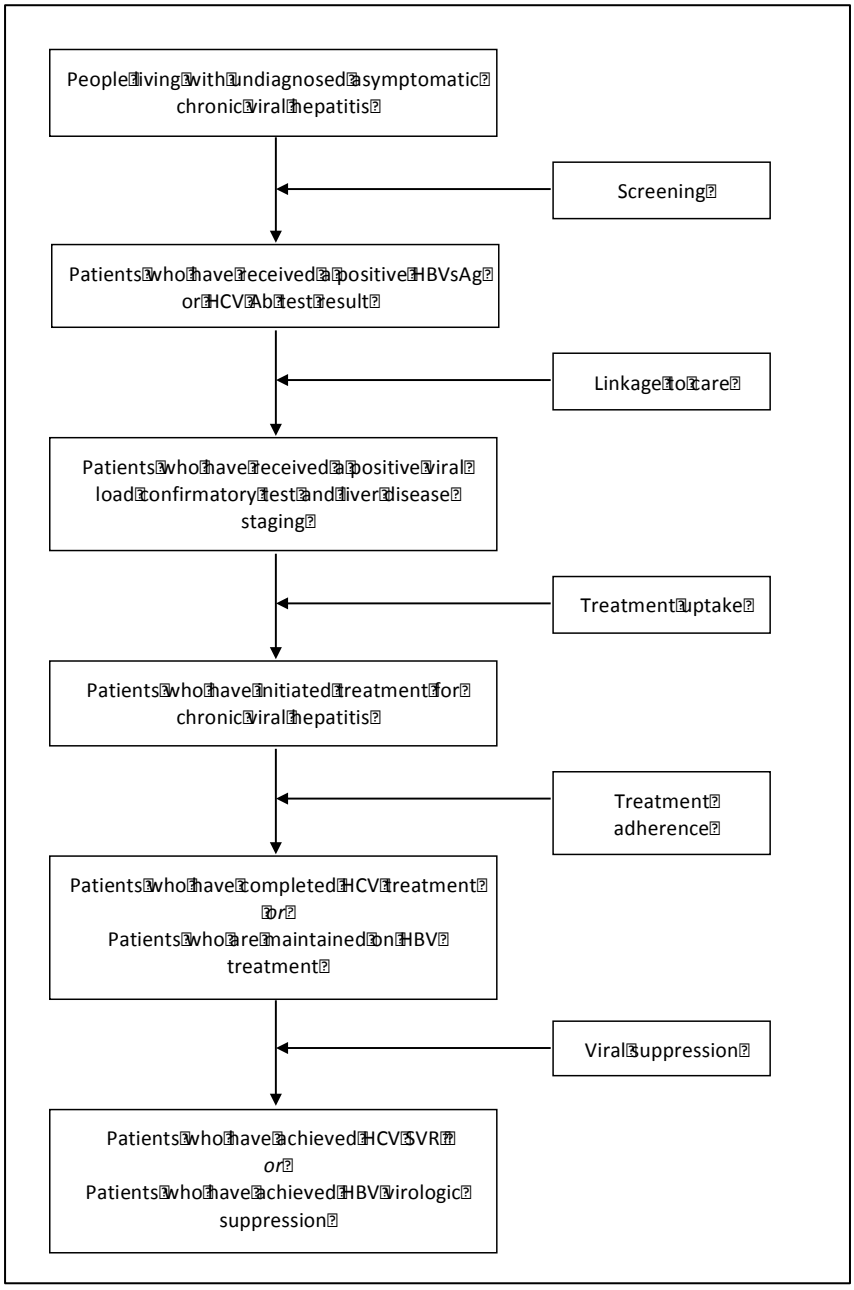


Fig. 1. Overview of the stages comprising the viral hepatitis treatment continuum, including testing, linkage to care, enrolment in care, treatment uptake, treatment adherence, and viral suppression.

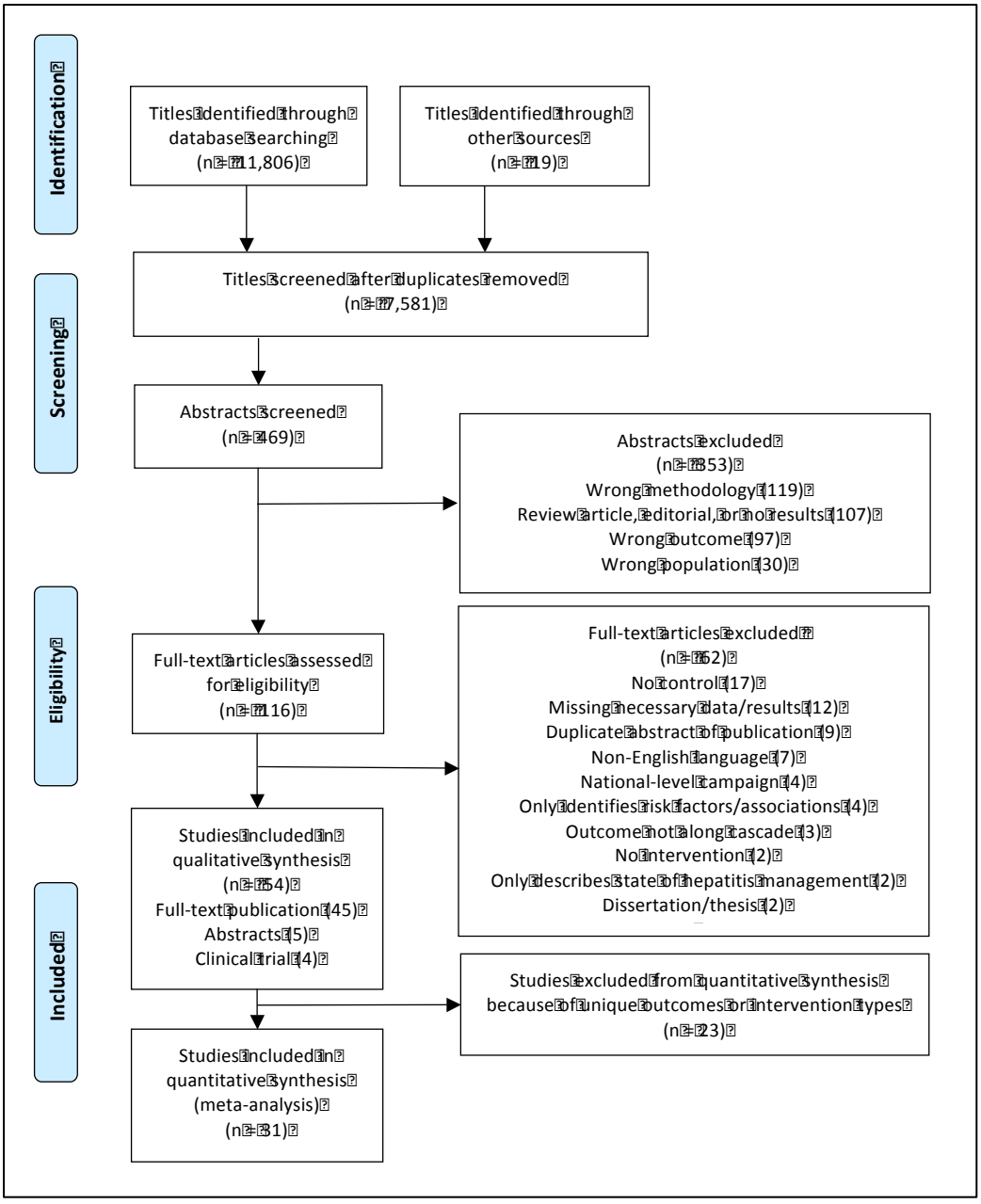


Fig. 2. PRISMA flow diagram outlining study selection for this systematic review of interventions to optimize retention across the chronic viral hepatitis continuum of care.

Author	Year	Publication type	Study design	HCV or HBV	Intervention type	Population	Location	Stages with reported outcomes	Sample size
Ahmed, I	2013	Full-text article	NRS	HCV	Coordinated care between mental health and treatment specialists with psychological therapy and counseling for patients with mental health and/or substance use comorbidities	Patients eligible for HCV treatment	United Kingdom	Treatment uptake Treatment adherence Treatment outcome	82
Arora, S.	2011	Full-text article	NRS	HCV	Training and support for primary care physicians	Patients on HCV treatment	United States	Treatment adherence Treatment outcome	407
Asthana, A	2012	Abstract	NRS	HBV	New facility testing protocol and provider education	Patients receiving chemotherapy	Australia	HBV screening	229
Bastani, R.	2015	Full-text article	Cluster RCT	HBV	Single culturally-tailored lay health worker educational session to improve HBV knowledge and promote testing	Korean Americans	United States	HBV screening	1123
Bonkovsky, H.	2008	Full-text article	RCT	HCV	Directly observed therapy	Patients on HCV treatment and enrolled in MMT	United States	Treatment adherence Treatment outcome	48
Bruce, RD	2012	Full-text article	RCT	HCV	Directly observed therapy	Patients on HCV treatment and enrolled in MMT	United States	Treatment uptake Treatment outcome	21
Cacoub, P	2008	Full-text article	NRS	HCV	Nurse-led therapeutic educational sessions	Patients on HCV treatment	France	Treatment adherence Treatment outcome	674
Carrion, JA	2013	Full-text article	NRS	HCV	Coordinated care between mental health and treatment specialists with psychological therapy and counseling for patients with mental health and/or substance use comorbidities	Patients on HCV treatment	Spain	Treatment adherence Treatment outcome	447
Chakrabarty, G	2013	Abstract	RCT	HBV	Bloodspot testing	Household or sexual contacts of patients with diagnosed chronic HBV	Not specified	HBV screening	79
Chen, MS	2013	Full-text article	RCT	HBV	Single culturally-tailored lay health worker educational session to improve HBV knowledge and promote testing	Hmong Americans	United States	HBV screening	260
Chen, WL	2014	Full-text article	RCT	HCV	Telecare therapeutic education and nursing support	Patients on HCV treatment	Taiwan	Treatment adherence Treatment outcome	298
Cioe, P	2013	Full-text article	NRS	HCV	Directly observed therapy	Patients on HCV treatment	United States	Treatment outcome	155
Craine, N	2014	Full-text article	Cluster RCT	HCV	Institutional adoption of bloodspot testing	Prisoners	United Kingdom	HCV screening	Not specified
Cullen, W	2006	Full-text article	Cluster RCT	HCV	Facilitated referral and scheduling to specialist visit by staff at site of established care with supplementary HCV education	Patients enrolled in MMT	Ireland	HCV screening HCV linkage to care Treatment uptake	196
Curcio, F	2010	Full-text article	NRS	HCV	Coordinated care between mental health and treatment specialists with psychological therapy and counseling for patients with mental health and/or substance use comorbidities	Patients on HCV treatment with substance use comorbidities	Italy	Treatment adherence Treatment outcome	48
Drainoni, M	2012	Full-text article	NRS	HCV	Clinician reminder to use screening algorithm with provider education	Patients attending urban clinics in Bronx, NY	United States	HCV Screening	8981
Evon, DM	2011	Full-text article	RCT	HCV	Psychological therapy and counseling for patients with mental health and/or substance use comorbidities without coordinated care	HCV+ patients with mental health and/or substance abuse comorbidities previously denied treatment	United States	HCV linkage to care Treatment uptake	101
Hagedorn, H	2007	Full-text article	NRS	Both	New testing protocol, patient education, counseling, and facilitated referral with provider education	Patients attending substance use disorder clinic	United States	HBV screening HCV screening HBV linkage to care HCV linkage to care	275
Helsper, C	2010	Full-text article	NRS	HCV	Regional education campaign for providers	Patients attending primary care practices	Netherlands	HCV screening	Not specified
Hickman, M	2008	Full-text article	Cluster RCT	HCV	Institutional adoption of bloodspot testing	Patients attending drug clinics or prisoners	United Kingdom	HCV screening	12350
Hirsch, A	2014	Full-text article	NRS	HCV	Expanded viral load confirmatory testing	Patients receiving care through the veteran's affairs system	United States	HCV linkage to care	3039
Ho, S	2015	Full-text article	RCT	HCV	Coordinated care between mental health and treatment specialists with psychological therapy and counseling for patients with mental health and/or substance use comorbidities and case management	HCV+ patients with mental health and/or substance abuse comorbidities	United States	Treatment uptake Treatment adherence Treatment outcome	363
Hsu, L	2013	Full-text article	RCT	HBV	Clinician reminder to use screening algorithm	Asian Americans	United States	HBV screening	175

Hussein, M	2010	Full-text article	NRS	HCV	Telecare therapeutic education and nursing support	Patients on HCV treatment	United States	Treatment adherence	9352
Juon, H	2014	Full-text article	Cluster RCT	HBV	Single culturally-tailored lay health worker educational session to improve HBV knowledge and promote testing	Asian Americans	United States	HBV screening	446
Knott, A	2006	Full-text article	NRS	HCV	Coordinated care between mental health and treatment specialists with psychological therapy and counseling for patients with mental health and/or substance use comorbidities	HCV+ patients with mental health and/or substance abuse comorbidities	United States	HCV linkage to care Treatment uptake Treatment adherence Treatment outcome	149
Koruk, I	2011	Full-text article	NRS	HBV	New testing protocol with provider education	Pregnant women	Turkey	HBV screening	36987
Krauskopf, K	2014	Abstract	Cluster RCT	HCV	Clinician reminder to use screening algorithm without provider education	Patients attending primary care clinic born between 1945 and 1965	United States	HCV screening	26697
Lan, CL	2012	Full-text article	NRS	HCV	Therapeutic and psychological educational sessions tailored to patients with substance use and/or mental health comorbidities	Patients on HCV treatment with alcohol dependence	France	Treatment outcome	146
Larrey, D	2011	Full-text article	RCT	HCV	Nurse-led therapeutic educational sessions	Patients on HCV treatment	France	Treatment adherence Treatment outcome	244
Lee, R	2010	Full-text article	NRS	HBV	New testing protocol with provider education	Patients on intravenous chemotherapy	Canada	HBV screening	285
Litwin, A	2012	Full-text article	NRS	HCV	Clinician reminder to use screening algorithm with provider education	Patients on HCV treatment	United States	HCV screening	25737
Lubega, S	2013	Full-text article	NRS	HCV	Nurse-led therapeutic educational sessions	Patients on HCV treatment	United States	Treatment uptake Treatment adherence Treatment outcome	118
Ma, G	2011	Full-text article	NRS	HBV	Targeted outreach with interactive education and counseling sessions by lay health workers and active CBO involvement	Korean Americans	United States	HBV screening	158
Masson, CL	2013	Full-text article	RCT	HCV	Facilitated referral and scheduling to specialist visit by staff at site of established care with motivational interviewing and case management	HCV+ patients enrolled in MMT	United States	HCV linkage to care	286
Matthews, H	2012	Abstract	NRS	HBV	Electronic patient referral system	Patients receiving antenatal care	United Kingdom	HBV linkage to Care	102
Merchant, R	2014	Full-text article	RCT	HCV	HCV educational sessions, pretest counseling, and on-site testing at healthcare facilities serving high-risk populations	Patients in emergency department with self-reported recent drug use	United States	HCV screening	395
Merck Sharp & Dohme Corp.	2007	Clinical trial	NRS	HCV	Nurse-led therapeutic educational sessions	Patients on HCV treatment	Poland	Treatment adherence Treatment outcome	99
Merck Sharp & Dohme Corp.	2009	Clinical trial	NRS	HCV	Psychological support	Patients on HCV treatment	Austria	Treatment adherence	601
Merck Sharp & Dohme Corp.	2009	Clinical trial	NRS	HCV	Psychological support	Patients on HCV treatment	France	Treatment adherence	568
Mostert, MC	2004	Full-text article	NRS	HBV	Patient educational letter and provider education	Patients with positive HBeAg result	Netherlands	HBV linkage to care	189
Neri, S	2010	Full-text article	RCT	HCV	Coordinated care between mental health and treatment specialists with psychological therapy and counseling for patients with mental health and/or substance use comorbidities	Patients on HCV treatment	Italy	Treatment adherence Treatment outcome	211
Ramsey, S.	2011	Full-text article	RCT	HCV	Therapeutic and psychological educational sessions tailored to patients with substance use and/or mental health comorbidities	Patients on HCV treatment and enrolled in MMT	United States	Treatment adherence	29
Reimer, J	2013	Full-text article	NRS	HCV	Therapeutic and psychological educational sessions tailored to patients with substance use and/or mental health comorbidities	Patients on HCV treatment and enrolled in OST	Germany	Treatment adherence Treatment outcome	189
Renou, C	2009	Abstract	NRS	HCV	Nurse-led therapeutic educational sessions	Patients on HCV treatment	France	Treatment adherence Treatment outcome	424
Rifai, MA	2006	Full-text article	NRS	HCV	Inpatient substance use treatment with HCV testing and therapeutic education, followed by psychological therapy and counseling for patients with mental health and/or substance use comorbidities throughout treatment	HCV+ patients with substance use comorbidities	United States	Treatment uptake Treatment outcome	158
Rosenberg, SD	2010	Full-text article	RCT	Both	HCV educational sessions, pretest counseling, and on-site testing at healthcare facilities serving high-risk populations	Patients with mental health and/or substance use comorbidities	United States	HBV screening HCV screening HCV linkage to care	236
Sahajian, F	2004	Full-text article	NRS	HCV	Regional education campaign for providers	Patients with HCV exposure risk factors	France	HCV screening	6104
Tait, JM	2009	Full-text article	NRS	HCV	Patient education, counseling, and facilitated referral, and therapeutic education	Patients with positive HCV serology result	United Kingdom	HCV linkage to care Treatment adherence Treatment outcome	1305

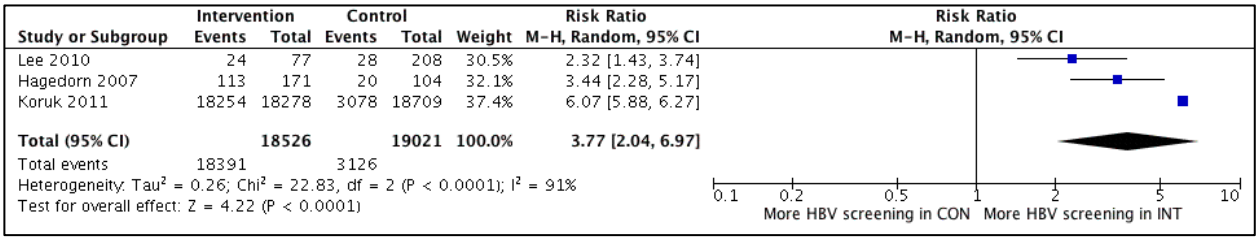
Taylor, V	2013	Full-text article	RCT	HBV	Single culturally-tailored lay health worker educational session to improve HBV knowledge and promote testing	Cambodian Americans	United States	HBV screening	250
Taylor, V	2011	Full-text article	Cluster RCT	HBV	Single culturally-tailored lay health worker educational session to improve HBV knowledge and promote testing	Asian Canadians	Canada	HBV screening	218
Taylor, V	2009	Full-text article	RCT	HBV	Single culturally-tailored lay health worker educational session to improve HBV knowledge and promote testing	Chinese Americans, Chinese Canadians	United States, Canada	HBV Screening	460
Teleen, Norman	2014	Clinical trial	NRS	HCV	Training and support for primary care physicians	Patients on HCV treatment	United States	Treatment adherence	197
Van Der Veen, Y	2013	Full-text article	RCT	HBV	Online educational website with culturally-tailored educational content	Turkish migrants in the Netherlands	Netherlands	HBV screening	1400

Table 1. Summary table of characteristics of included studies

5. Supplementary materials

1. 1. Additional comparisons, forest plots and GRADE tables

New institutional testing protocol for at-risk populations with education and testing promotion activities for providers vs. previous standard of care for HBV screening

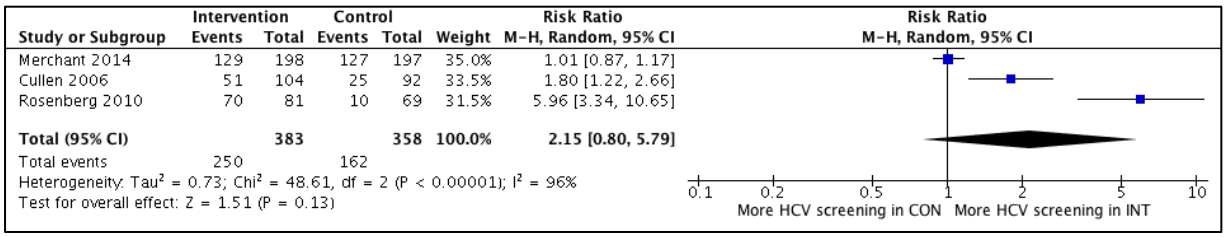


Quality assessment							No. of patients		Effect		Quality
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	New institutional testing protocol and provider education	Previous standard of care	Relative (95% CI)	Absolute (95% CI)	
HBV screening											
3	Before-after studies	Serious[1]	Serious[2]	Not serious	Serious[3]	None[4]	18391/18526 (99.3%)	3126/19021 (16.4%)	**RR 3.77** (2.04–6.97)	455 more per 1000 (from 171 more to 981 more)	⨁◯◯◯ Very low
								16.4%		456 more per 1000 (from 171 more to 982 more)	

1. 3/3 included studies did not employ methods to adjust for confounding potentially introduced by non-randomized study design.
2. All included studies report a risk ratio >1.0. However, I^2 = 91%. The high degree of heterogeneity may be due to the different at-risk populations under investigation between studies (pregnant women, patients starting chemotherapy, and patients with substance use comorbidities) or differences in the intensity of interventions between studies.

3. Although the pooled sample size exceeds the calculated OIS, the confidence interval is wide.
4. All included studies report a risk ratio >2.0. However, the pooled results have not been upgraded for large effect because the non-randomized design of the included studies introduces a significant possibility of confounding.

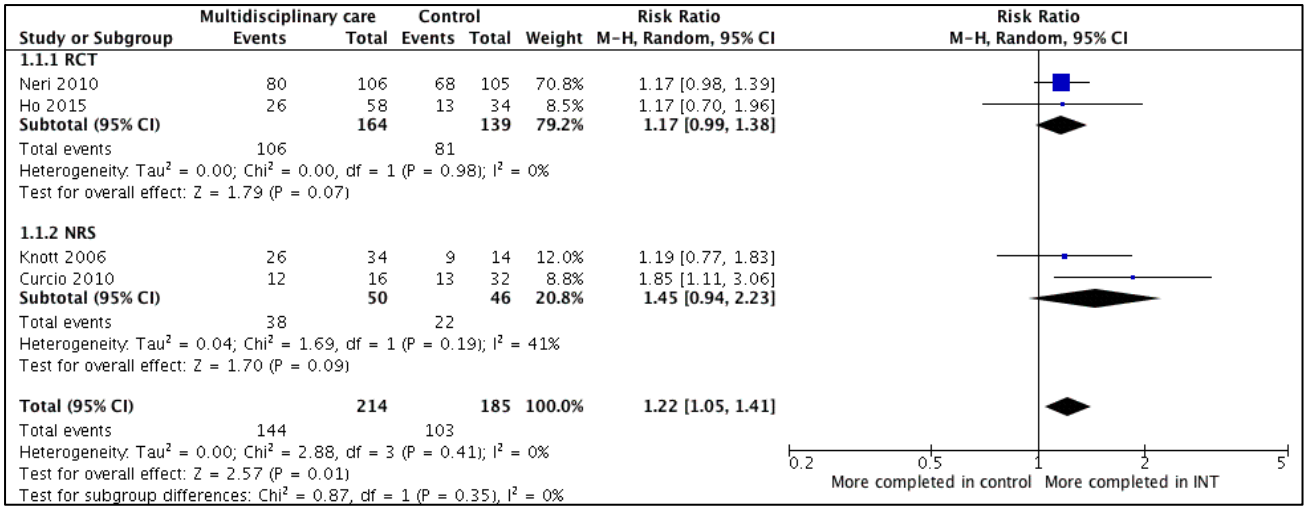
HCV educational sessions, pretest counselling, and on-site testing at healthcare facilities serving high-risk populations vs. no educational and counselling sessions for HCV screening



Quality assessment							No. of patients		Effect		Quality
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Facility-based education and counselling	No educational and counselling sessions	Relative (95% CI)	Absolute (95% CI)	
HCV screening											
3	Randomized trials	Not serious[1]	Serious[2]	Not serious	Serious[3]	None	250/383 (65.3%)	162/358 (45.3%) 27.2%	RR 2.15 (0.80–5.79)	520 more per 1000 (from 91 fewer to 1000 more) 312 more per 1000 (from 54 fewer to 1000 more)	⨁⨁◯◯ Low

1. Rosenberg (2010) is at high risk of detection bias because the control group outcome was self-reported. The introduced systematic bias would not necessarily exaggerate the reported effect size, so the quality of evidence was not downgraded.
2. One reported risk ratio is at 1.0 while two are > 1.0, and I^2 = 96%. The high degree of heterogeneity may be due to different study contexts. Cullen (2006) and Rosenberg (2010) examined interventions at facilities where patients had established care. Merchant (2014) examined an intervention at a hospital emergency department. Additionally, Merchant (2014) offered on-site testing to both intervention and control groups, while the other included studies did not.
3. Although the pooled sample size exceeds the calculated OIS of 193, the confidence interval is wide. Additionally, Cullen (2006) was a cluster RCT that did not account for clustering in its analysis. Consequently, this meta-analysis commits a unit-of-analysis error and produces over-precise results. No ICC was reported, so statistical methods could not be used to reduce the effective sample size of the cluster RCT.

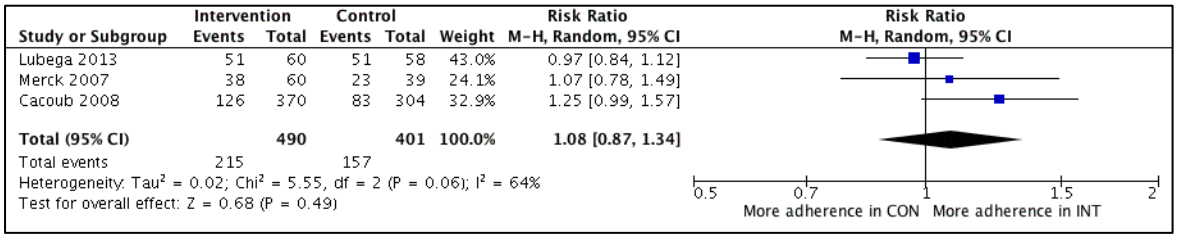
Coordinated care between mental health and treatment specialists with psychological therapy and counselling for patients with mental health and/or substance use comorbidities vs usual care for treatment completion



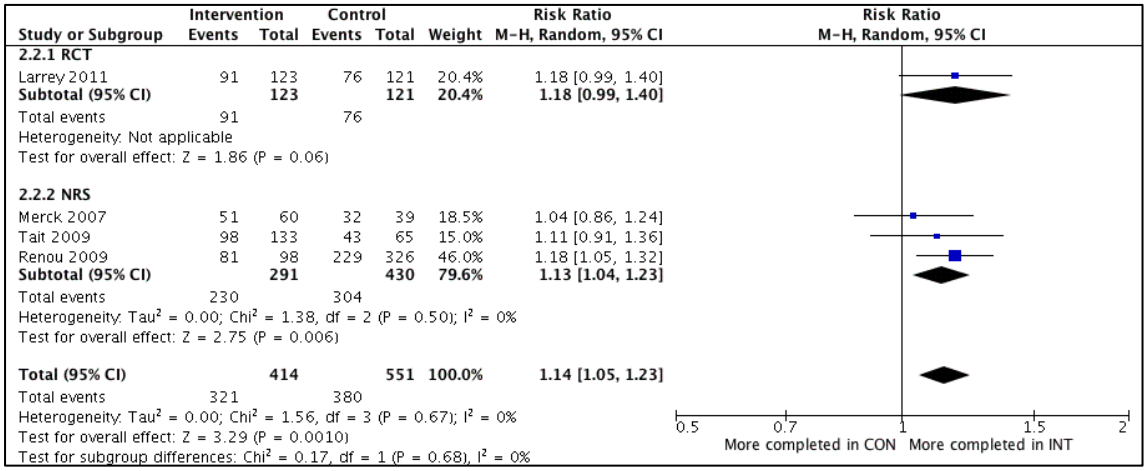
Quality assessment							No. of patients		Effect		Quality
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Coordinated care with psychological therapy and counselling	Usual care	Relative (95% CI)	Absolute (95% CI)	
Treatment completion											
4	Other design[1]	Serious[2]	Not serious	Not serious	Serious[3]	None	144/214 (67.3%)	103/185 (55.7%)	**RR 1.22** (1.05– 1.41)	122 more per 1000 (from 28 more to 228 more)	⊕◯◯◯ Very low
								52.5%		115 more per 1000 (from 26 more to 215 more)	

1. Neri (2010) and Ho (2015) are RCTs. Knott (2006) and Curcio (2010) are NRS.
2. Curcio (2010) was at high risk of performance bias because intervention and control treatment were delivered at significantly different institutions. Knott (2006) did not employ methods to adjust for confounding potentially introduced by its non-randomized study design.
3. The confidence interval is not wide. However, the OIS was calculated to be 553, and the pooled sample size did not meet this threshold.

Nurse-led therapeutic educational sessions with information on HCV infection, treatment, side effects, and/or adherence vs no therapeutic education for treatment adherence



Nurse-led therapeutic educational sessions with information on HCV infection, treatment, side-effects, and/or adherence vs no therapeutic education for treatment completion

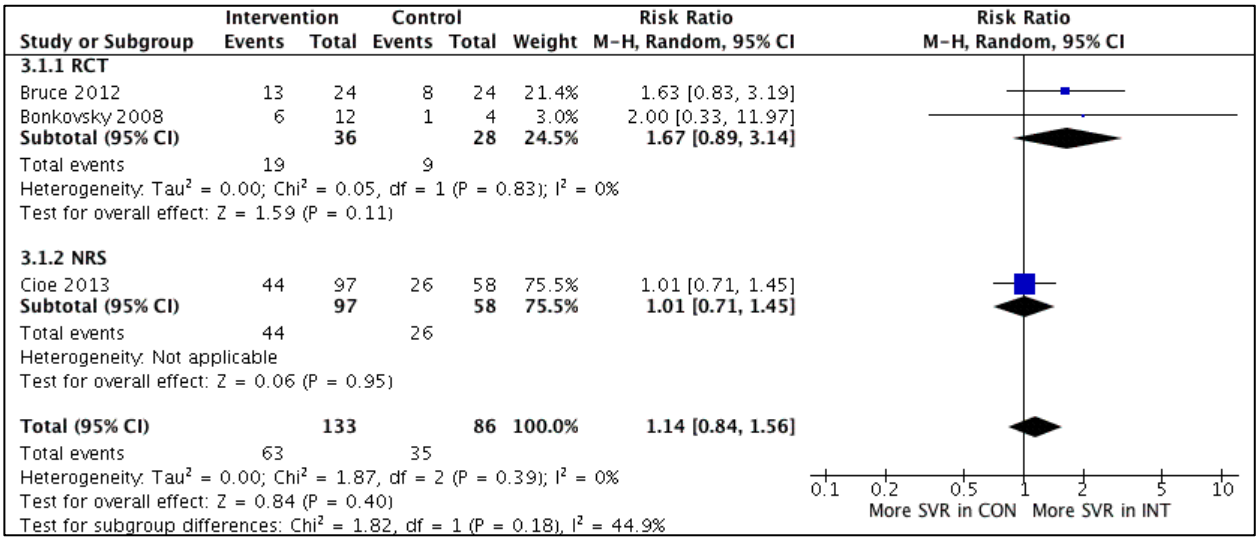


Quality assessment							No. of patients		Effect		Quality
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Therapeutic education	No specific education	Relative (95% CI)	Absolute (95% CI)	
Treatment adherence											
3	Cohort studies	Serious[1]	Serious[2]	Not serious	Serious[3]	None	215/490 (43.9%)	157/401 (39.2%) 59.0%	RR 1.08 0.87–1.34)	31 more per 1000 (from 51 fewer to 133 more) 47 more per 1000 (from 77 fewer to 200 more)	⨁◯◯◯ Very low
Treatment completion											
4	Other design[4]	Serious[5]	Not serious	Not serious	Not serious[6]	None	321/414 (77.5%)	380/551 (69.0%) 68.2%	**RR 1.14** (1.05 to 1.23)	97 more per 1000 (from 34 more to 159 more) 95 more per 1000 (from 34 more to 157 more)	⨁◯◯◯ Very low

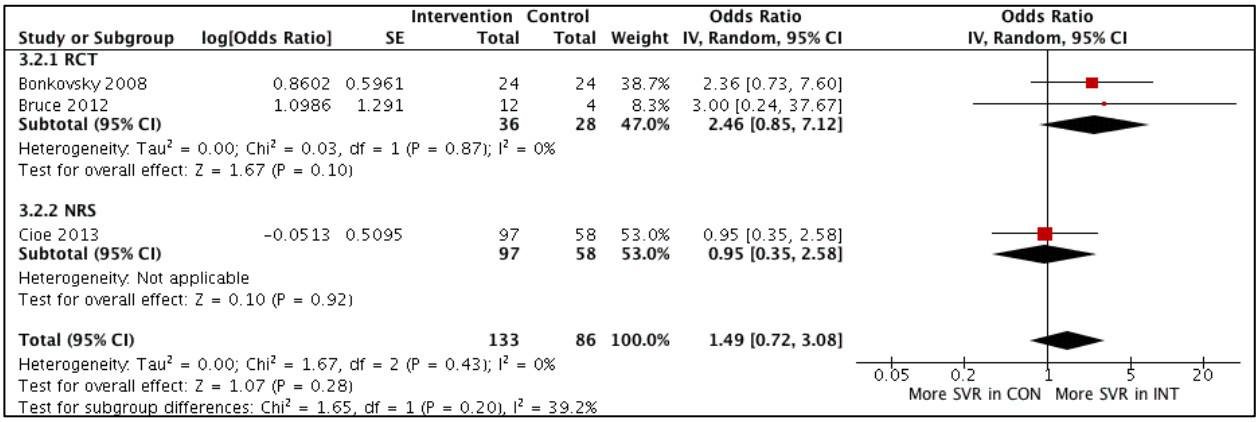
1. 3/3 studies did not employ methods to adjust for confounding potentially introduced by their non-randomized study design. Cacoub (2008) was also at high risk of performance bias because the intervention was not standardized, and at high risk of detection bias because the outcome was assessed through self-report.
2. Included studies report risk ratios on both sides of 1.0 and $I^2 = 64\%$.

3. The confidence interval is not wide. However, the OIS was calculated to be 3,481, and the pooled sample size did not meet this threshold.
4. Larrey (2011) is a RCT. Merck (2007), Tait (2009) and Renou (2009) are NRS.
5. Tait (2009) and Merck (2007) both did not employ methods to adjust for confounding potentially introduced by their non-randomized study design. Additionally, Tait (2009) was at high risk of performance bias due to study design.
6. The confidence interval is not wide. The OIS was calculated to be 861, and the pooled sample size exceeded the OIS.

Directly observed interferon therapy vs self-administered interferon therapy for SVR



Unadjusted results



Adjusted results

Quality assessment							No. of patients		Effect		Quality
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Directly observed therapy	Self-administered therapy	Relative (95% CI)	Absolute (95% CI)	
SVR											

Quality assessment							No. of patients		Effect		Quality
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Directly observed therapy	Self-administered therapy	Relative (95% CI)	Absolute (95% CI)	
3	Other design[1]	Serious[2]	Not serious	Not serious	Serious[3]	None	63/133 (47.4%)	35/86 (40.7%)	RR 1.14 (0.84–1.56)	57 more per 1000 (from 65 fewer to 228 more)	⨁◯◯◯ Very low
								33.3%		47 more per 1000 (from 53 fewer to 187 more)	
Adjusted SVR											
3	Other design[1]	Serious[4]	Not serious	Not serious	Serious[5]	None	–/133	–/86	OR 1.49 (0.72–3.08)	1 fewer per 1000 (from 1 fewer to 3 fewer)	⨁◯◯◯ Very low

1. Bruce (2012) and Bonkovsky (2008) are RCTs. Cioe (2013) is a NRS.
2. Bruce (2012) is at high risk of attrition bias and reporting bias. Bruce (2012) also had significant baseline differences between the intervention and control groups but methods were not used to adjust for potential confounding. Unadjusted results from Cioe (2013) were used in this meta-analysis that did not employ methods to adjust for confounding potentially introduced by its non-randomized study design.
3. The confidence interval is not wide. However, the OIS was calculated to be 1801, and the pooled sample size did not meet this threshold.
4. Bruce (2012) is at high risk of attrition bias and reporting bias. Bruce (2012) also had significant baseline differences between the intervention and control groups but methods were not used to adjust for potential confounding.
5. The confidence interval for the pooled adjusted results is wide.

Risk of bias tables

Risk of bias assessment for included studies with interventions for HBV screening

Author	Year	Outcome measure	Study design	Selection bias (random sequence)	Selection bias (allocation concealment)	Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias
Bastani	2015	HBV screening	Cluster RCT	Low risk	Low risk	Unclear risk	High risk	High risk	Unclear risk	Unclear risk
Juon	2014	HBV screening	Cluster RCT	Unclear risk	Unclear risk	Unclear risk	High risk	Unclear risk	Unclear risk	High risk
Taylor	2011	HBV screening	Cluster RCT	Unclear risk	Unclear risk	Low risk	High risk	High risk	Unclear risk	Unclear risk
Chakrabarty	2013	HBV screening	RCT	Unclear risk	Unclear risk	Unclear risk	High risk	High risk	Unclear risk	Unclear risk
Chen, MS	2013	HBV screening	RCT	Unclear risk	Unclear risk	Low risk	High risk	High risk	Low risk	Low risk
Hsu	2013	HBV screening	RCT	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	Unclear risk	Unclear risk
Rosenberg	2010	HBV screening	RCT	Unclear risk	Unclear risk	Low risk	High risk	Unclear risk	Low risk	Low risk
Taylor	2013	HBV screening	RCT	Low risk	Unclear risk	Low risk	High risk	High risk	Unclear risk	Low risk
Taylor	2009	HBV screening	RCT	Unclear risk	Unclear risk	Low risk	High risk	High risk	Unclear risk	Low risk
Van Der Veen	2013	HBV screening	RCT	Unclear risk	Unclear risk	Low risk	Low risk	Unclear risk	Unclear risk	Low risk
Asthana	2012	HBV screening	NRS	High risk	High risk	Unclear risk	Low risk	Unclear risk	Unclear risk	High risk
Hagedorn	2007	HBV screening	NRS	High risk	High risk	Unclear risk	Low risk	Unclear risk	Unclear risk	High risk
Koruk	2011	HBV screening	NRS	High risk	High risk	Unclear risk	Low risk	Unclear risk	Unclear risk	High risk
Lee	2010	HBV screening	NRS	High risk	High risk	Unclear risk	Low risk	Unclear risk	Unclear risk	High risk
Ma	2012	HBV screening	NRS	High risk	High risk	High risk	High risk	Unclear risk	Unclear risk	High risk

Risk of bias assessment for included studies with interventions for HCV screening

Author	Year	Outcome measure	Study design	Selection bias (random sequence)	Selection bias (allocation concealment)	Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias
Craine	2014	HCV screening	Cluster RCT	High risk	Unclear risk	Unclear risk	Low risk	High risk	Low risk	High risk
Cullen, W	2006	HCV screening	Cluster RCT	Low risk	Unclear risk	Low risk	Low risk	Low risk	Unclear risk	Unclear risk
Hickman	2008	HCV screening	Cluster RCT	Low risk	Unclear risk	Low risk	Low risk	Unclear risk	Unclear risk	High risk
Krauskopf	2014	HCV screening	Cluster RCT	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	High risk
Merchant	2014	HCV screening	RCT	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk
Rosenberg	2010	HCV screening	RCT	Unclear risk	Unclear risk	Low risk	High risk	Unclear risk	Low risk	Low risk
Drainoni	2012	HCV screening	NRS	High risk	High risk	High risk	Low risk	Low risk	Unclear risk	High risk
Hagedorn	2007	HCV screening	NRS	High risk	High risk	Unclear risk	Low risk	Unclear risk	Unclear risk	High risk
Helsper	2010	HCV screening	NRS	High risk	High risk	High risk	Low risk	Unclear risk	Unclear risk	High risk
Litwin	2012	HCV screening	NRS	High risk	High risk	Unclear risk	Low risk	Unclear risk	Unclear risk	Unclear risk
Sahajian	2004	HCV screening	NRS	High risk	High risk	High risk	Low risk	Unclear risk	Unclear risk	High risk

Risk of bias assessment for included studies with interventions for HBV linkage to care

Author	Year	Outcome measure	Study design	Selection bias (random sequence)	Selection bias (allocation concealment)	Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias
Matthews	2012	HBV linkage to care	NRS	High risk	High risk	Unclear risk	Low risk	Unclear risk	Unclear risk	High risk
Mostert	2004	HBV linkage to care	NRS	High risk	High risk	High risk	Low risk	Unclear risk	Unclear risk	High risk

Risk of bias assessment for included studies with interventions for HCV linkage to care

Author	Year	Outcome measure	Study design	Selection bias (random sequence)	Selection bias (allocation concealment)	Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias
Cullen, W	2006	HCV linkage to care	Cluster RCT	Low risk	Unclear risk	Low risk	Low risk	Low risk	Unclear risk	Unclear risk
Evon	2011	HCV linkage to care	RCT	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Masson	2013	HCV linkage to care	RCT	Low risk	Low risk	Low risk	Low risk	Unclear risk	Low risk	Low risk
Rosenberg	2010	HCV linkage to care	RCT	Unclear risk	Unclear risk	Low risk	High risk	Unclear risk	Low risk	Low risk
Hagedorn	2007	HCV linkage to care	NRS	High risk	High risk	Unclear risk	Low risk	Unclear risk	Unclear risk	High risk
Hirsch	2014	HCV linkage to care	NRS	High risk	High risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	High risk
Knott	2006	HCV linkage to care	NRS	High risk	High risk	Unclear risk	High risk	Low risk	Unclear risk	Unclear risk
Tait	2009	HCV linkage to care	NRS	High risk	High risk	High risk	Low risk	Unclear risk	Unclear risk	High risk

Risk of bias assessment for included studies with interventions for treatment uptake

Author	Year	Outcome measure	Study design	Selection bias (random sequence)	Selection bias (allocation concealment)	Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias
Cullen, W	2006	Treatment uptake	Cluster RCT	Low risk	Unclear risk	Low risk	Low risk	Low risk	Unclear risk	Unclear risk
Bruce	2012	Treatment uptake	RCT	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	High risk	Unclear risk
Evon	2011	Treatment uptake	RCT	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Ho	2015	Treatment uptake	RCT	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Ahmed	2013	Treatment uptake	NRS	High risk	High risk	Unclear risk	Low risk	Low risk	Unclear risk	High risk
Knott	2006	Treatment uptake	NRS	High risk	High risk	Unclear risk	Low risk	Low risk	Unclear risk	Unclear risk
Lubega	2013	Treatment uptake	NRS	High risk	High risk	Unclear risk	Low risk	Unclear risk	Unclear risk	Unclear risk
Rifai	2006	Treatment uptake	NRS	High risk	High risk	High risk	Low risk	Low risk	Unclear risk	Unclear risk

Risk of bias assessment for included studies with interventions for treatment adherence and completion

Author	Year	Outcome measure	Study design	Selection bias (random sequence)	Selection bias (allocation concealment)	Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias
Bonkovsky	2008	Adherence	RCT	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	Unclear risk	Low risk
Chen, WL	2014	Adherence	RCT	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	Unclear risk	Low risk
Ho	2015	Adherence	RCT	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Larrey	2011	Adherence	RCT	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Unclear risk	Low risk
Neri	2010	Adherence	RCT	Low risk	Unclear risk	Low risk	Unclear risk	Low risk	Unclear risk	Low risk
Ramsey	2011	Adherence	RCT	Low risk	Unclear risk	Low risk	Low risk	Unclear risk	Low risk	Unclear risk
Ahmed	2013	Adherence	NRS	High risk	High risk	High risk	Low risk	Low risk	Unclear risk	High risk
Arora	2011	Adherence	NRS	High risk	High risk	High risk	Low risk	Low risk	Unclear risk	High risk
Cacoub	2008	Adherence	NRS	High risk	High risk	High risk	High risk	Unclear risk	Unclear risk	Unclear risk
Carrion	2013	Adherence	NRS	High risk	High risk	Unclear risk	Unclear risk	Low risk	Low risk	High risk
Curcio	2010	Adherence	NRS	High risk	High risk	High risk	Low risk	Low risk	Unclear risk	Unclear risk
Hussein	2010	Adherence	NRS	High risk	High risk	High risk	Low risk	Unclear risk	Unclear risk	Unclear risk
Knott	2006	Adherence	NRS	High risk	High risk	Unclear risk	Low risk	Low risk	Unclear risk	High risk
Lubega	2013	Adherence	NRS	High risk	High risk	Unclear risk	Low risk	Low risk	Unclear risk	High risk
Merck	2007	Adherence	NRS	High risk	High risk	Unclear risk	Unclear risk	Low risk	Low risk	High risk
Merck (Austria)	2009	Adherence	NRS	High risk	High risk	Unclear risk	Unclear risk	Low risk	Low risk	High risk
Merck (France)	2009	Adherence	NRS	High risk	High risk	Unclear risk	Unclear risk	Low risk	Low risk	High risk
Reimer	2013	Adherence	NRS	High risk	High risk	High risk	Low risk	Low risk	Low risk	Unclear risk
Renou	2009	Adherence	NRS	High risk	High risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Tait	2009	Adherence	NRS	High risk	High risk	High risk	Low risk	Unclear risk	Unclear risk	High risk
Teleen	2014	Adherence	NRS	High risk	High risk	Unclear risk	Low risk	Low risk	Low risk	High risk

Risk of bias assessment for included studies with interventions for viral suppression

Author	Year	Outcome measure	Study design	Selection bias (random sequence)	Selection bias (allocation concealment)	Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias
Bonkovsky	2008	SVR	RCT	Unclear risk	Unclear risk	Low risk	Low risk	Unclear risk	Unclear risk	Low risk
Bruce	2012	SVR	RCT	Unclear risk	Unclear risk	Low risk	Low risk	High risk	High risk	Unclear risk
Chen, WL	2014	SVR	RCT	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	Unclear risk	Low risk
Ho	2015	SVR	RCT	Low risk	Low risk	Low risk	Low risk	Unclear risk	Low risk	Low risk
Larrey	2011	SVR	RCT	Unclear risk	Unclear risk	Unclear risk	Low risk	Unclear risk	Unclear risk	Low risk
Neri	2010	SVR	RCT	Low risk	Unclear risk	Low risk	Low risk	Unclear risk	Unclear risk	Low risk
Ahmed	2013	SVR	NRS	High risk	High risk	High risk	Low risk	Unclear risk	Unclear risk	High risk
Arora	2012	SVR	NRS	High risk	High risk	High risk	Low risk	Low risk	Unclear risk	Unclear risk
Cacoub	2008	SVR	NRS	High risk	High risk	High risk	Low risk	Low risk	Unclear risk	Unclear risk
Carrion	2013	SVR	NRS	High risk	High risk	Unclear risk	Low risk	Unclear risk	Low risk	Unclear risk
Cioe	2013	SVR	NRS	High risk	High risk	Unclear risk	Low risk	Low risk	Unclear risk	Unclear risk
Curcio	2010	SVR	NRS	High risk	High risk	High risk	Low risk	Unclear risk	Unclear risk	Unclear risk
Knott	2006	SVR	NRS	High risk	High risk	Unclear risk	Low risk	Unclear risk	Unclear risk	High risk
Lan	2012	SVR	NRS	High risk	High risk	Unclear risk	Low risk	Low risk	High risk	Unclear risk
Lubega	2013	SVR	NRS	High risk	High risk	Unclear risk	Low risk	Unclear risk	Unclear risk	Unclear risk
Merck	2007	SVR	NRS	High risk	High risk	Unclear risk	Low risk	High risk	Low risk	High risk
Reimer	2014	SVR	NRS	High risk	High risk	High risk	Low risk	Low risk	Low risk	Unclear risk
Renou	2009	SVR	NRS	High risk	High risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Rifai	2006	SVR	NRS	High risk	High risk	High risk	Low risk	Low risk	Unclear risk	Unclear risk
Tait	2009	SVR	NRS	High risk	High risk	High risk	Low risk	Unclear risk	Unclear risk	High risk

2. 3. Cochrane risk of bias tool rubric

Domain	Support for judgement	Review authors' judgement
Selection bias		
Random sequence generation	Describe the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups.	Selection bias (biased allocation to interventions) due to inadequate generation of a randomized sequence.
Allocation concealment	Describe the method used to conceal the allocation sequence in sufficient detail to determine whether intervention allocations could have been foreseen in advance of, or during, enrolment.	Selection bias (biased allocation to interventions) due to inadequate concealment of allocations prior to assignment
Performance bias		
Blinding of participants and	Describe all measures used, if any, to blind	Performance bias due to knowledge of

Domain	Support for judgement	Review authors' judgement
personnel: *Assessments should be made for each main outcome (or class of outcomes)*	study participants and personnel from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective.	the allocated interventions by participants and personnel during the study
Detection bias		
Blinding of outcome assessment: *Assessments should be made for each main outcome (or class of outcomes)*	Describe all measures used, if any, to blind outcome assessors from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective.	Detection bias due to knowledge of the allocated interventions by outcome assessors
Attrition bias		
Incomplete outcome data: *Assessments should be made for each main outcome (or class of outcomes)*	Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. State whether attrition and exclusions were reported, the numbers in each intervention group (compared with total randomized participants), reasons for attrition/exclusions where reported, and any re-inclusions in analyses performed by the review authors.	Attrition bias due to amount, nature or handling of incomplete outcome data
Reporting bias		
Selective reporting	State how the possibility of selective outcome reporting was examined by the review authors and what was found.	Reporting bias due to selective outcome reporting
Other bias		
Other sources of bias	State any important concerns about bias not addressed in the other domains in the tool. If particular questions/entries were pre-specified in the review's protocol, responses should be provided for each question/entry.	Bias due to problems not covered elsewhere in the table

PICO table

PICO	
P	Individuals living with chronic hepatitis B or C (diagnosed or undiagnosed) or providers caring for these patients.
I	Operational interventions delivered in conjunction with screening, care, or treatment of hepatitis
C	Standard of care or no intervention
O	Retention and progression along the continuum of care

-

Search strategy

Potential search terms:

Population:

Hepatitis B OR HBV OR CHB

Hepatitis C OR HCV OR CHC

Chronic viral hepatitis

Intervention:

Intervention

Counselling

Education or educate

Teach

Training

Program

Engagement

Alcohol and reduce, reduction, cessation

Outcome:

Screen OR screened OR screening

Test OR tested OR testing

Linking OR linkage

Refer OR referral

Uptake

Retain OR retained OR retention

Adherence OR adhere

Compliance OR comply

PubMed search strategy:

(Hepatitis B OR HBV OR CHB[tiab] OR Hepatitis C OR HCV OR CHC[tiab] OR chronic viral hepatitis[tiab] OR chronic viral hepatitis[mh]) AND (Intervention[tiab] OR counselling[tiab] OR education[tiab] OR educate[tiab] OR teach[tiab] OR training[tiab] OR program[tiab] OR Engagement[tiab] OR (alcohol[tiab] AND (reduce OR reduction OR cessation OR decrease)) AND (Uptake[tiab] OR Adherence[tiab] OR adhere[tiab] OR Compliance[tiab] OR comply[tiab] OR retain[tiab] OR retained[tiab] OR Retention[tiab] OR Screen[tiab] OR screened[tiab] OR screening[tiab] OR test[tiab] OR tested[tiab] OR testing[tiab] OR Linkage[tiab] OR linking[tiab] OR refer[tiab] OR Referral[tiab])

Additional information on study selection, inclusion criteria and exclusion criteria

Authors of included abstracts were contacted to determine whether the same data had been later published as a full-text article in a peer-reviewed journal, in which case the abstract would be excluded and full-text article included.

Study selection proceeded in three stages. First, two reviewers screened titles obtained from the initial search strategy according to standard inclusion and exclusion criteria. Second, abstracts for all titles identified for further review were assessed independently by two reviewers for inclusion. If there was disagreement, a third reviewer determined final inclusion. Finally, full texts for all abstracts identified for further review were assessed independently by two reviewers for inclusion. If there was disagreement, a third reviewer determined final inclusion.

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ANNEX 6. Predictive modelling analysis

6.1. Testing strategies for hepatitis B and C infection - predictive modelling

6.2. Decision analysis of strategies to identify hepatitis C – predictive modelling

Diagnostic accuracy of a one or two serological test strategy

Effect of prevalence and sensitivity of assay used on outcomes using a single serological testing strategy for HBV infection.

Table 7.1 Scenario 1 Population 1000; **Assay A: Sensitivity 98%, Specificity 99%**

Prevalence	True positive	False negative	False positives	True negatives	Total tests	PPV	NPV	Ratio of TP:FP
10.0%	98	2	9	891	1000	91.59%	99.78%	10.89
2.0%	20	0	20	970	1000	66.67%	99.96%	2.00
0.4%	4	0	20	986	1000	28.24%	99.99%	0.39

Scenario 2 Population 1000; **Assay A: Sensitivity 90%, Specificity 99%**

Prevalence	True Positive	False negative	False positives	True negatives	Total tests	PPV	NPV	Ratio of TP:FP
10.0%	90	10	9	891	1000	90.91%	98.89%	10.00
2.0%	18	2	10	970	1000	64.75%	99.79%	1.84
0.4%	4	0	10	986	1000	26.55%	99.96%	0.36

Scenario 3 Population 1000; **Assay A: Sensitivity 98%, Specificity 98%**

Prevalence	True Positive	False negative	False positives	True negatives	Total tests	PPV	NPV	Ratio of TP:FP
10.0%	98	2	18	882	1000	84.48%	99.77%	5.44
2.0%	20	0	20	960	1000	50.00%	99.96%	1.00
0.4%	4	0	20	976	1000	16.44%	99.99%	0.20

Scenario 4 Population 1000; **Assay A: Sensitivity 80%, Specificity 99%**

Prevalence	True Positive	False negative	False positives	True negatives	Total tests	PPV	NPV	Ratio of TP:FP
10.0%	80	20	9	891	1000	89.89%	97.80%	8.89
2.0%	16	4	10	970	1000	62.02%	99.59%	1.63
0.4%	3	1	10	986	1000	24.32%	99.92%	0.32

Effect of prevalence and sensitivity of assay used on outcomes using a two serological testing strategy for HBV infection.

Table 7.2.Scenario 5 Population 1000; **Assay A: Sensitivity 90.0%, Specificity 99%; Assay B: Sensitivity 90.00%, Specificity 99%**

Prevalence	True Positive	False negative	False positive	True negative	Total tests (A+B)	PPV	NPV	Ratio of TP:FP	Observed sensitivity	Observed specificity
10.0%	81	19	0	900	1099	99.89%	97.93%	900.00	81.00%	99.99%
2.0%	16	4	0	980	1028	99.40%	99.61%	165.31	81.00%	99.99%
0.4%	3	1	0	996	1014	97.02%	99.92%	32.53	81.00%	99.99%

Scenario 6 Population 1000; **Assay A: Sensitivity 98.0%, Specificity 99%; Assay B: Sensitivity 98.00%, Specificity 99%**

Prevalence	True Positive	False negative	False positive	True negative	Total tests (A+B)	PPV	NPV	Ratio of TP:FP	Observed sensitivity	Observed specificity
10.0%	96	4	0	900	1107	99.91%	99.56%	1067.11	96.04%	99.99%
2.0%	19	1	0	980	1029	99.49%	99.92%	196.00	96.04%	99.99%
0.4%	4	0	0	996	1014	97.47%	99.98%	38.57	96.04%	99.99%

Condition: Assay B was performed on all individuals reactive in assay A. Assay B performance is considered independent of assay A.
Outcome of two test strategy: when assay A+ and B+ = positive; assay A- or A+ B- = negative

Effect of prevalence and sensitivity of assay used on outcomes using a single serological testing strategy for HCV infection.

Table 8.2. Scenario 1 Population 1000; **Assay A: Sensitivity 99%, Specificity 99%**

Prevalence	True Positive	False negative	False positives	True negatives	Total tests	PPV	NPV	Ratio of TP:FP
40.0%	396	4	6	594	1000	98.51%	99.33%	66.00
10.0%	99	1	9	891	1000	91.67%	99.89%	11.00
2.0%	20	0	10	970	1000	66.89%	99.98%	2.02
0.4%	4	0	10	986	1000	28.45%	100.00%	0.40

Scenario 2 Population 1000; **Assay A: Sensitivity 98%, Specificity 99%**

Prevalence	True Positive	False negative	False positives	True negatives	Total tests	PPV	NPV	Ratio of TP:FP
40.0%	392	8	6	594	1000	98.49%	98.67%	65.33
10.0%	98	2	9	891	1000	91.59%	99.78%	10.89
2.0%	20	0	10	970	1000	66.67%	99.96%	2.00
0.4%	4	0	10	986	1000	28.24%	99.99%	0.39

Scenario 3 Population 1000; **Assay A: Sensitivity 99.5%, Specificity 98.0%**

Prevalence	True Positive	False negative	False positives	True negatives	Total tests	PPV	NPV	Ratio of TP:FP
40.0%	396	4	12	588	1000	97.06%	99.32%	33.00
10.0%	99	1	18	882	1000	84.62%	99.89%	5.50
2.0%	20	0	20	960	1000	50.25%	99.98%	1.01
0.4%	4	0	20	976	1000	16.58%	100.00%	0.20

Effect of prevalence and sensitivity of assay used on outcomes using a two serological testing strategy for HCV infection.

Table 8.3. Scenario A Population 1000**; Assay A: Sensitivity 98%, Specificity 99%; Assay B Sensitivity 98%, Specificity 98%**

Prevalence	True Positive	False negative	False positive	True negative	Total tests (A+B)	PPV	NPV	Ratio of TP:FP	Observed sensitivity	Observed specificity
40.0%	384	16	0	600	1398	99.97%	97.43%	3201.33	96.04%	99.98%
10.0%	96	4	0	900	1107	99.81%	99.56%	533.56	96.04%	99.98%
2.0%	19	1	0	980	1029	98.99%	99.92%	98.00	96.04%	99.98%
0.4%	4	0	0	996	1014	95.07%	99.98%	19.29	96.04%	99.98%

Condition: Assay B was performed on all individuals reactive in assay A. Assay B performance is considered independent of assay A.
Outcome of two test strategy: when assay A+ and B+ = positive; assay A- or A+ B- = negative

Annex 6.1

Testing strategies for hepatitis B and C infection

Predictive Modelling

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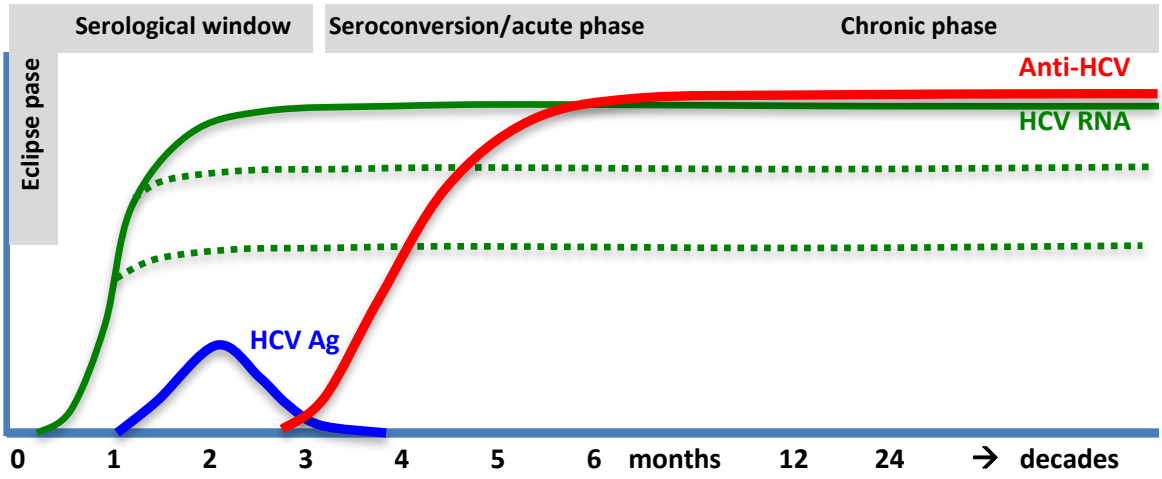
Hepatitis C

1. Diagnostic background to HCV infection

Unlike HIV, infection with HCV does not inevitably lead to long-term chronic infection, the body spontaneously clearing infection in a substantial minority of cases. Estimates of clearance vary, but appear to occur in 15–25% of HCV infections. The majority of spontaneous clearances appear to arise in the first few months of infection. Clearance of infection, whether spontaneous or as a result of antiviral therapy, does not appear to signify protection from reinfection.

The exact time course of virological and immunological markers of infection in HCV, particularly during the first months of infection, has not been easy to define because of the difficulties of pinpointing the time of exposure in community cases and of obtaining suitable specimens at frequent intervals. Nonetheless, several studies on plasma/blood donors, recipients and exposed health-care workers have provided estimates that should be sufficient to inform testing strategies. As illustrated below (Fig. 1), following an initial "eclipse phase" of 1–2 weeks when no virological or serological markers of infection may be detected, there is typically a substantial viræmia that is present in the absence of an anti-HCV antibody response for a further 6–10 weeks. During this "serological window" it has been shown that free (i.e. not complexed with anti-HCV antibody) core antigen of HCV (HCV Ag) can be detected in a proportion of cases employing combined anti-HCV/HCV Ag immunoassays.

Fig. 1. Approximate time course for HCV virological and serological markers in chronic HCV infection.

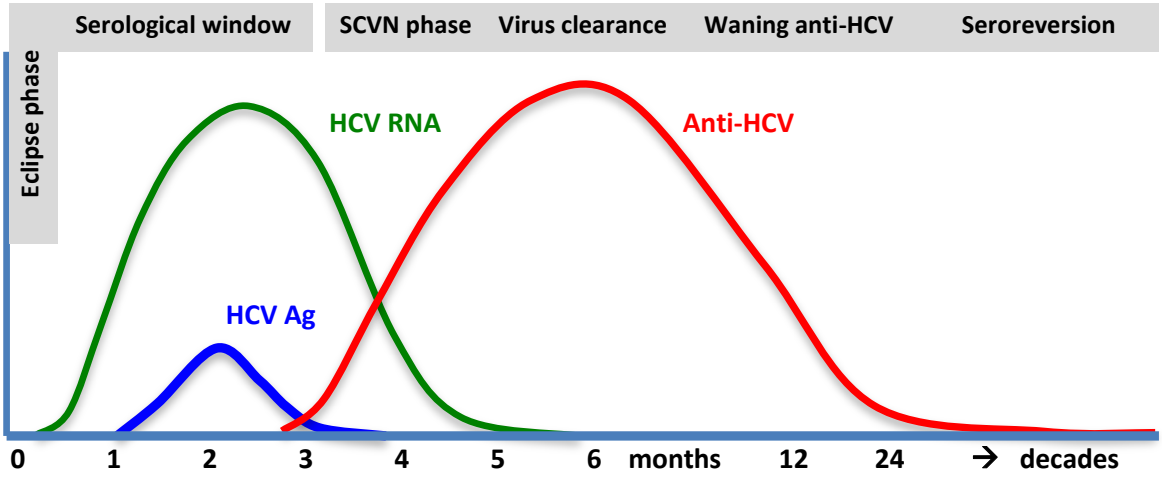


Following the development of the anti-HCV response, HCV Ag becomes complexed with anti-HCV antibody such that its presence cannot reliably be detected without a dissociation step to release HCV Ag from immune complexes prior to an immunoassay for HCV Ag. A majority of HCV infections become established as long-term chronic infections, persisting for decades, with an increased risk of

severe liver disease. Such individuals also present a source of infection to others. There are rare reports of persistent "occult" HCV infection, i.e. HCV RNA positive/anti-HCV negative.

In a substantial minority of cases, usually considered to be 15–25%, HCV fails to establish as a long-term chronic infection and the individual spontaneously clears infection. It is thought that this mostly happens during the first 6 months of infection; active infection persisting beyond that time usually associated with long-term carriage. Individuals who clear the infection usually also exhibit slowly waning anti-HCV antibody levels, presumably due to the removal of the antigenic stimulus to the immune system (Fig. 2). In turn, this leads to low-level anti-HCV antibody levels that may not be consistently detected by all anti-HCV test devices. It is likely that such low-level reactivities are in the main associated with waning anti-HCV levels following virus clearance rather than with a relatively brief period during the early stages of anti-HCV seroconversion.

Fig. 2. Hypothetical time course for HCV virological and serological markers in self-resolving HCV infection. Timings are ill-defined and may vary widely.



These two likely outcomes in untreated individuals complicate the matter of identifying those who would benefit from HCV-specific care, including antiviral therapy. Unlike HIV, a simple and inexpensive anti-HCV antibody test will not alone identify those with active infection. Furthermore, there is at present no simple and immediate prognostic test to differentiate between those who are in the first months of infection, a substantial minority of whom will eradicate their infection without therapeutic intervention, and those who are already in an established chronic state of HCV infection. There is evidence that, as with HIV, the avidity of anti-HCV antibody in the first months after seroconversion is low, and thus those with high avidity antibody would be more predictive of chronic infection. However, there is currently no commercial anti-HCV avidity method available.

2. Aim of testing

The preferred testing strategy will depend on the aims of the testing programme as well as factors such as assay performance, technical feasibility, access and cost.

1. **Identify all anti-HCV positive individuals**

   This scenario employs the most widely available and least expensive diagnostics. It would not, however, detect recent infections in the period prior to anti-HCV seroconversion. If the ultimate aim is to offer antiviral treatment to those with an active HCV infection, anti-HCV positive individuals, or at least a blood specimen, would then have to be referred to a facility able to carry out the necessary procedures to determine whether HCV treatment was needed, and then to monitor its success.

   Strategy: Anti-HCV test (RDT or EIA) with or without a different anti-HCV test

2. **Identify all active HCV infections**

   This approach would ascertain whether individuals are carrying replicating HCV. The current cost and complexity of tests for HCV RNA would probably preclude their use as a screening tool in all but the highest prevalences. Consequently, the primary screening test would likely be an anti-HCV test, which would fail to detect acute infections in the seroconversion window phase. This would be partially offset by the use of a combined anti-HCV/HCV Ag assay. Identification of active HCV infection would require the application of a test for HCV RNA, though an immunoassay that is able to detect immune-complexed HCV Ag may be sufficiently accurate. Early infections, a minority of which would have gone on to be spontaneously eradicated by the host would also be treated under this approach.

   Strategy: Anti-HCV test (RDT or EIA) or combined anti-HCV + HCV Ag test followed by an HCV RNA test or an HCV Ag test

3. **Identify chronic HCV infections**

   This approach would need to go a further step to aim 2, by incorporating the means to distinguish chronic from acute infection. Such a test would permit immediate treatment of those identified as having a chronic infection and deferral of those with a recent acute HCV infection. At present, though, there is no appropriate and validated laboratory marker to distinguish chronic from acute HCV infection. While a combination of clinical and non-virological laboratory tests might identify those in need of immediate treatment, others may have to be deferred for 3–6 months to allow a re-test to exclude the minority whose infection cleared spontaneously. Dependent on the costs of antiviral drugs, the risks of unnecessary delay to treatment in those that would benefit from it and of loss to follow up would probably exclude this approach at this time.

3.HCV infections in a community

Many factors will influence the status of HCV infection in any given community, including prevalence, incidence, risk behaviours, harm reduction interventions, access to health care, and specific HCV diagnosis and treatment. If anti-HCV prevalence is known and incidence has been estimated it may be reasonable to make crude estimates of the numbers of cases at the several stages of HCV infection. Taking a reasonably straightforward example of a concentrated epidemic, such as has occurred widely in persons who inject drugs (PWID), it is commonplace to find an ongoing outbreak with anti-HCV prevalence in the region of 40–60% and an annual incidence of approximately 10%. Applying this to a population of PWID of 100 000 one can deduce the following approximations:

Anti-HCV positive (45%)	= N × P	= 100k × 0.45	=	**45 000**
Acute HCV (RNA/Ag only)	= (N – (N × P)) × (W × I)	= (100k – 45k) × (0.2 × 0.1)	=	**1 100**
Eclipse cases (no marker)	= (N – (N x P)) × (E × I)	= (100k – 45k) × (0.04 × 0.1)	=	**220**
Seropos HCV (Ab + RNA/Ag)	= (N × P × (1 – C))	= (100k × 0.45 × (1 – 0.2))	=	**36 000**
Chronic HCV (Ab + RNA + >6 m)	= (N × P × (1 – C)) – (N × P × C) – (N × P × I × 0.5)			
		= 36k – 9k – 2.25k	=	**24 750**

where: N = population; P = anti-HCV prevalence; W = seroconversion window (estimated 10 weeks); E = eclipse phase (estimated 2 weeks); I = annual HCV incidence; C = proportion of resolved HCV infection

In the above model population, there are at least 46 320 individuals who have been infected with HCV. This does not include a further group with resolved infection who will have seroreverted to an anti-HCV negative status. Employing the above estimates, one can then approximate the effectiveness of different strategies to detect HCV infection.

Strategies and algorithms

It was recognized soon after HIV diagnostics became available in the mid-1980s that the application of a combination of HIV tests would need to be employed to minimize the risk of inaccurate results. At that time, the diagnosis was almost entirely based on the detection of anti-HIV antibodies, and false-positive reactions were common. Within several years a very wide range of anti-HIV diagnostics became available, often employing a range of constructions (e.g. indirect, competitive, immunometric) and different sources of HIV antigens (e.g. viral, peptides, recombinants). While many countries adopted an approach of EIA screen followed by an immunoblot EIA (western blot) or indirect immunofluorescence, which were both expensive and imperfect, others recognized that a simpler and more economic approach employing a carefully selected combination of screening tests could achieve equivalent or better accuracy. This led to the development of WHO guidance that outlined several testing strategies. Each strategy was based on the prevalence of HIV in the community being tested and the expected accuracy of the individual tests employed. The strategies were generic, not specifying individual diagnostic products or types, but providing a decision flow chart and guidance about how to select a combination of diagnostic devices to establish and validate a local algorithm employing specific diagnostic tests.

4.Strategies under consideration to identify individuals infected with HCV

Aim 1: Identify anti-HCV positive individuals

The two strategies employed to generate the outcomes in the following tables are as follows:

Fig. 3: Single anti-HCV test

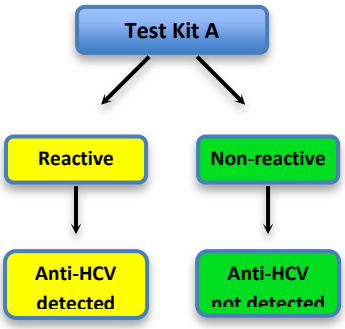
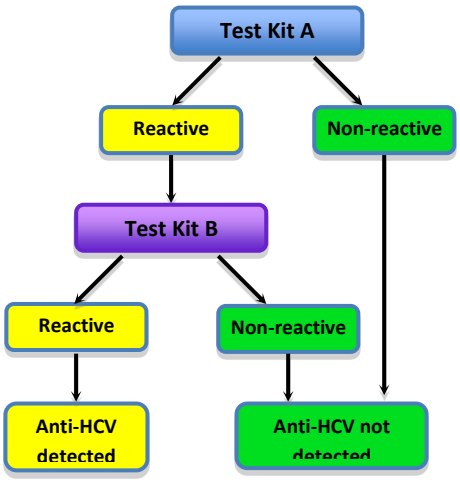


Fig. 4. Two independent anti-HCV tests in sequence



Employing the simple **Single-test strategy** for the detection of anti-HCV antibodies, Table 1 demonstrates hypothetical outcomes based on a range of typical performance characteristics for anti-HCV tests employed by trained individuals within an appropriate quality system. The estimates have been prepared for a range of prevalences, reflecting a concentrated epidemic such as is found among PWIDs (45%) through to a low endemicity setting among a general population, say of northern Europe (0.4%).

Table 1. Outcomes for single-test strategy based on typical estimates of test accuracy

Anti-HCV Prevalence: 45%		Population		100,000								
Test A		Test B										
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
1.000	0.995	n/a	n/a	45,000	0	275	54,725	100,000	0.994	1.000	164	1.000	0.995
0.995	0.995	n/a	n/a	44,775	225	275	54,725	100,000	0.994	0.996	163	0.995	0.995
0.995	0.980	n/a	n/a	44,775	225	1,100	53,900	100,000	0.976	0.996	41	0.995	0.980
0.980	0.980	n/a	n/a	44,100	900	1,100	53,900	100,000	0.976	0.984	40	0.980	0.980
Anti-HCV Prevalence: 10%		Population		100,000								
Test A		Test B										
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
1.000	0.995	n/a	n/a	10,000	0	450	89,550	100,000	0.957	1.000	22	1.000	0.995
0.995	0.995	n/a	n/a	9,950	50	450	89,550	100,000	0.957	0.999	22	0.995	0.995
0.995	0.980	n/a	n/a	9,950	50	1,800	88,200	100,000	0.847	0.999	5.5	0.995	0.980
0.980	0.980	n/a	n/a	9,800	200	1,800	88,200	100,000	0.845	0.998	5.4	0.980	0.980
Anti-HCV Prevalence: 2%		Population		100,000								
Test A		Test B										
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
1.000	0.995	n/a	n/a	2,000	0	490	97,510	100,000	0.803	1.000	4.1	1.000	0.995
0.995	0.995	n/a	n/a	1,990	10	490	97,510	100,000	0.802	1.000	4.1	0.995	0.995
0.995	0.980	n/a	n/a	1,990	10	1,960	96,040	100,000	0.504	1.000	1.0	0.995	0.980
0.980	0.980	n/a	n/a	1,960	40	1,960	96,040	100,000	0.500	1.000	1.0	0.980	0.980
Anti-HCV Prevalence: 0.4%		Population		100,000								
Test A		Test B										
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
1.000	0.995	n/a	n/a	400	0	498	99,102	100,000	0.445	1.000	0.8	1.000	0.995
0.995	0.995	n/a	n/a	398	2	498	99,102	100,000	0.444	1.000	0.8	0.995	0.995
0.995	0.980	n/a	n/a	398	2	1,992	97,608	100,000	0.167	1.000	0.2	0.995	0.980
0.980	0.980	n/a	n/a	392	8	1,992	97,608	100,000	0.164	1.000	0.2	0.980	0.980

The outcomes illustrate the strong influence of prevalence and assay specificity on positive predictive value (PPV). At high prevalences, the use of a highly specific single screening test yields a PPV in excess of 0.99, and the ratio of true positive (TP) : false positive (FP) results is high (164), but nonetheless there would be a small but substantial number of false-positive diagnoses. At the lowest prevalences (e.g. 0.4%), PPV might be expected to fall below 0.5, yielding more false-positive diagnoses than true ones, particularly if the test specificity falls below 0.995. Even in middling prevalences, the PPV will struggle to exceed 0.9 should the specificity performance of the test employed fall below 0.99. The negative predictive value (NPV) is generally high except in high-prevalence populations (e.g. 45%) when a test with relatively poor sensitivity (<99%) is employed.

As discussed above (Strategies and algorithms), the use of a second diagnostic test is able to improve the diagnostic accuracy. While repeating testing of a reactive specimen by the original test can provide some gains in specificity, in a quality-controlled environment, these gains are generally modest. This is because in such a context false-positive reactions are generally due to real and reproducible reactions between factors in the specimen and materials incorporated in the diagnostic device. In circumstances in which initial reactions are not reproducible it is usually a failure of the quality system such as training, mistakes, failure to follow defined procedures, and instrument calibration and maintenance. In such circumstances the solution is to invest in training and the quality system; repeating unreliable tests is no substitute.

The choice of the second test product employed in the local algorithm is critical to satisfactory outcomes. It is not uncommon for different products from the same manufacturer to use assay components in common; also, different manufacturers or distributors can source the same raw materials, or even the entire device, from the same third party manufacturer/supplier. In such cases, there is an increased likelihood that false reactions in the primary screening test will arise also in the second assay, and this will erode the PPV of the applied algorithm (discussed in more detail below). Consequently, test devices employed should be sourced from a reputable accredited manufacturer, and most critically the translation of the **Testing strategy**, which is generic, into a **Local algorithm** that employs specific test devices, needs carefully to be piloted and validated before being put into routine clinical use.

(It is understood) that the Guidelines Group's current thinking is that a two-test strategy would follow that outlined in Fig. 4. Unlike HIV testing in which discordant results would generally be considered "indeterminate", and undergo further testing, the finding of a negative reaction in the second test (Test Kit B), performed only on those individuals reactive in the initial test (Test Kit A), would be interpreted as an absence of anti-HCV. Such findings are most likely to be non-specific, but even if the reaction did reflect the presence of true, but low level, anti-HCV reactivity, as discussed above, this is more likely to reflect the waning antibodies associated with cleared HCV infection than with a brief phase early in HCV infection, during seroconversion.

Hypothetical outcomes of the two-test strategy are given in Table 2, employing the same notional anti-HCV prevalences as in Table 1. To keep the tables relatively simple, only two of the four sets of illustrative sensitivity and specificity employed for Test Kit A in Table 1 have been employed. Similarly, only two sets of sensitivity and specificity measures have been employed for Test Kit B when calculating the expected outcomes.

Table 2: Outcomes for two-test strategy based on typical estimates of test accuracy (see Fig. 4)

Anti-HCV Prevalence: 45%		Population		100,000									
Test Kit A		Test Kit B											
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
1.000	0.995	1.000	0.995	45,000	0	1	54,999	145,275	1.000	1.000	32,727	1.000	1.000
1.000	0.995	0.980	0.980	44,100	900	6	54,995	145,275	1.000	0.984	8,018	0.980	1.000
0.980	0.980	1.000	0.995	44,100	900	6	54,995	145,200	1.000	0.984	8,018	0.980	1.000
0.980	0.980	0.980	0.980	43,218	1,782	22	54,978	145,200	0.999	0.969	1,964	0.960	1.000

Anti-HCV Prevalence: 10%		Population		100,000									
Test Kit A		Test Kit B											
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
1.000	0.995	1.000	0.995	10,000	0	2	89,998	110,450	1.000	1.000	4,444	1.000	1.000
1.000	0.995	0.980	0.980	9,800	200	9	89,991	110,450	0.999	0.998	1,089	0.980	1.000
0.980	0.980	1.000	0.995	9,800	200	9	89,991	111,600	0.999	0.998	1,089	0.980	1.000
0.980	0.980	0.980	0.980	9,604	396	36	89,964	111,600	0.996	0.996	267	0.960	1.000

Anti-HCV Prevalence: 2%		Population		100,000									
Test Kit A		Test Kit B											
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
1.000	0.995	1.000	0.995	2,000	0	2	97,998	102,490	0.999	1.000	816	1.000	1.000
1.000	0.995	0.980	0.980	1,960	40	10	97,990	102,490	0.995	1.000	200	0.980	1.000
0.980	0.980	1.000	0.995	1,960	40	10	97,990	103,920	0.995	1.000	200	0.980	1.000
0.980	0.980	0.980	0.980	1,921	79	39	97,961	103,920	0.980	0.999	49	0.960	1.000

Anti-HCV Prevalence: 0.4%		Population		100,000									
Test Kit A		Test Kit B											
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
1.000	0.995	1.000	0.995	400	0	2	99,598	100,898	0.994	1.000	161	1.000	1.000
1.000	0.995	0.980	0.980	392	8	10	99,590	100,898	0.975	1.000	39	0.980	1.000
0.980	0.980	1.000	0.995	392	8	10	99,590	102,384	0.975	1.000	39	0.980	1.000
0.980	0.980	0.980	0.980	384	16	40	99,560	102,384	0.906	1.000	10	0.960	1.000

Across all prevalences, the introduction of a second test kit to be applied to all specimens reactive in the initial test kit provides substantial potential gains in the PPV of this strategy. Even at the lowest prevalence shown here (0.4%), employing two test kits with relatively low specificity (0.98), the PPV was vastly improved, converting a PPV of 0.164 in the one-test strategy to 0.906. In all but the highest population prevalences, the use of less sensitive test kits in the two-test strategy had only a modest impact on NPVs. In all but the highest prevalences, the calculations demonstrate the potential for substantial gains in diagnostic accuracy in return for a modest investment in a second, independent, test kit to be applied to initially reactive individuals. For example, taking a model population of 100 000 with an anti-HCV prevalence of 2%, the PPV of an algorithm employing two independent test kits with modest sensitivities and specificities of 0.98 is improved from 0.500 to 0.980 in return for the supplementary use of <4000 of Test Kit B.

Selection of test kits for local algorithm

As discussed above, it is of critical importance that the two test kits selected for a local algorithm are chosen with great care. It must be remembered that the sensitivity and specificity estimates claimed by manufacturers and even those of independent evaluations should be based on, or approximate to, the testing of a panel of samples representative of an unbiased and unselected population of positives and negatives. This can be difficult to achieve, and sometimes some bias may be introduced either unintentionally or intentionally, such as including only strongly positive samples. There can also be regional differences due to prevalent genotypes, transmission patterns, the maturity of an outbreak or age at acquisition. However, when applying a testing strategy such as

that illustrated in Fig. 4, it is critical to recognize that the population of samples that is selected for testing in Test Kit B has been highly selected by the application of Test Kit A. Under such circumstances, the performance claims made by the manufacturer of Kit A, or described in an independent evaluation, are unlikely to apply. In particular, the occurrence of false-positive reactions in Test Kit A will generally increase the risk of a false-positive reaction also in Test Kit B. Consequently, in this algorithmic approach the specificity claims are likely to be misleading and better than will be achieved when applied to individuals who were falsely reactive on Test Kit A. This phenomenon has been observed when applying HIV testing algorithms, with shared false-positive reactions observed frequently between particular diagnostic products. Similarly, when employing two kits in an algorithm, each with a sensitivity of 0.995 on a "random" sample, it is probable that the 1 in 200 true positives not detected by Test Kit A will be the same sample that would be missed by Test Kit B, rather than one of the other 199, and therefore the sensitivity on the sample selected by Test Kit A is likely to be closer to 1.00 than 0.995. The outcomes illustrated in **Table 2** are based on an assumption that the two test kits employed act independently and that false reactions arise at random, such a reaction in Test Kit A not increasing the likelihood of a false reaction in Test Kit B. In **Table 3,** the performance characteristics of Test Kit B have been adjusted to take into account the potential impact of selection by Test Kit A of the individuals to be tested by Test Kit B. The modified sensitivity and specificity of Test Kit B have been adjusted to 0.999 and 0.850, reflecting the selection bias, including a 15% increased risk of false-positive reactions in Test Kit B.

Table 3: Outcomes for a two-test strategy taking into account the theoretical impact on the performance characteristics Test Kit B introduced by selecting the individuals to be tested by first applying Test Kit A

Anti-HCV Prevalence: 45%		Population		100,000									
Test Kit A		Test Kit B											
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
0.995	0.995	0.999	0.850	44,730	270	41	54,959	145,050	0.999	0.995	1,084	0.994	0.999
0.995	0.980	0.999	0.850	44,730	270	165	54,835	145,875	0.996	0.995	271	0.994	0.997
Anti-HCV Prevalence: 10%		**Population**		**100,000**									
Test Kit A		**Test Kit B**											
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
0.995	0.995	0.999	0.850	9,940	60	68	89,933	110,400	0.993	0.999	147	0.994	0.999
0.995	0.980	0.999	0.850	9,940	60	270	89,730	111,750	0.974	0.999	37	0.994	0.997
Anti-HCV Prevalence: 2%		**Population**		**100,000**									
Test Kit A		**Test Kit B**											
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
0.995	0.995	0.999	0.850	1,988	12	74	97,927	102,480	0.964	1.000	27	0.994	0.999
0.995	0.980	0.999	0.850	1,988	12	294	97,706	103,950	0.871	1.000	7	0.994	0.997
Anti-HCV Prevalence: 0.4%		**Population**		**100,000**									
Test Kit A		**Test Kit B**											
Sens	Spec	Sens	Spec	True Positives	False Negatives	False Positives	True negatives	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.
0.995	0.995	0.999	0.850	398	2	75	99,525	100,896	0.842	1.000	5	0.994	0.999
0.995	0.980	0.999	0.850	398	2	299	99,301	102,390	0.571	1.000	1	0.994	0.997

While PPV holds up well for the highest prevalences of anti-HCV, at lower prevalences the PPV will fall substantially, particularly if the specificity of Test Kit A is relatively poor. On the other hand, recognizing the consequence to the sensitivity of Test Kit B when testing the subpopulation selected by the use of Test Kit A, gives rise to improved NPVs, even at higher prevalences.

In summary, particularly for resource-poor settings, testing strategies must be simple, inexpensive, practically feasible and produce clear categorical results. The use of a two-test strategy

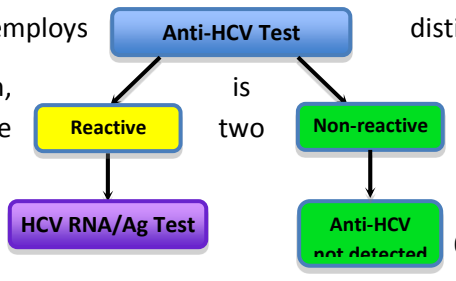
has the potential to provide accurate anti-HCV results, substantially reducing the numbers of individuals who would be inappropriately referred on to more specialist services, with the concomitant burdens on scarce health-are resources, of inappropriate interventions and on individuals well-being. The two tests employed in such a strategy, to form part of the local algorithm, must be selected with great care, validated locally, and their output monitored for adverse events. The use of combinations of assays without such care may provide a false sense of security, but will lead to greater costs both to the health-care system and patients, but also to a potential loss of credibility of any associated programme.

Aim 2: Detection of active HCV infection

While the use of a two-test strategy has the potential to improve the accuracy of anti-HCV detection, it is recognized that for a programme whose aims are primarily to treat and cure individuals with chronic HCV infection, further diagnostic tests would be needed before initiating an expensive course of antiviral therapy. Estimates vary, and may depend on differences in the pattern, timing and maturity of the epidemic in different regions, but as discussed earlier, 10–25% of HCV infections spontaneously resolve, as evidenced by the proportion of anti-HCV positive individuals that lack HCV RNA. If such individuals can be identified at the time of initial testing this would reduce the burden on more expensive parts of the health-care system as well as reduce unnecessary anxiety among those who no longer have active HCV infection. Furthermore, one could take the view that if the aim of any HCV screening programme is to identify individuals who need antiviral therapy then even the most sensitive and specific anti-HCV test strategy might, with that goal in mind, achieve a specificity of only 0.75–0.90, begging the question as to whether there is any value in undertaking a second anti-HCV test to improve the PPV of anti-HCV screening. In view of the relatively small number of second anti-HCV tests that would be needed at lower prevalences, there may be a case for including the second anti-HCV test kit, should a test for HCV RNA, or possibly HCV Ag, not be feasible. It should be borne in mind that even this approach would fail to identify those in the pre-seroconversion phases of HCV infection, a majority of whom would, without therapeutic intervention, go on to develop a long-term chronic HCV infection, and be a potential source of new infections in their contacts. It is likely, though, that the opportunity to detect such incident infections will be rare with the exception of populations with higher prevalences of infection practising high-risk behaviours. The premium cost of a test for HCV RNA, or even that for sequestered HCV Ag, plus the equipment requirements for such assays that prevail at present, would preclude testing for HCV RNA or HCV Ag as a primary test in low- and middle-income settings. Therefore, this aim would probably be best met by a two-test strategy, the first being to screen for the presence of anti-HCV, and the second to test the anti-HCV reactive specimens for the presence of HCV RNA or HCV Ag, and this should prove feasible once the promise of low-technology tests for HCV RNA or HCV Ag become a reality. At present, though, in many resource-poor settings, testing for HCV RNA or HCV Ag may not be available in all settings in which anti-HCV screening might be desirable.

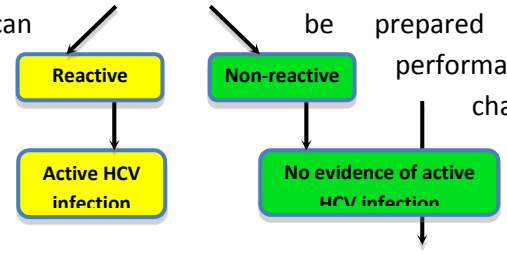
A potentially important benefit of a test strategy that detects different markers of infection, anti-HCV and HCV RNA, and most importantly, employs distinct technologies, immunoassay and nucleic acid amplification, is that there will be minimal risk of confounding between the two

Fig. 5. Two-test strategy to identify active HCV infection



diagnostic tests, providing added assurance of the reliability of testing.

As with the two-test anti-HCV strategy, tables can be prepared to generate hypothetical outcomes based on expected performance characteristics of the assays employed. The principles and underlying calculations are the same. However, the aim of this strategy is to detect active HCV infection, and therefore performance characteristics of the test kits involved should be adjusted to reflect this application. For example, although a test kit for anti-HCV antibodies might have an associated specificity claim of 0.995, when detecting individuals who have active HCV infection its specificity might be more modest, in the range 0.75–0.9. The influence of several of these variables is summarized in Table 4.



To limit the number of variables, and for the purposes of the model outcomes given in **Table 4**, it is assumed that the anti-HCV screening test has the same performance characteristics (sensitivity 0.995; specificity 0.990) for each of the three hypothetical algorithms. In each algorithm "virus" test kits would subsequently be applied to the samples that were reactive in the anti-HCV screening test.

Three model performance characteristics have been applied: the first reflecting the hypothetical performance of a "licensed" laboratory nucleic acid amplification test (NAT) kit for HCV RNA, with sensitivity 0.995 and specificity 0.990; the second a notional "field-based" NAT kit or a laboratory-based HCV Ag kit, with sensitivity 0.960 and specificity 0.970; and the third, a rapid diagnostic test (RDT) for HCV antigen (HCV Ag). The effect of anti-HCV prevalence in the population screened (0.45; 0.1; 0.02; 0.004) and that of spontaneous viral clearance (anti-HCV +ve/HCV RNA or Ag –ve), employing a low and a high estimate (10%; 25%), on predictive values in each of the algorithms were explored.

Table 4: Impact of model anti-HCV prevalence, HCV clearance & assay performance on PPV & NPV of a 2-test strategy employing an anti-HCV test kit, reactive persons then being tested by a test kit for

Anti-HCV (Test A)	HCV viral (Test B)	Anti-HCV prevalence	PPV 10% HCV resolved	PPV 25% HCV resolved	NPV 10% HCV resolved	NPV 25% HCV resolved
sensitivity 0.995; specificity 0.990 (Typical approved anti-HCV test)	sensitivity 0.995; specificity 0.990 (e.g. Lab-based PCR)	0.45	0.999	0.996	0.993	0.995
		0.10	0.998	0.995	0.999	0.999
		0.02	0.993	0.990	1.000	1.000
		0.004	0.972	0.964	1.000	1.000
	sensitivity 0.960; specificity 0.970 (e.g. Field PCR or Lab-based HCV Ag)	0.45	0.996	0.989	0.970	0.978
		0.10	0.993	0.986	0.996	0.996
		0.02	0.980	0.970	0.999	0.999
		0.004	0.917	0.897	1.000	1.000
	sensitivity 0.800; specificity 0.970 (e.g. HCV Ag RDT)	0.45	0.995	0.987	0.878	0.905
		0.10	0.992	0.983	0.980	0.984
		0.02	0.976	0.964	0.996	0.997
		0.004	0.902	0.879	0.999	0.999

More comprehensive findings from which Table 4 is drawn can be found in Appendix 1.

As would be expected, as prevalence falls the PPV also falls. Conversely, NPVs increase as prevalences fall. PPVs were slightly worse when a larger proportion of HCV had spontaneously resolved but, on the other hand, NPVs were marginally better. The application of a two-test algorithm ensures that PPV was maintained well above 0.9, except for the lowest prevalences (≤0.004).

The model shows that the accuracy of the "virus" test (HCV RNA/ HCV Ag) would have a substantial influence both on PPV and NPV, e.g. the most accurate algorithm would be expected to achieve a PPV of 0.990 in a population with a 2% anti-HCV prevalence, of which 25% had resolved infection, yet the least accurate approach might achieve only 0.964. Translating this into diagnoses, employing the better algorithm, 10 false-positive diagnoses of active HCV infection might arise in a population of 1000 in which there are actually 15 real active HCV infections; the worse algorithm might yield 36 false diagnoses of active HCV, nearly 2.5 times the number of real active infections.

At high anti-HCV prevalences, the use of an insensitive device (e.g. sensitivity 0.8) to identify active HCV infection, hypothetically possibly an HCV Ag RDT (to the best of the author's knowledge no example is yet on the market), could fail to identify a considerable proportion of individuals with active HCV, the NPV falling to 0.878 in a population with a 45% anti-HCV prevalence, of which 10% had resolved infection, translating to approximately 12% of active infection going undetected. At lower prevalences, the NPV holds up well, but this masks the finding that the use of such a low sensitivity device would lead to approximately 20% of the active infections going undetected whatever the prevalence.

Hepatitis B

1.Diagnostic background to HBV infection

Despite the small size of the hepatitis B virus (HBV, the infectious virion, "Dane Particle", is 42 nm diameter) and of its genome (just over 3200 base pairs), it is a persistent and pervasive pathogen that causes a wide range of public health, disease, therapeutic and diagnostic problems. The genome is used very efficiently to generate several gene products, mostly utilizing some overlapping regions that control replication and provide the structural proteins for a new infectious virus, as well as help suppress host immune surveillance, and after many years of chronic infection, cause liver disease and hepatic cancer. The viral proteins, and the host response to them, together provide the basis for diagnostic tests that permit the identification of acute and chronic HBV infection, as well as resolved infection. An almost universal marker of active HBV infection is hepatitis B surface antigen (HBsAg) (Figs 1 & 2), although in "occult" or "cryptic" HBV infections, HBsAg levels can dip below the level of detection of HBsAg immunoassays.

Fig. 1. Acute HBV infection with recovery

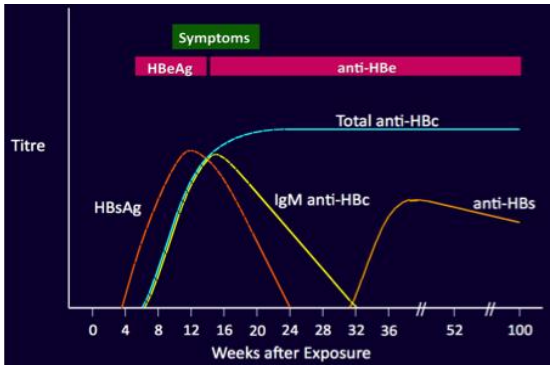
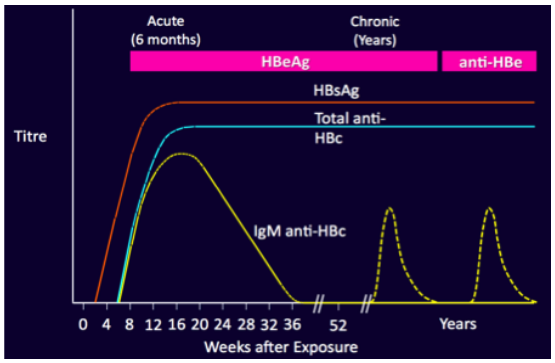


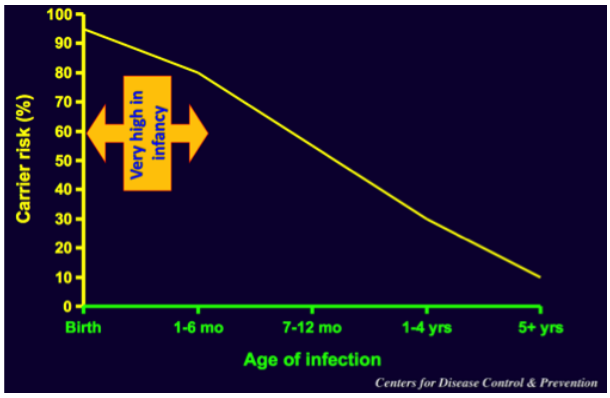
Fig. 2. Chronic HBV infection



The appearance of HBsAg in blood is soon followed by hepatitis B e antigen (HBeAg), which is a marker of high levels of viral replication. In acute HBV infection that resolves, HBeAg seroconverts relatively early to anti-HBe, but in chronic HBV infection, seroconversion to anti-HBe may be delayed for many years, HBeAg may persist, or neither may be detectable. An anti-HBc response occurs relatively soon, often within a week or two after the emergence of HBsAg, and is typified by a profound IgM anti-HBc response, which may wane approximately 6 months later.

Differentiating between primary acute and chronic HBV infection is important because in general the risk of HBV infection becoming chronic is inversely proportional to age, with approximately 90% of children infected in the neonatal period going on to develop chronic HBV, falling to 10% or less by school age (**Fig. 3**).

Fig. 3. Risk of chronicity by age of HBV infection



While IgM anti-HBc has long been employed as a means of differentiating between acute and chronic HBV infection, its reappearance during "flares" in chronic infection (**Fig. 2**) make it an unreliable indicator of recent primary HBV infection. A potential consequence of misinterpreting a reactive IgM anti-HBc result is that a patient with chronic infection, experiencing flares in liver disease, may not be offered timely treatment and, although they could be re-examined several months later to confirm the original diagnosis of acute HBV, a substantial proportion of such patients may be lost to follow up. Anti-HBc avidity has also been used as a means of distinguishing acute from chronic HBV infection, the early anti-HBc response being of low avidity; however, there is no licensed commercial avidity test currently available.

In recent years, the detection and quantification of HBV DNA has been increasingly used either to supplement or replace HBeAg testing as a more direct and accurate measure of active HBV replication. Sensitive nucleic acid tests (NATs) for HBV DNA are able to detect infection early in the acute phase through to resolution, and are now commonly used as a supplement to HBsAg testing for blood and plasma donors, detecting HBV DNA in occasional HBsAg-negative donors. The widespread need for and provision of NAT facilities for the care of HIV patients should facilitate access to NAT for HBV.

2.Aim of testing

As for HCV, the preferred testing strategy will depend on the aims of the testing programme as well as factors such as assay performance, technical feasibility, access and cost. As a rule, it should be feasible to translate the strategy into a simple and inexpensive local algorithm.

1. **Identify all HBsAg-positive individuals**

   This scenario employs the most widely available and least expensive diagnostics. It would not detect recent infections in the brief period prior to HBsAg seroconversion. However, it is expected that a large majority of such infections in adults will be self-limiting. If the ultimate aim is to offer antiviral treatment to those with an active HBV infection, anti-HBV-positive individuals, or at least a blood specimen, would then have to be referred to a facility able to carry out the necessary procedures to determine whether HBV treatment was needed, and then to monitor its success.

Strategy: HBsAg test (RDT or EIA) with or without a different HBsAg test.

2. Identify chronic HCV infections

This approach would ascertain whether individuals are carrying replicating HCV. The current cost and complexity of tests for HCV RNA would probably preclude their use as a screening tool in all but areas with the highest prevalence. Consequently, the primary screening test would likely be an anti-HCV test, which would fail to detect acute infections in the seroconversion window phase. This would be partially offset by the use of a combined anti-HCV/HCV Ag assay. Identification of active HCV infection would require the application of a test for HCV RNA, though an immunoassay that is able to detect immune-complexed HCV Ag may be sufficiently accurate. Early infections, a minority of which would have gone on to be spontaneously eradicated by the host, would also be treated under this approach.

Strategy: Anti-HCV test (RDT or EIA) or combined anti-HCV + HCV Ag test followed by an HCV RNA test or an HCV Ag test.

3. Identify chronic HCV infections

This approach would need to go a further step to aim 2, by incorporating the means to distinguish chronic from acute infection. Such a test would permit immediate treatment of those identified as having a chronic infection and deferral of those with a recent acute HCV infection. At present, though, there is no appropriate and validated laboratory marker to distinguish chronic from acute HCV infection. While a combination of clinical and non-virological laboratory tests might identify those in need of immediate treatment, others may have to be deferred for 3–6 months to allow a re-test to exclude the minority whose infection cleared spontaneously. Dependent on the costs of antiviral drugs, the risks of unnecessary delay to treatment in those who would benefit from it and of loss to follow up would probably exclude this approach at this time.

Appendix 1: Outcomes from Two-Test Strategy to Identify Active HCV Infection

Population: 100,000		Anti-HCV Test			HCV RNA or Ag Test		Strategy Outcomes									
% Resolved HCV	Anti-HCV prevalence	Sens	Spec	Effective anti-HCV PPV	Sens	Spec	True Pos	False Neg	False Pos	True neg	Total Tests	PPV	NPV	Ratio of TP:FP	Overall sens.	Overall spec.	Population
10%	0.45	0.995	0.990	**0.889**	0.995	0.990	40,096	404	51	59,450	145,348	**0.999**	**0.993**	794	0.990	0.999	100000
	0.10	0.995	0.990	**0.825**	0.995	0.990	8,910	90	19	90,981	110,855	**0.998**	**0.999**	469	0.990	1.000	100000
	0.02	0.995	0.990	**0.603**	0.995	0.990	1,782	18	12	98,188	102,971	**0.993**	**1.000**	151	0.990	1.000	100000
	0.004	0.995	0.990	**0.257**	0.995	0.990	356	4	10	99,630	101,394	**0.972**	**1.000**	34	0.990	1.000	100000
	0.45	0.995	0.990	**0.889**	0.960	0.970	38,686	1,814	152	59,349	145,348	**0.996**	**0.970**	255	0.955	0.997	100000
	0.10	0.995	0.990	**0.825**	0.960	0.970	8,597	403	57	90,943	110,855	**0.993**	**0.996**	151	0.955	0.999	100000
	0.02	0.995	0.990	**0.603**	0.960	0.970	1,719	81	35	98,165	102,971	**0.980**	**0.999**	49	0.955	1.000	100000
	0.004	0.995	0.990	**0.257**	0.960	0.970	344	16	31	99,609	101,394	**0.917**	**1.000**	11	0.955	1.000	100000
	0.45	0.995	0.990	**0.889**	0.800	0.970	32,238	8,262	152	59,349	145,348	**0.995**	**0.878**	213	0.796	0.997	100000
	0.10	0.995	0.990	**0.825**	0.800	0.970	7,164	1,836	57	90,943	110,855	**0.992**	**0.980**	126	0.796	0.999	100000
	0.02	0.995	0.990	**0.603**	0.800	0.970	1,433	367	35	98,165	102,971	**0.976**	**0.996**	40	0.796	1.000	100000
	0.004	0.995	0.990	**0.257**	0.800	0.970	287	73	31	99,609	101,394	**0.902**	**0.999**	9	0.796	1.000	100000
25%	0.45	0.995	0.990	**0.740**	0.995	0.990	33,413	337	118	66,132	145,381	**0.996**	**0.995**	283	0.990	0.998	100000
	0.10	0.995	0.990	**0.687**	0.995	0.990	7,425	75	34	92,466	110,863	**0.995**	**0.999**	218	0.990	1.000	100000
	0.02	0.995	0.990	**0.502**	0.995	0.990	1,485	15	15	98,485	102,973	**0.990**	**1.000**	100	0.990	1.000	100000
	0.004	0.995	0.990	**0.214**	0.995	0.990	297	3	11	99,689	101,395	**0.964**	**1.000**	27	0.990	1.000	100000
	0.45	0.995	0.990	**0.740**	0.960	0.970	32,238	1,512	354	65,896	145,381	**0.989**	**0.978**	91	0.955	0.995	100000
	0.10	0.995	0.990	**0.687**	0.960	0.970	7,164	336	102	92,398	110,863	**0.986**	**0.996**	70	0.955	0.999	100000
	0.02	0.995	0.990	**0.502**	0.960	0.970	1,433	67	44	98,456	102,973	**0.970**	**0.999**	32	0.955	1.000	100000
	0.004	0.995	0.990	**0.214**	0.960	0.970	287	13	33	99,667	101,395	**0.897**	**1.000**	9	0.955	1.000	100000
	0.45	0.995	0.990	**0.740**	0.800	0.970	26,865	6,885	354	65,896	145,381	**0.987**	**0.905**	76	0.796	0.995	100000
	0.10	0.995	0.990	**0.687**	0.800	0.970	5,970	1,530	102	92,398	110,863	**0.983**	**0.984**	59	0.796	0.999	100000
	0.02	0.995	0.990	**0.502**	0.800	0.970	1,194	306	44	98,456	102,973	**0.964**	**0.997**	27	0.796	1.000	100000
	0.004	0.995	0.990	**0.214**	0.800	0.970	239	61	33	99,667	101,395	**0.879**	**0.999**	7	0.796	1.000	100000

Annex 6.2

Decision analysis of strategies to identify hepatitis C

Predictive Modelling

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September 2015

1.Executive summary

We use a decision model to evaluate the positive predictive value (PPV), negative predictive value (NPV), percentage of HCV cases identified, cost of screening, and cost per case of HCV identified of 22 HCV testing strategies in three prevalence settings (2.3%, 10% and 40). The analysis answers the following four questions about HCV testing strategies

1. **What is the best test for exposure to HCV, an EIA or RDT?**

   In general, either RDT or EIA provide similar testing outcome and cost. Choice of which test to use should depend on in-country costs and previous investments. In very high prevalence settings, however, loss to follow up between tests for exposure and active infection results in missed cases of HCV. In such settings, it may be best to not use either test, and to instead employ a one-test strategy that uses nucleic acid amplification testing (NAT) or HCV core antigen (HCV core Ag) to directly assess for active HCV infection without previous tests for exposure.

2. **Is it beneficial to use two tests for exposure to minimize false-positive results?**

When it is possible to reflexively conduct repeat testing for HCV exposure using the same specimen, it can be cost saving to employ two tests for exposure in lower (2.3%) and high (10%) prevalence settings. If reflex testing is not possible, however, then there is little value added by the two tests for exposure strategy. In very high prevalence settings (40%), loss to follow up results in strategies that test directly for active HCV infection (no test for exposure) being preferred.

3. **Among those with a positive test for exposure to HCV, what is the best test for assessing active infection, qualitative HCV RNA, quantitative HCV RNA, or HCV core Ag?**

At current cost, qualitative RNA emerges as the preferred test for active HCV infection. Quantitative RNA is slightly less sensitive than qualitative RNA, and therefore results in slightly more false-negative results at similar cost. The difference in testing outcomes is small, however, and countries with previous investment in quantitative RNA platforms, as well as those that can leverage existing HIV testing infrastructure to conduct quantitative HCV RNA testing should do so. Further, if countries anticipate needing quantitative RNA for monitoring HCV therapy, then quantitative RNA should likely be used for HCV testing as well. At its current approximate cost, HCV core Ag results in worse testing accuracy and higher cost than either quantitative or qualitative RNA.

4. **How does HCV prevalence impact the choice of testing strategy?**

Prevalence is central to the choice of testing strategy. There is no one strategy that is appropriate to all prevalence settings. In lower-prevalence settings, the preferred testing strategies limit false-positive results. It can be efficient to use two tests for HCV exposure (assuming that they can be done reflexively and on one specimen). In contrast, in very high-prevalence settings, preferred strategies are sensitive and minimize false-negative results. In such settings, the high probability of being infected, as well as the potential for missed cases due to loss to follow up, results in strategies that directly test for active infection (no test for exposure) being preferred.

2.Introduction

Hepatitis C virus (HCV) is a global public health burden and major cause of morbidity and mortality, including liver failure and hepatocellular carcinoma.[1, 2] With current global HCV seroprevalence estimated to be 2.8%, or more than 185 million infected individuals worldwide,[3] and new high-efficacy, low-duration therapy available,[4] there is a need to optimize testing strategies for the detection of HCV.

Because 10–25% of persons infected with HCV clear the virus without treatment, diagnosis of active HCV infection is typically a two-step process. First, a person is screened for exposure to HCV, typically with an HCV antibody (Ab) test. Those who test positive for exposure then undergo assessment for HCV viral replication, usually with nucleic acid testing (NAT), to confirm the diagnosis of active HCV. This two-step process has multiple barriers to implementation such as loss to follow up between testing for exposure and confirmation of active viral replication, missed pre-seroconversion infection, and limited access to costly NAT techniques, all of which allow for potential missed diagnosis and treatment.

There are a variety of modalities for screening for HCV exposure, including enzyme-linked immune assays (EIA), and rapid diagnostic tests (RDT). There are also a variety of tests for assessing viral replication, including qualitative and quantitative HCV RNA, as well as a newer testing modality, HCV core antigen (HCV core Ag). It is possible to combine these tests in a large number of permutations to create strategies with either one or two steps in the process of assessing HCV exposure and active infection. The sequence of testing has major implications for the likelihood of missing cases of HCV and of having false-positive test results, as well as directly impacting costs.

The large number of potential strategies for testing generates four important questions about how to best use available assays to identify chronic HCV infection:

1. What is the best test for exposure to HCV, an EIA or RDT?
2. Is it beneficial to use two tests for exposure to minimize false-positive results?
3. Among those with a positive test for exposure to HCV, what is the best test for assessing active infection, qualitative HCV RNA, quantitative HCV RNA, or HCV core Ag?
4. How does HCV prevalence impact the choice of testing strategy?

In this report, we use decision analytic methods to demonstrate the positive and negative predictive values of a wide array of possible testing strategies in three prevalence settings. We also estimate the average cost of each testing strategy, and the cost per case of HCV detected. The data are useful in making decisions about investment in HCV screening and can provide guidance in choosing the appropriate testing strategy for a given testing venue.

Clarification of terminology used in this report

Test for exposure – These are assays that test for the presence of anti-HCV antibody in a specimen. A positive test result indicates that a person has been previously exposed to HCV virus, but cannot confirm that the person has active infection. Patients with positive test for exposure require additional testing for active HCV infection.

*Test for active infection*_ – although most people exposed to HCV establish a chronic infection, approximately 25% spontaneously clear their infection during the acute phase. Such patients will have a positive test for exposure, even though they do not have active HCV. Tests for active

infection assay either nucleic acids (viral load) or presence of HCV core Ag to determine whether a person has active HCV replication and therefore chronic HCV.

EIA – enzyme-linked immune assay is one modality for testing for the presence of HCV antibody (HCV Ab) in a specimen. EIA can be used as a test for exposure.

RDT – rapid diagnostic tests are another modality for testing for the presence of HCV Ab in a specimen. RDT can be used as a test for exposure.

Qualitative RNA – a nucleic acid amplification test (NAT) can be used as a test for active infection. The results of a qualitative RNA are binary (positive/negative) and do not provide detail about the degree of viraemia.

*Quantitative RNA*_ – a nucleic acid amplification test (NAT) that can be used as a test for active infection. The results of a quantitative RNA provide detail about the number of copies of HCV RNA present in every ml of specimen.

HCV core Ag – a test that assays the presence of HCV core antigen (HCV core Ag) in a specimen. HCV core Ag is a product of viral replication. The test can be used as a test of active infection.

Two-step strategy – a testing strategy that first employs tests for exposure to identify patients who are likely HCV-infected and rule out the majority of patients who are not infected. Next, patients with a positive test for exposure undergo testing for active HCV infection. This strategy is used most frequently around the world, as it reduces the need for more costly tests for active infection.

One-step strategy – a strategy that does not employ any test for exposure, but instead directly assesses for active HCV infection in all patients.

Tests in series – when conducting two tests for HCV exposure, it is possible to perform them in series or in parallel. Conducting tests in series means that the first test is performed and if the result is negative, the test is complete and it is considered a negative test. If the first test is positive, only then is the second test performed. If the second test is positive, then the two-test procedure is interpreted as positive.

Considerations for testing strategies

Several dynamics impact the outcomes of screening and should inform decision-making about HCV testing:

1. *Prevalence in a population* – prevalence is central in thinking about HCV testing. In low-prevalence settings, the potential for false-positive tests for exposure to HCV is high. In such settings, it may be beneficial to require two tests for exposure in an effort to minimize false positive test results. In high-prevalence settings, however, the larger risk is missing cases of active HCV infection. In those venues, it is likely optimal to choose strategies that maximize sensitivity and HCV case detection. In some cases, it may even be efficient to forgo all screening for exposure and move directly to confirmation of active viral replication. The role of prevalence in determining outcomes and cost is so great, that it is not possible to consider how to test for HCV without contextualizing the decision to a specific prevalence setting.
2. *Cost of diagnostic assays* – tests for exposure to HCV are substantially less costly than tests for confirmation of active infection. As a result, it is typically useful to use a two-step strategy for HCV testing. First, a low cost test for exposure rules out the majority of patients who have never been exposed to HCV. Second, those with a reactive test for exposure

undergo more costly testing for active infection. In some low-prevalence settings, the relative cost of testing for exposure and active replication may result in it being efficient to use two tests for exposure before acquiring testing for active infection.

3. *Presence of existing laboratory infrastructure* – previous investments in testing infrastructure can be large. Further, some countries have existing infrastructure for HIV testing that they could leverage for HCV testing. This analysis does not consider previous investment or start-up costs. Small differences in the test characteristics of given strategies should not lead countries to abandon existing platforms for testing or duplicate testing infrastructure.
4. *Loss to follow up rates and rates of linkage to care* – the cascade of care is important for the cost–effectiveness of efforts to treat HCV, and poor follow up can reduce reach and effectiveness of testing.[6] Reducing the number of steps required to diagnose HCV limits the number of times an individual must present and re-present to care and likely improves diagnosis rates. When it is possible to acquire the results of testing for HCV exposure quickly, such that the patient can undergo testing for exposure and replication at the same visit, follow up will improve and more cases will be identified. Further, in some very high-prevalence settings, a one-step strategy may be appropriate.
5. *Availability of highly effective new therapies* – whereas historically it was very difficult to treat HCV, new therapies are oral and provide cure rates approaching 100% with very low toxicity.[7–9] The ability to treat and cure HCV in the vast majority of cases identified and started on therapy increases the urgency of designing testing strategies that maximize case identification. Implementation of HCV screening and treatment could reduce the global prevalence of chronic HCV infection.
6. *The cost of new therapies* – while new treatments to cure HCV are exciting, they are costly and are not available in all settings.[10] Some low- and middle-income countries have had success negotiating the cost of new therapy, with 12-week treatment course priced at <$1000 in Egypt and $150 in India, for example.[11] Still, the cost of therapy makes accurate diagnosis essential. False-positive screening results will lead to patients who are not infected with HCV being treated with costly drugs.

The optimal screening strategy for a given setting will balance the costs associated with false-negative and false-positive test results.

3.Methods

Overview

This decision analysis demonstrates the test characteristics of a variety of strategies for testing for HCV and estimates costs of screening. We consider 22 testing strategies evaluated in three prevalence settings: 2.3%, 10%, and 40%. The data demonstrate the trade-offs between false-positive and false-negative results, provide projections of the direct cost of HCV testing in different settings, and are useful for decision-making about investment in screening platforms and modalities. We seek to answer the following four questions:

1. What is the best test for exposure to HCV, an EIA or RDT?
2. Is it beneficial to use two tests for exposure to minimize false-positive results?
3. Among those with a positive test for exposure to HCV, what is the best test for assessing active infection, qualitative HCV RNA, quantitative HCV RNA, or HCV core Ag?
4. How does HCV prevalence impact the choice of testing strategy?

Strategies

A number of testing strategies have been evaluated in the literature, and a recently completed systematic review provides an overview of different strategy configurations.[12] We include three broad types of strategies in our model: (1) those with a single test of HCV exposure followed by testing for active HCV infection, (2) those with two tests of exposure followed by testing for active HCV infection, and (3) those that use a single test to simultaneously assess for both exposure and active infection (Table 1 and Fig. 1).

Strategies that employ a single test of exposure first test for the presence of HCV Ab using either EIA or RDT. If non-reactive, we conclude that there is no serological evidence for exposure to HCV, and no further testing occurs. If the assay is reactive, the patient advances to the next step of testing and undergoes an additional test for the presence of active HCV replication, which can be evaluated with a qualitative RNA assay, a quantitative RNA assay, or an HCV core Ag.

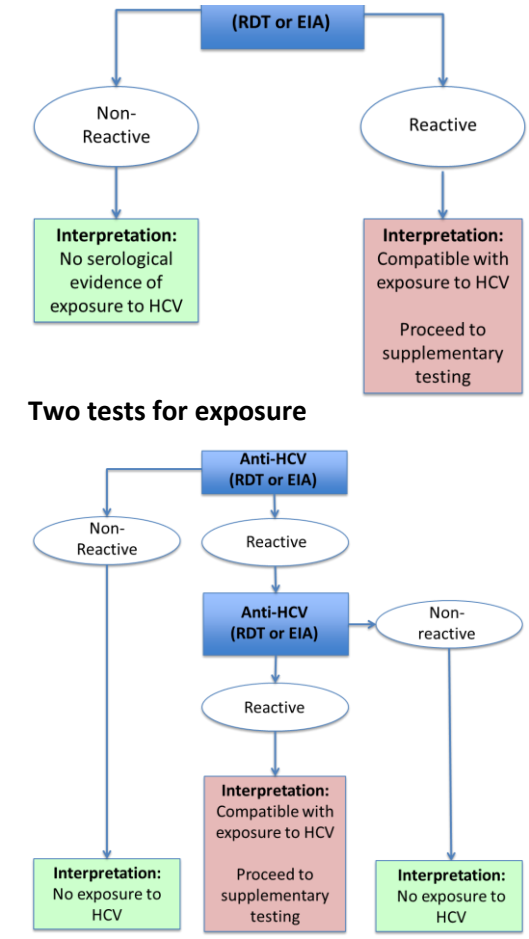
Strategies that employ two tests for exposure typically use those tests in series. First, test the sample with an anti-HCV assay such as EIA or RDT. If the result is non-reactive, there is no serological evidence of exposure to HCV and no further testing occurs. If the first test for exposure is positive, then the laboratory reflexively conducts a second anti-HCV assay on the same specimen (in our model we assume a single sample is collected for the two tests for exposure strategies and so can be tested again without the patient needing to return). If the second anti-HCV assay is non-reactive, we conclude no evidence of exposure to HCV and no further testing occurs. If positive, the patient proceeds to testing for the detection of active HCV infection with either a core antigen, qualitative RNA, or quantitative RNA assay.

Strategies that use a single test to assess for both exposure and active infection employ one of the three tests for active HCV replication (qualitative HCV RNA, quantitative HCV RNA, or HCV core Ag) without previous testing. Positive results indicate both exposure and active infection. Negative results do not provide information about exposure, but confirm that there is no active infection.

Table 1. HCV testing strategies

Test for exposure	Test for active infection
Single test for exposure	
EIA	Antigen
EIA	Antigen/Qualitative RNA
EIA	Antigen/Quantitative RNA
EIA	Qualitative RNA
EIA	Quantitative RNA
RDT	Antigen
RDT	Antigen/Qualitative RNA
RDT	Antigen/Quantitative RNA
RDT	Qualitative RNA
RDT	Quantitative RNA
Two tests for exposure	
EIA/EIA	Antigen
EIA/EIA	Qualitative RNA
EIA/EIA	Quantitative RNA
EIA/RDT	Antigen
EIA/RDT	Qualitative RNA

Fig. 1. HCV testing strategies



Model structure

We use a deterministic decision model (Excel, Microsoft Corporation, Seattle, WA) to assess the performance of 22 testing strategies in three prevalence settings. The model simulates the testing experience and outcomes of a cohort of patients characterized by a given prevalence of active HCV infection. Among those with chronic HCV, the probability of a positive test result is a function of test sensitivity, whereas among those without active HCV, the probability of a positive test result is a function of 1-specificity. Outcomes from the model include positive predictive value (PPV) of the entire testing strategy, negative predictive value (NPV) of the strategy, the percentage of all HCV cases identified, the cost of testing, and the cost per case of HCV identified.

Model inputs

Inputs for the model include estimates of screening and diagnostic test characteristics, the costs of screening tests, and the probability that patients return to care when asked to navigate a multiple-step screening strategy (Table 2).

Diagnostic performance: whenever possible, test characteristics in this decision analysis reflect the pooled sensitivity and specificity reported by the recently completed systematic reviews and meta-analyses commissioned ahead of the global HCV screening guidance meeting.[12–14]

When data are not available from meta-analyses, we identify parameter estimates from the medical literature[6, 15, 16] and the expert opinion of the research.

Table 2. Selected model inputs

Variable	Value	Sensitivity range	Source
Test sensitivity			
RDT	93.0	91.0–95.0	13
EIA	99.5	98.0–99.9	13
Antigen	93.4	88.7–96.2	14
Quantitative RNA	96.0	90.0–99.0	15
Qualitative RNA	98.0	92.9–99.4	16
Test Specificity			
RDT	98.0	97.0–99.0	13
EIA	89.1	78.0–95.0	13
Antigen	98.7	96.9–99.4	14
Quantitative RNA	99.0	95.0–99.9	15
Qualitative RNA	99.0	96.9–99.2	16
Cascade of Care			
Accept test	90%	–	17
Return for results of test for exposure	74%	–	6
Test costs			
RDT	$2	$1–$3	18

EIA	$2	$1–$3	Assumption*
Quantitative RNA	$25	$13–$38	[18]
Qualitative RNA	$25	$13–$38	Assumption*
Antigen	$35	$18–$53	[18]
Fixed costs			
Laboratory screening	$15	$8–$23	Assumption*
Point-of-care screening	$20	$10–$30	

RDT: rapid diagnostic test, EIA: enzyme-linked immunoassay

*Assumptions based on expert opinion of the research team

Costs of testing: the specific cost of each testing strategy in every country likely differs and is changing over time. To estimate costs for various screening and diagnostic platforms we use the recently published Médecins Sans Frontières (MSF) product guide,[18] as well as expert opinion of the research team. Costs in this analysis are intended to provide a general idea of the relative cost per testing strategy and cost per identified case for different testing strategies. Actual costs will be specific to individual countries based on purchase volume, currently held testing equipment, and other factors. Consequently, the preferred testing strategy may differ between countries.

Analytic approach

We first established a base case scenario, which is meant to reflect the current standard of care:

1. We assume that testing is performed in a centralized reference laboratory with a delay of at least one day in obtaining the results of tests for HCV exposure such that patients must re-present to the site of screening for testing for active HCV infection. Some individuals are lost to follow up when they fail to return for testing.
2. Those who are lost to follow-up are counted as negative results, affecting the NPV of testing. Additionally, those lost do not accrue costs related to testing for active HCV infection.
3. For strategies that use two tests for HCV exposure, we assume that the tests are performed in series, and that they are performed on the same specimen. As a result, patients who have a negative first test for exposure accrue no additional testing costs.

We used the model to estimate PPV, NPV, percentage of cases identified, and costs in three prevalence settings.

Next, we considered a scenario in which the results of testing for exposure to HCV are available quickly, such that the person being screened can undergo testing for active HCV infection at the same visit, eliminating the need to return for a second step in the testing cascade.

Finally, we conducted a series of one-way sensitivity analyses, in which we ranged test characteristics and costs to explore critical dynamics that influence decision-making, as well as assess for the potential impact of uncertainty in the input parameters on the results.

Because the large number of possible testing strategies makes interpretation difficult, we first eliminate strategies that are clearly not a good use of limited testing resources. First, we eliminate all strategies with test performance below a threshold of 95% PPV and NPV. Next we identify strategies that have both lower NPV and PPV than an alternative, as well as a higher cost of testing. Such strategies would result in a greater number of false positives AND false negatives than another option, while increasing cost. No matter what the cost of HCV therapy, or of untreated HCV, such strategies are never preferred and can be considered "inefficient".

When we eliminate strategies with unacceptable performance, as well as those that were inefficient, the remaining strategies represent tradeoffs between PPV, NPV, and cost. Without formal cost–effectiveness results, it is not possible to make quantitative conclusions about which strategy is preferred. We provide tables, however, that clearly illustrate those trade-offs, likely allow for qualitative conclusions, and that can inform rational discussion and policy-making.

4.Results

Which screening test should be preferred, RDT or EIA?

Summary: in general, either RDT or EIA provide similar testing outcomes and cost. Choice of which test to use should depend on in country costs and previous investments. In very high-prevalence settings, however, it may be best to not use either test, and to instead employ a one-test strategy that uses NAT or HCV core Ag to directly assess for active HCV infection without previous tests for exposure.

EIA is a slightly more sensitive test than RDT, but is also less specific. In lower-prevalence settings (2.3%), strategies that utilize EIA result in slightly more false-positive results (lower PPV) and slightly fewer false-negative results (higher NPV) than strategies that employ RDT, at a similar cost for testing. Without formal cost–effectiveness assessments, it is not possible to quantitatively conclude whether screening strategies should focus on minimizing false-positive or false-negative results. Generally speaking, however, there are multiple strategies using either RDT or EIA with PPV and NPV >99% that are likely appropriate for testing. The choice of EIA or RDT should be based largely on cost, in-country feasibility, and previous investments in technologies that may be leveraged for HCV screening.

In the higher-prevalence setting (10%), strategies using either RDT or EIA had similar performance and cost. The choice of which platform to use for two tests for exposure should be a function of specific, in-country costs, and previous investments in technology that could be leveraged for HCV screening, while a single EIA may not be an efficient option at this higher prevalence.

At very high prevalence (40%), assumptions about loss to follow up between testing for HCV exposure and active infection have an increasingly large impact on NPV. With our assumed loss to follow up (26%), only strategies that employ a single test for active infection have NPV >95%. Further, such one-step strategies identify a substantially greater proportion of HCV infections.

What is the value added by performing a second test for exposure?

Summary: when it is possible to reflexively conduct repeat testing for HCV exposure using the same specimen, it can be cost saving to employ two tests for exposure in lower (2.3%) and high-prevalence (10%) settings. If reflex testing is not possible, however, then there is little value added by the two tests for exposure strategy. In very high-prevalence settings (40%), loss to follow up results in strategies that test directly for active HCV infection (no test for exposure) being preferred.

In low-prevalence settings (2.3%), employing a second test for exposure improves PPV slightly and reduces costs by minimizing the need for more costly testing for active HCV infection. Similarly, at higher prevalence (10%), the addition of a second test for exposure improves PPV, has minimal impact in NPV, and reduces cost.

It is important to note, however, that the model assumes reflexive testing on one specimen such that patients who have a negative first test for exposure accrue no cost for additional testing. If in reality, a two-test strategy for exposure requires the collection of two specimens, or if there are overhead costs associated with the second test for exposure, then the two-test for exposure strategy likely increases cost with little gain in performance.

Finally, in the 40% prevalence setting, with 26% of individuals not returning for results of the test for exposure and confirmatory testing, our model demonstrates that the only strategies with NPV >95% are strategies that test directly for active HCV infection.

What diagnostic test is preferred, quantitative HCV RNA, qualitative HCV RNA, or HCV core Ag?

Summary: at current cost, qualitative RNA emerges as the preferred test for active HCV infection. Quantitative RNA is slightly less sensitive than qualitative RNA, and therefore results in slightly more false-negative results at similar cost. The difference in testing outcomes is small, however, and countries with previous investment in quantitative RNA platforms, as well as those that can leverage existing HIV testing infrastructure to conduct quantitative HCV RNA testing should do so. Further, if countries anticipate needing quantitative RNA for monitoring HCV therapy, then quantitative RNA should likely be used for HCV testing as well. At its current approximate cost, HCV core Ag results in worse testing accuracy and higher cost than either quantitative or qualitative RNA.

HCV core Ag has lower sensitivity and specificity than HCV RNA and is costly. As a result, under the base case assumptions about core Ag cost, all strategies that used core Ag as a test for active infection had lower PPV and NPV at a higher cost than alternative strategies that utilized either quantitative or qualitative HCV RNA. Thus, assuming current cost of core Ag ($35),[18] it is not an efficient test for active HCV infection. If, however, HCV core Ag becomes less expensive, it could be a very economically attractive option. In the base case analysis, core Ag was more costly than HCV RNA ($35 vs $25). When core Ag became less costly than RNA ($15 vs $25) it became very appealing, despite having slightly worse test characteristics. For example, with a less costly core Ag test in a 2.3% prevalence setting, two RDTs followed by confirmatory diagnosis using HCV core Ag had the highest PPV (100%) and outstanding NPV (99.1%) with the lowest cost of screening ($17.42). Thus, if countries are able to negotiate substantially lower core Ag prices, or the technology evolves to a lower cost platform, core Ag could be an excellent choice for screening.

Comparing quantitative RNA to qualitative RNA reveals no large differences in screening performance or cost. As a result, choice between these tests should likely be based entirely by in-country feasibility and price.

At higher HCV prevalence such as 10%, it is clearly preferred to use the most sensitive diagnostic test possible. Using a less sensitive, more specific diagnostic test leads to false-negative results while preventing very few false-positive outcomes. Therefore, qualitative HCV RNA emerges as preferred. With the base case cost assumptions, strategies that used quantitative RNA or HCV core Ag provided worse test performance often with higher cost compared to using qualitative HCV RNA.

In a high 40% prevalence setting, nearly every individual with positive tests for HCV is a "true positive". As a result, diagnostic testing should be as sensitive as possible in order to avoid false-negative results. This dynamic is clear in the results of the decision analysis, in which a single qualitative RNA (which is the most sensitive RNA test) is preferred to a single quantitative RNA.

How does HCV prevalence impact the choice of testing strategy?

Summary: as demonstrated above, prevalence is central to the choice of testing strategy. There is no one strategy that is appropriate to all prevalence settings. In lower-prevalence settings, the preferred testing strategies limit false-positive results. It can be efficient to use two tests for HCV exposure (assuming that they can be done reflexively and on one specimen). In contrast, in very high-prevalence settings, preferred strategies are sensitive and minimize false-negative results. In such settings, the high probability of being infected, as well as the potential for missed cases due to loss to follow up, results in strategies that directly test for active infection (no test for exposure) being preferred.

Scenario in which results of tests for exposure are available quickly such that the patient can undergo testing for exposure and active infection at the same visit

Same-day results reporting reduces loss to follow up because patients with positive tests for exposure do not need to return to the screening site for testing for active infection. This dynamic is most apparent in very high-prevalence settings (40%). With base case assumptions about loss to follow up, all of the two-step strategies resulted in missed cases of HCV and low NPV. As a result, strategies that test directly for active HCV infection were preferred. When patients can undergo testing for exposure and active infection without the need to leave the testing site and return at another time, many more strategies become viable with PPV and NPV greater than 95% and lower cost per case identified (Tables 6–8). Whenever possible, but especially in very high-prevalence settings, it is always preferred to use same-day tests that allow for testing for exposure and active infection at the same visit.

How to screen summary

Integrating all of the scenarios and data above reveals that while not every HCV testing approach is efficient, there are multiple possible strategies for testing for HCV exposure and active infection that countries can employ to test for HCV. The choice of EIA or RDT is not highly significant and should be based on in-country cost and feasibility considerations. In lower-prevalence settings, it is potentially cost saving to use two tests for exposure before testing for active infection, but only when the two tests can be conducted reflexively and on the same specimen. In the highest-prevalence settings, avoiding missed cases becomes the dominating

consideration. If possible, it is best to test for exposure and active replication at one visit. If such same-day testing is not feasible, then it is best to employ a one-step strategy that tests directly for active HCV infection without testing for exposure.

Table 3. Base case analysis, testing for HCV in a 2.3% prevalence setting

Test(s) for exposure	Test for active infection	PPV	NPV	Cost/ strategy	Cases identified	Cost/ identified
RDT/RDT	Qualitative RNA	100.0%	99.1%	$17.42	56.2%	$1213
RDT/RDT	Quantitative RNA	100.0%	99.1%	$17.42	55.3%	$1232
RDT/RDT	Antigen	100.0%	99.1%	$17.57	53.8%	$1278
RDT/EIA	Qualitative RNA	99.9%	99.2%	$17.48	60.1%	$1138
RDT/EIA	Quantitative RNA	99.9%	99.1%	$17.48	59.2%	$1156
RDT/EIA	Antigen	99.9%	99.1%	$17.65	57.6%	$1200
EIA/EIA	Qualitative RNA	99.5%	99.0%	$17.70	64.3%	$1078
EIA/EIA	Quantitative RNA	99.5%	99.0%	$17.70	63.3%	$1094
EIA/EIA	Antigen	99.3%	99.0%	$17.96	61.6%	$1141
RDT	Qualitative RNA	99.1%	98.7%	$17.76	60.4%	$1151
RDT	Quantitative RNA	99.1%	98.7%	$17.76	59.5%	$1169
RDT	Antigen	98.7%	98.7%	$18.06	57.9%	$1222
RDT	Antigen/Qualitative RNA	98.0%	98.8%	$18.44	61.8%	$1167
RDT	Antigen/Quantitative RNA	97.9%	98.8%	$18.44	61.8%	$1168
EIA	Qualitative RNA	95.4%	96.5%	$19.39	64.6%	$1175
EIA	Quantitative RNA	95.4%	96.5%	$19.39	63.6%	$1193
EIA	Antigen	93.9%	96.5%	$20.35	61.9%	$1287
EIA	Antigen/Qualitative RNA	90.4%	96.6%	$22.32	66.2%	$1320
EIA	Antigen/Quantitative RNA	90.4%	96.6%	$22.32	66.1%	$1322
Qualitative RNA		69.7%	99.9%	$40.00	87.8%	$1784
Quantitative RNA		69.3%	99.9%	$40.00	86.4%	$1812
Antigen		62.8%	99.8%	$50.00	84.1%	$2328

HCV: hepatitis C virus, RDT: rapid diagnostic test, EIA: enzyme-linked immunoassay

Table 4. Base case analysis, testing for HCV in 10% prevalence setting

Test(s) for exposure	Test for active infection	PPV	NPV	Cost/ strategy	Cases identified	Cost/ identified
RDT/EIA	Qualitative RNA	100.0%	96.4%	$18.94	60.1%	$284
RDT/EIA	Quantitative RNA	100.0%	96.3%	$18.94	59.2%	$288
RDT/EIA	Antigen	100.0%	96.1%	$19.64	57.6%	$307
RDT/RDT	Qualitative RNA	100.0%	96.0%	$18.78	56.2%	$301
RDT/RDT	Quantitative RNA	100.0%	95.9%	$18.78	55.3%	$306
RDT/RDT	Antigen	100.0%	95.7%	$19.42	53.8%	$325
EIA/EIA	Qualitative RNA	99.9%	96.6%	$19.25	64.3%	$269
EIA/EIA	Quantitative RNA	99.9%	96.5%	$19.25	63.3%	$274
EIA/EIA	Antigen	99.8%	96.3%	$20.06	61.6%	$293
RDT	Qualitative RNA	99.8%	96.0%	$19.05	60.4%	$284
RDT	Quantitative RNA	99.8%	95.9%	$19.05	59.5%	$288
RDT	Antigen	99.7%	95.7%	$19.87	57.9%	$309
RDT	Antigen/Qualitative RNA	99.6%	96.1%	$20.32	61.8%	$296
RDT	Antigen/Quantitative RNA	99.6%	96.1%	$20.32	61.8%	$296
EIA	Qualitative RNA	99.0%	94.2%	$20.66	64.6%	$288
EIA	Quantitative RNA	99.0%	94.1%	$20.66	63.6%	$292
EIA	Antigen	98.6%	93.9%	$22.12	61.9%	$322
EIA	Antigen/Qualitative RNA	97.8%	94.4%	$24.03	66.2%	$327
EIA	Antigen/Quantitative RNA	97.8%	94.4%	$24.03	66.1%	$327
Qualitative RNA		91.5%	99.7%	$40.00	87.8%	$410
Quantitative RNA		91.4%	99.6%	$40.00	86.4%	$417
Antigen		88.9%	99.3%	$50.00	84.1%	$535

HCV: hepatitis C virus, RDT: rapid diagnostic test, EIA: enzyme-linked immunoassay

Table 5. Base case analysis, testing for HCV in 40% prevalence setting

Test(s) for exposure	Test for active infection	PPV	NPV	Cost/ strategy	Cases identified	Cost/ identified
Qualitative RNA		98.5%	98.3%	$40.00	87.8%	$103
Quantitative RNA		98.5%	97.4%	$40.00	86.4%	$104
Antigen		98.0%	95.7%	$50.00	84.1%	$134
EIA/EIA	Qualitative RNA	100.0%	83.7%	$25.26	64.3%	$88
EIA/EIA	Quantitative RNA	100.0%	83.2%	$25.26	63.3%	$90
EIA	Antigen/Qualitative RNA	99.6%	82.6%	$30.68	66.2%	$104
EIA	Antigen/Quantitative RNA	99.6%	82.5%	$30.68	66.1%	$104
EIA/EIA	Antigen	100.0%	82.4%	$28.25	61.6%	$103
RDT	Antigen/Qualitative RNA	99.9%	82.3%	$27.62	61.8%	$100
RDT	Antigen/Quantitative RNA	99.9%	82.3%	$27.62	61.8%	$101
RDT/EIA	Qualitative RNA	100.0%	81.8%	$24.61	60.1%	$92
EIA	Qualitative RNA	99.8%	81.8%	$25.57	64.6%	$89
RDT	Qualitative RNA	100.0%	81.6%	$24.10	60.4%	$90
RDT/EIA	Quantitative RNA	100.0%	81.4%	$24.61	59.2%	$94
EIA	Quantitative RNA	99.8%	81.3%	$25.57	63.6%	$90
RDT	Quantitative RNA	100.0%	81.1%	$24.10	59.5%	$91
RDT/EIA	Antigen	100.0%	80.6%	$27.36	57.6%	$107
EIA	Antigen	99.8%	80.4%	$29.00	61.9%	$105
RDT	Antigen	100.0%	80.3%	$26.95	57.9%	$105
RDT/RDT	Qualitative RNA	100.0%	80.0%	$24.10	56.2%	$97
RDT/RDT	Quantitative RNA	100.0%	79.5%	$24.10	55.3%	$98
RDT/RDT	Antigen	100.0%	78.8%	$26.66	53.8%	$111

HCV: hepatitis C virus, RDT: rapid diagnostic test, EIA: enzyme-linked immunoassay

Table 6. Scenario assuming same-day results for tests for exposure (decreased loss to follow up) in a 2.3% prevalence setting

Test(s) for exposure	Test for active infection	PPV	NPV	Cost/ strategy	Cases identified	Cost/ identified
RDT/RDT	Qualitative RNA	100.0%	99.6%	$17.55	84.3%	$905
RDT/RDT	Quantitative RNA	100.0%	99.6%	$17.55	83.0%	$919
RDT/RDT	Antigen	100.0%	99.5%	$17.75	80.8%	$955
RDT/EIA	Qualitative RNA	99.9%	99.8%	$17.63	90.2%	$850
RDT/EIA	Quantitative RNA	99.9%	99.7%	$17.63	88.8%	$863
RDT/EIA	Antigen	99.9%	99.7%	$17.87	86.4%	$899
EIA/EIA	Qualitative RNA	99.5%	99.9%	$17.93	96.5%	$808
EIA/EIA	Quantitative RNA	99.5%	99.9%	$17.93	95.0%	$820
EIA/EIA	Antigen	99.3%	99.8%	$18.27	92.5%	$859
RDT	Qualitative RNA	99.1%	99.8%	$18.02	90.7%	$864
RDT	Quantitative RNA	99.1%	99.7%	$18.02	89.3%	$878
RDT	Antigen	98.7%	99.7%	$18.43	86.9%	$923
RDT	Antigen/Qualitative RNA	98.0%	99.8%	$18.95	92.8%	$887
RDT	Antigen/Quantitative RNA	97.9%	99.8%	$18.95	92.8%	$888
EIA	Qualitative RNA	95.4%	99.9%	$20.23	97.0%	$907
EIA	Quantitative RNA	95.4%	99.9%	$20.23	95.5%	$921
EIA	Antigen	93.9%	99.8%	$21.53	92.9%	$1 007
EIA	Antigen/Qualitative RNA	90.4%	100.0%	$24.19	99.3%	$1 059
EIA	Antigen/Quantitative RNA	90.4%	100.0%	$24.19	99.2%	$1 060
Qualitative RNA		69.7%	99.9%	$40.00	97.5%	$1 784
Quantitative		69.3%	99.9%	$40.00	96.0%	$1 812

RNA					
Antigen	62.8%	99.8%	$50.00	93.4%	$2 328

HCV: hepatitis C virus, RDT: rapid diagnostic test, EIA: enzyme-linked immunoassay

Table 7. Scenario assuming same-day results for tests for exposure (decreased loss to follow up) in a 10% prevalence setting

Test(s) for Exposure	Test for active infection	PPV	NPV	Cost/ Strategy	Cases Identified	Cost/ Identified
RDT/EIA	Qualitative RNA	100.0%	98.9%	$19.55	90.2%	$217
RDT/EIA	Quantitative RNA	100.0%	98.8%	$19.55	88.8%	$220
RDT/EIA	Antigen	100.0%	98.5%	$20.50	86.4%	$237
RDT/RDT	Qualitative RNA	100.0%	98.3%	$19.34	84.3%	$229
RDT/RDT	Quantitative RNA	100.0%	98.1%	$19.34	83.0%	$233
RDT/RDT	Antigen	100.0%	97.9%	$20.21	80.8%	$250
EIA/EIA	Qualitative RNA	99.9%	99.6%	$19.96	96.5%	$207
EIA/EIA	Quantitative RNA	99.9%	99.5%	$19.96	95.0%	$210
EIA/EIA	Antigen	99.8%	99.2%	$21.06	92.5%	$228
RDT	Qualitative RNA	99.8%	99.0%	$19.78	90.7%	$218
RDT	Quantitative RNA	99.8%	98.8%	$19.78	89.3%	$221
RDT	Antigen	99.7%	98.6%	$20.89	86.9%	$240
RDT	Antigen/Qualitative RNA	99.6%	99.2%	$21.48	92.8%	$231
RDT	Antigen/Quantitative RNA	99.6%	99.2%	$21.48	92.8%	$232
EIA	Qualitative RNA	99.0%	99.7%	$21.94	97.0%	$226
EIA	Quantitative RNA	99.0%	99.5%	$21.94	95.5%	$230
EIA	Antigen	98.6%	99.2%	$23.92	92.9%	$257
EIA	Antigen/Qualitative RNA	97.8%	99.9%	$26.50	99.3%	$267
EIA	Antigen/Quantitative RNA	97.8%	99.9%	$26.50	99.2%	$267
Qualitative RNA		91.5%	99.7%	$40.00	97.5%	$410
Quantitative RNA		91.4%	99.6%	$40.00	96.0%	$417

Antigen		88.9%	99.3%	$50.00	93.4%	$535

HCV: hepatitis C virus, RDT: rapid diagnostic test, EIA: enzyme-linked immunoassay

Table 8: Scenario assuming same-day results for tests for exposure (decreased loss to follow up) in a 40% prevalence setting

Test(s) for exposure	Test for active infection	PPV	NPV	Cost/ Strategy	Cases Identified	Cost/ Identified
EIA/EIA	Qualitative RNA	100.0%	97.7%	$27.88	96.5%	$72
EIA/EIA	Quantitative RNA	100.0%	96.8%	$27.88	95.0%	$73
EIA/EIA	Antigen	100.0%	95.2%	$31.92	92.5%	$86
RDT	Antigen/Qualitative RNA	99.9%	95.4%	$31.35	92.8%	$84
RDT	Antigen/Quantitative RNA	99.9%	95.4%	$31.35	92.8%	$84
EIA	Qualitative RNA	99.8%	98.0%	$28.59	97.0%	$74
EIA	Quantitative RNA	99.8%	97.1%	$28.59	95.5%	$75
EIA	Antigen	99.8%	95.5%	$33.22	92.9%	$89
EIA	Antigen/Qualitative RNA	99.6%	99.6%	$35.49	99.3%	$89
EIA	Antigen/Quantitative RNA	99.6%	99.5%	$35.49	99.2%	$89
Qualitative RNA		98.5%	98.3%	$40.00	97.5%	$103
Quantitative RNA		98.5%	97.4%	$40.00	96.0%	$104
Antigen		98.0%	95.7%	$50.00	93.4%	$134
RDT	Qualitative RNA	100.0%	94.1%	$26.60	90.7%	$73
RDT/EIA	Qualitative RNA	100.0%	93.9%	$27.03	90.2%	$75
RDT	Quantitative RNA	100.0%	93.3%	$26.60	89.3%	$74
RDT/EIA	Quantitative RNA	100.0%	93.1%	$27.03	88.8%	$76
RDT	Antigen	100.0%	91.9%	$30.44	86.9%	$88
RDT/EIA	Antigen	100.0%	91.7%	$30.74	86.4%	$89
RDT/RDT	Qualitative RNA	100.0%	90.5%	$26.35	84.3%	$78
RDT/RDT	Quantitative RNA	100.0%	89.8%	$26.35	83.0%	$79
RDT/RDT	Antigen	100.0%	88.6%	$29.81	80.8%	$92

HCV: hepatitis C virus, RDT: rapid diagnostic test, EIA: enzyme-linked immunoassay

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ANNEX 7. Summary of Declared Interests

Participants	Declared interests	Declared amount	Level of participation
Isabelle Andrieux-Meyer	*Personal*: None *Institutional*: Employer (MSF) has received a UNITAID grant HIV-HCV grant to the value of 14 Million USD (current).	US$14 million	Full
Lara Tavoschi	*Personal:* None *Institutional:* Participation in the meeting covered by ECDC The outcomes of the meeting will substantially impact ECDC's work on the subject.		Full
Manal El-Sayed	*Personal:* Travel support from MSD and Roche Institutional: None	Not declared	Full
Charles Gore	*Personal*: None *Institutional*: President of World Hepatitis Alliance that receives grants and other support from BMS, Boehringer Ingelheim, Janssen, Roche, Roche diagnostics, Merck, GSK, Gilead, AbbVie, Abbott, Achillion, Bayer, Novartis	not declared; but all the funding for the organization comes from these firms	Full
Richard Njouom	*Personal*: None *Institutional*: None		Full
Margaret Hellard (Chair)	*Personal*: Research support from Gilead and Abbvie to support an investigator initiated independent research study. Provided expert opinion to Australian Parliamentary committee on hepatitis. *Institutional*: None	3.5 million plus drugs (research support was not for income)	Full
Niklas Luhmann	*Personal*: None *Institutional*: None		Full
Teri Roberts	*Personal*: None *Institutional*: None		Full
Jacinto Amandua	*Personal: None* *Institutional: None*		Full
John Parry	Personal: Expert advisor on other WHO panels (diagnostics and testing technologies) Institutional: Past research on DBS testing supported by Gilead (no longer current, study complete).	£30,000 (no longer current)	Full
Khwairakpam Giten Singh	*Personal: None* *Institutional: None*		Full

Roger Chou (methodologist)	*Personal*: None *Institutional*: None		Full
Michael Ninburg	*Personal*: None *Institutional*: None		Full
Trevor Peter	*Personal*: None *Institutional*: None		Full

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