STORAGE OF INACTIVATED OIL ADJUVANTED FOOT-AND-MOUTH DISEASE VACCINE AT LOW TEMPERATURE[1]

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SHORT COMMUNICATION

Because of the instability of foot-and-mouth disease (FMD) vaccines, even under conditions of continuous refrigeration, it has been impossible to prepare a standard reference vaccine, which can be used for a prolonged period of time. The best approximation for such a standard vaccine with present technology, would probably be the formulation of a vaccine from purified known antigens, stored at very low temperatures. However, the required manipulation and formulation always would cast some doubts regarding its value as a reference reagent.

In April 1980 the Pan American Foot-and-Mouth Disease Center (PAFMDC) stored a batch of about 3000 dosis of inactivated oil-adjuvanted FMD vaccine at -70°C. This vaccine was prepared 8 months earlier and had been kept at +4°C until the moment of freezing. Table 1 shows the infectivity and complement fixation (CF) titers of the antigens. The vaccine was formulated according to the standard methods used at that time at the PAFMDC. Briefly, the antigens were inactivated with binary ethylenimine (BEI) (*1*); the oil phase consisted of 90% Marcol 52 and 10% Arlacel A (Atlas); equal parts of the aqueous phase and the oil phase were emulsified by means of semi industrial emulsification equipment developed by the PAFMDC in collaboration with a local firm.

The results of vaccine potency tests at 30 and 180 days post-vaccination (DPV) are given in Table 2. In addition 3 months after formulation, the vaccine was applied in 14 young cattle six to eight months old. The sera of these cattle were assayed by the mouse protection test (*2*) 30, 90 and 180 DPV (Table 3) with results expressed as the mean expected percentage of protection (EPP) (*3, 4*). Cattle challenge test results and serological data indicated that the vaccine was of very satisfactory quality. After a storage period of 4 years and 3 months at -70°C some of the vaccine was placed for 24 hours at +4°C and then used to vaccinate 22 cattle similar to those in the earlier test. The results of the mouse protection tests of sera collected at 30, 90 and 190 DPV are also shown in

TABLE 1. *Infectivity and complement fixation titers of the antigens used for the formulation of the inactivated FMD vaccine*

Antigens	ID_{50}/ml	CF titers
O_1 Campos	7.5	1/20
A_{24} Cruzeiro	8.2	1/18
C_3 Resende	8.2	1/22

TABLE 2. *FMD vaccine potency test. 50% cattle protective dose*

	Number of cattle protected/vaccinated	
Dilution[a]	30 DPV[b]	180 DPV
1/1	5/5	5/6
1/4	5/5	6/6
1/16	5/5	4/5
1/64	4/4	3/6
1/256	2/5	1/6
Controls	0/2	0/2

[a] Diluted in emulsion without antigens.

[b] DPV = Days post-vaccination.

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Table 3. These results demonstrate that oil-adjuvanted FMD vaccine can be stored at -70°C without detectable loss of potency for a prolonged period of time. Such vaccine could serve as a reference vaccine for the purpose of standardization in vaccine control procedures.

TABLE 3. *Mean expected percentage of protection*

	Days post-vaccination					
	30	90	180	30	90	180
Sera	Vaccinated in 1979			Vaccinated in 1984		
O_1 Campos	95.8	85.5	53.9	98.2	95.4	76.5
A_{24} Cruzeiro	94.3	94.8	87.5	98.6	97.0	91.7
C_3 Resende	97.7	78.2	62.9	97.5	92.8	75.1

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