MONTANIDE 888 EMULSIFYING AGENT FOR PREPARATION OF OIL-ADJUVANTED FOOT-AND-MOUTH DISEASE VACCINE

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SHORT COMMUNICATION

The production of oil-adjuvanted vaccines (Freund's incomplete adjuvant) requires the use of an antigen in aqueous suspension, a light mineral oil and an emulsifying agent that permits dispersion of the inactivated antigen (aqueous suspension) throughout the mineral oil in order to form a stable water-in-oil emulsion.

The Pan American Foot-and-Mouth Disease Center (PAFMDC) uses antigen produced in BHK_{21}, Clon 13 cell cultures on monolayers or in suspension (*1*), and a USP light mineral oil (Marcol 52[3]). The emulsifying agent originally used was mannide monooleate (Arlacel A[4]), but it was later replaced by monoanhydrous oleate and deanhydromannide (Montanide 80[5]). Comparative tests with vaccines conducted at the PAFMDC showed that Montanide 80 possessed the same properties as Arlacel A (*6*) and is guaranteed by more complete technical information from the manufacturer.

The formulation of the vaccine produced at the PAFMDC contains 50% aqueous phase and 50% oily phase composed of Marcol 52 (90%) and emulsifying agent (10%). The mixture is thoroughly emulsified by a mechanical process in an industrial emulsifier[6] especially developed for the purpose. The end-product has a high viscosity level.

In order to resolve that problem, and by request of the PAFMDC, SEPPIC developed anhydromannide octodocenate, a new lipophilic emulsifying agent having a 5.0 hydrophilic/lipophilic balance (HLB). The emulsifying agent, Montanide 888[7], meets all the physical and biological tests required for products of this class. It also permits the production of easier to handle low-viscosity, water-in-oil primary emulsions.

The viscosity of the vaccine prepared with Montanide 888, measured in the Brokkfield LVT viscosity meter with the sample at 25°C and the machine set at 12 RPM, is approximately 300 cps. This is significantly lower than the 2400 cps recorded for the vaccines prepared with Arlacel A or Montanide 80. The viscosity meter was set at the same speed and temperature for all cases.

When samples of both vaccines are centrifuged at 1000 g for 60 minutes, similar results are recorded: no antigen is separated to the bottom of the tube, but the slight separation observed in the upper part is somewhat less and also more turbid in the preparation with Montanide 888. Both vaccines exceed 2 years' stability at 4°C. Stability at 37°C is greater than 6 months for the vaccines with Montanide 888 and is approximately 30 days for the vaccines prepared with Montanide 80.

In a preliminary test conducted at the Pan American Foot-and-Mouth Disease Center, a vaccine prepared with a sample of Montanide 888 was compared with a vaccine prepared with Montanide 80. Both vaccines were administered to young cattle and produced similar immunological performance during 6 months post-vaccination. The first vaccine was less viscous (*3*).

In the research carried out, immunological performance and stability were checked through several experiments in which vaccines produced from four different batches of Montanide 888

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[3] Marcol 52 – Exxon Corporation, U.S.A.

[4] Arlacel A – ICI American Inc. Atlas Chemicals Division, U.S.A.

[5] Montanide 80 – SEPPIC, Paris, France.

[6] Caldeiraria e Mecânica INOX. Mauá, SP, Brasil.

[7] Montanide 888 – SEPPIC, Paris, France.

were compared with vaccines produced with the same antigenic composition and the same mineral oil, but taking a known batch of Montanide 80 as reference. In some tests the vaccines were prepared in 2.0-liter volumes in a benchtop emulsifier[8], while others were produced in 360-liter volumes in the industrial emulsifier mentioned above.

In experiment 2 the vaccines with both emulsifying agents were produced with the aqueous phase formed of inactivated virus adsorbed to aluminum hidroxide (*2*). The final preparation had one part aqueous phase per two parts oily phase.

The tests were performed on 9- to 18-month-old susceptible cattle that had never been vaccinated against foot-and-mouth disease. The serum protection test as per Cunha *et al.* (*4*) was used to assess immunity, which was expressed in expected percentages of protection (EPP) according to Gomes and Astudillo (*5*).

The guinea pig tests utilized PD_{50} in guinea pigs vaccinated intramuscularly with 1/20th of the bovine dose with vaccines diluted in active diluent (emulsion equal to the vaccine, but without virus). They were challenged at 30 days post-vaccination.

The results observed in the six tests in cattle (Table 1) show no significant differences between the two emulsifying agents, whether in recently prepared vaccines or those stored at 4°C for up to 24 months. This observation holds true for both the oil-adjuvanted vaccines and those prepared with antigen adsorbed in aluminum hydroxide (oil/hydroxide). The values recorded in guinea pigs always average higher than the values of vaccines prepared with Montanide 80.

As a consequence of these tests, the PAFMDC began to use Montanide 888 in the preparation of its oil-adjuvanted vaccines, as of September 1982. Excellent results have been recorded in the regular vaccine-control tests and in the serological response of field cattle.

[8]Silverson-Machine (Sales) Ltd. London, England.

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TABLE 1. *Comparison between Montanide 888 and Montanide 80. Tests with fresh vaccines and vaccines stored at 4°C for up to 24 months*

Experiment No.	Vaccines	Type of vaccine Bovine dose	Months stored at 4°C	EPP[a] Virus O 30[c]	O 90	A 30	A 90	C 30	C 90	$GPPD_{50}$[b] Virus O 30[c]	A 30	C 30
1	Vaccine No. 1 Mont. 80	Oily 5 ml	1	79	60	85	70	93	72	32	64	51
	Vaccine No. 2 Mont. 888 (Part. 1)	Oily 5 ml	1	90	88	86	93	97	92	79	128	64
	"	"	24	99	99	92	90	99	97	–	–	–
2	Vaccine No. 3 Mont. 80	Oil/ hydroxide 2.0 ml	9	84	76	94	75	95	75	27	32	12
	Vaccine No. 4 Mont. 888 (Part. 1)	Oil/ hydroxide 2.0 ml	9	93	89	93	71	98	92	32	27	19
	"	"	24	99	98	97	89	99	90	–	–	–
3	Vaccine No. 5 Mont. 80	Oily 5.0 ml Ind. Prod.	3	99	83	91	90	99	83	110	128	32
	Vaccine No. 6 Mont. 888 (Part. 2)	Oily 5.0 ml Ind. Prod.	3	90	86	82	85	97	85	128	200	128
4	Vaccine No. 7 Mont. 80	Oily 5.0 ml	2	77	76	80	69	95	88	–	–	–
	Vaccine No. 8 Mont. 888 (Part. 3)	Oily 5.0 ml	2	83	93	72	84	99	96	–	–	–
5	Vaccine No. 9 Mont. 80	Oily 5,0 ml Ind. Prod.	1	98	–	96	–	98	–	≥32	≥32	16
	Vaccine No. 10 Mont. 888 (Part. 3)	Oily 5.0 ml Ind. Prod.	1	99	–	99	–	98	–	≥32	≥32	≥32
6	Vaccine No. 11 Mont. 80	Oily 5.0 ml Ind. Prod.	1	98	98	94	93	99	97	16	≥32	≥32
	Vaccine No. 12 Mont. 888 (Part. 4)	Oily 5.0 ml Ind. Prod.	1	99	99	97	99	99	99	≥32	≥32	16

[a] Expected percentage of protection.
[b] Guinea pig protective dose 50%.
[c] Days postvaccination.