

## STRATIFIED AND CRYOGENICALLY STORED SACS VACCINES, A NOVEL FORMULATING PROCEDURE FOR EXTENDING THE SHELF-LIFE OF EMERGENCY FOOT-AND-MOUTH DISEASE VACCINES

*P.V. Barnett & R.J. Statham*

**Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Woking,  
Surrey GU24 0NF, United Kingdom**

### Summary

Strategic reserves of foot-and-mouth disease (FMD) antigen have become an integral part of FMD control policy for many countries. They are based on two principles, ready formulated vaccine stored at +4°C, or concentrated antigen preparations held at ultra-low temperature for later formulation. However, the latter is more economical, since ready formulated vaccine, based on oil or aluminium hydroxide/saponin adjuvants, requires regular replacement. This is primarily the result of the vaccine's limited shelf-life, nominally 18 months at +4°C.

Montanide ISA 206 and ISA 25, two >ready-to-formulate= oil adjuvants which can be used in all target species, are ideal for emergency vaccination.

A novel approach of layering the individual components of FMD vaccine in the same primary container and then storing the product at ultra-low temperature is described. This avoids the detrimental effect on potency, normally observed with frozen formulated FMD vaccine. The implications of substantially extending the products shelf-life for emergency vaccination strategy are discussed.

### Introduction

The principle of storing concentrated FMD antigen over liquid nitrogen for later formulation was originally established by Denmark, a non-FMD vaccinating country. The United States set up a similar reserve of FMD antigen concentrates in 1980, to which Canada and Mexico later subscribed and the antigens held by this North American Vaccine Bank (NAVB), if required, would be formulated by the commercial sector. In 1985 the International Vaccine Bank (IVB) was established at Pirbright in the United Kingdom (UK) by a consortium consisting of the UK, Australia, New Zealand, Finland, Ireland, Norway and Sweden. Malta later joined the IVB as an associate member in 1995. However, unlike the NAVB, the IVB has the convenience of its own manufacturing facility allowing vaccine to be formulated and despatched within days of a request. Indeed, early in the 2001 FMD outbreak in the United Kingdom, and at the request of the UK Ministry of Agriculture, Fisheries and Food, the IVB was, for the first time, called upon to produce 500,000 bovine doses of aluminium hydroxide/saponin adjuvanted O1 Manisa vaccine over a 12 day period. The more recent establishment of a European Community FMD antigen reserve and many other examples of individual countries assigning their own FMD reserves, which are maintained commercially or through government support (Ryan, 1999; Garland, 1997; Callis, 1994), underline the increasing popularity of antigen banks.

Conventional formulated FMD vaccine, either oil or aqueous, have a limited shelf life, normally 18-24 months at +4°C and it has been demonstrated that aqueous vaccines prepared from

commercial antigen concentrates are considerably less stable when stored at +4°C (Doel and Pullen, 1990).

Work at the Institute for Animal Health, Pirbright, has shown that a reduction in potency occurs when oil adjuvanted vaccines are stored at either –20°C and –70°C (unpublished data), and therefore neither type of formulation can be frozen under these conditions without detrimental effect.

Montanide ISA 206 and ISA 25 are two >ready-to-formulate= oil adjuvants (SEPPIC, France) which are effective in cattle, pigs and sheep and capable of promoting early protective responses (Doel et al., 1994; Cox et al., 1999; Salt et al., 1995; Salt et al., 1998) making them ideal for use as emergency vaccines. Their potential is enhanced by the simplicity of formulation into oil emulsion vaccines, requiring no complicated high-shear emulsification equipment. We have been monitoring the oil adjuvant component (Montanide ISA 206), which, in an attempt to extend its shelf-life (nominally 2 years at +4°C), has unconventionally been stored at –20°C. This batch, lot 3001, currently in its eighth year of storage, is still a viable component, as quality control tests undertaken by the commercial suppliers, SEPPIC, have shown that two critical parameters, the acid and peroxide values, are still within acceptability limits.

Recent studies on the IVB's antigen concentrates have also established that the shelf-life of these preparations are likely to be well in excess of 15 years (Barnett and Statham, 1998).

Given that both the oil adjuvant and the antigen component can be maintained appropriately at low temperature, and that the 'ready-to-formulate' adjuvant readily forms a stable emulsion, we examined the possibility of extending the shelf-life of the final product by a novel process. Here we describe a procedure applying the main components of FMD vaccine as stratified layers in the same primary container and storing at ultra-low temperature.

## **Materials and Methods**

### **Vaccine preparation**

Vaccine formulations, incorporating FMDV O<sub>1</sub> Lausanne inactivated antigen as either water-in-oil-in-water (W/O/W) emulsion with Montanide ISA 206, or as a oil-in-water (O/W) emulsion with Montanide ISA 25, were prepared conventionally (Barnett et al., 1996), or by a novel procedure, using antigen concentrate held by the IVB over liquid nitrogen with a PD<sub>50</sub> value of 41 per bovine dose. The formulated vaccine contained 5.62 [µ]g of 146S antigen per 2ml bovine dose.

The novel formulation procedure (see Figure 1) involved 4 main steps as follows:-

1. Oil adjuvants Montanide ISA 206 or 25, at the required volume, were aliquoted into the desired primary container, placed in the ultra-low temperature gaseous phase of liquid nitrogen, and snap frozen.
2. The frozen oil adjuvant is then momentarily removed from the low temperature environment and the prerequisite volume of aqueous buffer is carefully layered onto the top of the frozen oil adjuvant to form two distinguishable layers or stratifications. This is immediately and carefully returned to the ultra-low temperature gaseous phase of liquid nitrogen to snap freeze the aqueous buffer.
3. The frozen oil adjuvant and aqueous buffer layers are again momentarily removed from the low temperature environment and the prerequisite volume of concentrated antigen is then layered on top

of the frozen buffer. This is immediately returned to the ultra-low temperature environment to snap freeze the antigen concentrate.

4. When required, the stratified and cryogenically stored (SACS) vaccine are thawed at room temperature, mixed by simply agitation, and administered into the target host.

### **In vivo potency tests**

Vaccine preparations were tested in female Duncan-Hartley guinea pigs, approximately 400-500 gm in weight. Each group of five animals received a specific volume of vaccine of either 1ml, 0.33ml or 0.11ml, administered subcutaneously. Animals were challenged 28 days postvaccination with  $3 \times 10^3$  ID<sub>50</sub> of the homologous guinea pig adapted virus, injected by the intraplantar route. All animals were monitored closely for 7-10 days, and immunised guinea pigs were considered protected if the virus failed to be generalised beyond the challenge site.

Later experiments incorporated dilutions of vaccine instead of the reduced volume dose described previously. Essentially vaccines were diluted in a similarly formulated vaccine that did not contain the antigen component so that the antigen but not the adjuvant was diluted. The dilution range used was three-fold from neat to 1/81. Again animals were challenged 28 days post-vaccination with  $3 \times 10^3$  ID<sub>50</sub> of the homologous guinea pig adapted virus, injected by the intraplantar route and monitored as described previously. This dilution range allowed the potency (PD<sub>50</sub>) of the vaccine to be calculated by the method of Karber (Karber, 1931).

### **Results**

In the first trial, SACS vaccines based on either Montanide ISA 206 or ISA 25 were examined for their stability at ultra-low temperature over a 40 month period. Using a divided dose regime results were encouraging showing that in the absence of any loss in vaccine potency the procedure was not detrimental to either adjuvanted formulation (Table 1).

**Table 1 Potency of SACs vaccines based on Montanide ISA 25 (oil-in-water) and 206 (water-in-oil-in-water) adjuvanted vaccines following long term storage at ultra-low temperature**

Vaccine	0 day			5 months			7 months			40 months		
	1.0ml	0.33ml	0.11 ml	1.0ml	0.33ml	0.11ml	1.0ml	0.33ml	0.11 ml	1.0ml	0.33 ml	0.11 ml
<b>SACs ISA 206</b>	100*	100	100	100	100	100	100	100	90	100	100	100
<b>SACs ISA 25</b>	100	100	100	100	100	100	100	100	100	ND	ND	ND

\* Percentage of guinea-pigs protected per dosage group.

ND - Not determined

In a second trial, SACS vaccine's based on Montanide ISA 206 or ISA 25 were diluted in similarly treated vaccine without the antigen component and compared to the PD<sub>50</sub> value of conventionally formulated vaccines (Table 2).

**Table 2 Potency (PD50) estimation of SACs ISA 206 and ISA 25 vaccines**

Vaccine	Dilutions						PD <sub>50</sub> value
	1/1	1/3	1/9	1/27	1/81	Control	
<b>SACs ISA 206</b>	100	100	100	100	75	0	<b>106.5**</b>
<b>SACs ISA 25</b>	100	100	100	60	60	0	<b>58.2</b>

\* Figures show the percentage of guinea-pigs protected per dosage group.

\*\*This compares with conventionally made oil vaccine using the same batch of Montanide ISA 206 with a PD<sub>50</sub> value of 46.71, which was performed on a separate occasion (data not shown).

Using the two mineral oil adjuvants in a third trial, SACs vaccine when thawed mixed and subsequently stored at +4°C were shown to still remain potent after 7 months (Table 3). This compared well to previous observations on conventionally formulated emergency vaccines composed of the same adjuvants (Barnett et al., 1996)

**Table 3 Potency of SACs vaccines based on Montanide ISA 25 (oil-in-water) and 206 (Water-in-oil-inwater) adjuvanted vaccines following thawing, mixing and storage at +4°C for up to 7 months**

Vaccine	0 day			4 months			7 months		
	1.0ml	0.33ml	0.11ml	1.0ml	0.33ml	0.11ml	1.0ml	0.33ml	0.11ml
<b>SACs ISA 206</b>	100	100	100	100	100	100	100	100	100
<b>SACs ISA 25</b>	100	100	100	100	100	100	100	100	100

\* Figures show the percentage of guinea-pigs protected per dosage group.

## Conclusion

In order to be credible, countries free of FMD that maintain the option to vaccinate in the event of an outbreak, must be able to access sufficient FMD vaccine within days of making such a request. This is only possible if they are members of an antigen or vaccine bank underlining their important supporting role in the control of FMD. These banks are based on a) concentrated antigen which can be rapidly formulated into vaccine, and/or b) formulated vaccines for immediate use. Of vital importance is the locality of stored antigens, since the need to formulate may require antigen to be returned to the original manufacturer, which in an emergency would further delay its production. Even if the antigens are held in the commercial sector, delay following a request for the supply of emergency vaccine might still occur if the manufacturer is currently in production, and should the facility be available for manufacture the time to produce the vaccine may be at best 24-48 hours. Such delays in the production and despatch of emergency vaccine to control an outbreak inevitably leads to wider spread of the disease and further difficulty in its control. Formulated vaccine would of course allow for immediate access. However, beside the wasteful and uneconomic implications resulting from regular replacement of the vaccine, it may not always contain the most suitable strain to deal with an outbreak. Even when an appropriate vaccine is

produced in an emergency, such as the 500,000 bovine doses of O<sub>1</sub> Manisa vaccine formulated by the IVB from a commercial antigen concentrate, a major disadvantage is the short shelf-life. Given these considerations a fully formulated vaccine that can be stored for an indefinite period of time has many benefits including:-

1. Readily available vaccine on request, alleviating delays in despatch.
2. No requirement once formulated for accessibility to a **manufacturing facility**.
3. Quality control, sterility and efficacy, at the required standards, performed well in advance of its possible use.
4. Cold-chain requirement less critical in transit as the vaccine could be shipped as it is thawing and hand mixed prior to application.
5. Economical, alleviating the need to replace vaccine components such as adjuvant on a regular basis.
6. Full drawing rights or dose requirement could be available in >one-hit= without the need to do further manufacturing runs.
7. Accessibility, could be strategically stored in various locations ready for immediate use.

Using a novel approach of layering the individual components of FMD vaccine in the same primary container and then storing at ultra-low temperature, these so-called stratified and cryogenically stored (SACS) vaccines appear to make these list of benefits more realistic. The results of experiments indicate that applying this methodology to Montanide 25 or 206 oil based vaccines has no detrimental effect to the potency or stability of the final product or indeed its shelf-life following reconstitution and storage at +4°C. More significantly the process maintained the potency of fully formulated FMD vaccine after several years of storage at ultra-low temperature, well in excess of the 12-18 month shelf-life period of conventional FMD vaccine kept +4°C .

Consideration will of course have to be undertaken on the optimum manufacturing approach for this type of vaccine, including the most suitable primary containers and method of labelling. Nevertheless, this approach offers many advantages over the existing system, not only in the context of FMD but also other vaccines including those based on attenuated strains, in order to improve their shelf-life characteristics and immediate accessibility.

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