

## **FFECT OF STORAGE TEMPERATURE ON THE KEEPING QUALITY F OIL ADJUVANTED FMD VACCINE**

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### **SUMMARY**

In this study, inactivated oil FMD vaccine adjuvanted with ISA 206 was prepared . Guinea pig potency testing was applied to determine the ultimate shelf life of oil adjuvanted FMD vaccine at different conditions of storage temperatures. Our results revealed that guinea pig potency of oil FMD vaccine stored at 4°C was 40.7, 40.7, 31.6, 20, 20, 15.8, 10.47, 1.58 at 0, 3, 6,12,15,18, 21, 24 months respectively. While if the vaccine bottle was left at room temperature its guinea pig potency estimates was 40.7, 40.7, 20, 15.8, 1.99 at 0, 1, 2, 3, 4 days respectively. From the previous results, it was found that the ultimate shelf life of oil FMD vaccine is not less than 18 months at 4 °C.While if the vaccine bottle is left at room temperature its shelf will be markedly decreased to not more than 3 days. So FMD vaccine must be properly shipped, stored and handled at 4°C.

### **INTRODUCTION**

Foot and Mouth Disease is highly contagious viral disease of domesticated and wild cloven-hoofed animals.FMD virus has the potential to cause explosive epidemics of FMD because of its diverse host range, low infectious dose requirement, high quantity of virus excretion,multiple routes of transmission and short incubation period (Salt et.al., 1998). The virus that causes FMD belongs to the Aphthovirus genus of family Picornaviridae that are non- enveloped ,icosahedral viruses with positive sense RNA genomes, the genus consists of seven serotypes O,A,C, SAT1,SAT2, SAT3 and Asia 1 (John et. al., 2004) .Of these, serotype O is the most prevalent and is considered endemic in parts of south America, the Middle East , Africa and Asia.( Samuel et. Al., 1999).

Vaccination constitutes an important control policy for FMD in affected areas with advanced eradication programs as well as in free regions that decide to use immunization as a control measure after a recent introduction of the disease (Bergmann et al., 2003).

The shelf life of inactivated FMD vaccine indicated by the manufacturer is usually 12 months under the specified conditions of storage (4°C) (Bartel and Vreeswijk 1991). Inactivated FMD vaccine must be properly shipped, stored and handled to avoid loss of their biological activity. In Egypt, where the vaccine is transported over long distances and the difference in climatic conditions between Upper and Lower Egypt, it is difficult to meet the specified conditions of storage.

The aim of this work is to study the ultimate shelf life of oil FMD vaccine as well as the effect of exposure the vaccine to elevated temperatures for varying periods on the potency of the vaccine.

## MATERIALS AND METHODS

### 1. Baby hamster kidney cells (BHK21 clone 13):

The cells were propagated at FMD department, Abbasia, Cairo, using Minimum Essential Medium (MEM) with Earl's salts with 8-10% sterile newborn calf serum.

**2. Virus:** The virus used in this study was the locally isolated FMD virus type O1/1993. Viral stock of O1 was prepared according to Ubertini

et al., (1967) by infecting BHK21 cell monolayer. Virus were collected from the supernatant and cleared by centrifugation (3000 rpm). The cytopathic effect of FMDV on BHK21 cells appeared 24 hours after infection was determined by TCID<sub>50</sub> of Reed and Muench (1938) analysis.

Viral stocks were stored at -70°C. The titer of virus used for vaccine preparation was 10<sup>8</sup> TCID<sub>50</sub>/ml and 8 complement fixing unit.

**3. Inactivated oil FMD vaccine:** The vaccines were formulated according to Barnett et al., (1996). FMD virus type O1/93 inactivated by binary ethylenimine was stirred into an equal volume of Montanide ISA206 to form a water in-oil-in-water blend. Sterility and safety of the prepared vaccine was done according to OIE manual (2000)

The Montanide ISA206 was kindly supplied from Seppic, France.

### 4. Animals:

Guinea Pigs: Healthy adult guinea pigs, of 400-500 gm body weight each, were used for vaccine potency.

### 5. Vaccine Potency:

The potency for each vaccine was tested in guinea pigs according to Black et al., (1985). The doses which have 1/4, 1/16, 1/64/1/256 concentration of the original dose (i.e., four fold dilution) were used and 0.5 ml from each dilution was inoculated.

ed into each group of guinea pigs by subcutaneous route. 21 days post inoculation; the animals were challenged by 10,000 ID<sub>50</sub> guinea pig-adapted virus by intradermal route. All animals checked daily for signs of generalization for 3-5 days. Guinea pig protective dose<sub>50</sub> (GPPD<sub>50</sub>) was calculated according to Reed and Muench (1938).

#### Experimental Design:

Inactivated oil FMD vaccines adjuvanted with

#### RESULTS

ISA 206 were formulated. The potency tests in guinea pigs of the prepared vaccine were applied shortly after formulation and the vaccines were subsequently held at 4°C. Representative samples for testing were collected every 3 months of storage at 4°C and tested for their potency in guinea pigs. Also some samples were exposed to 25°C for varying periods of time and tested for their potency.

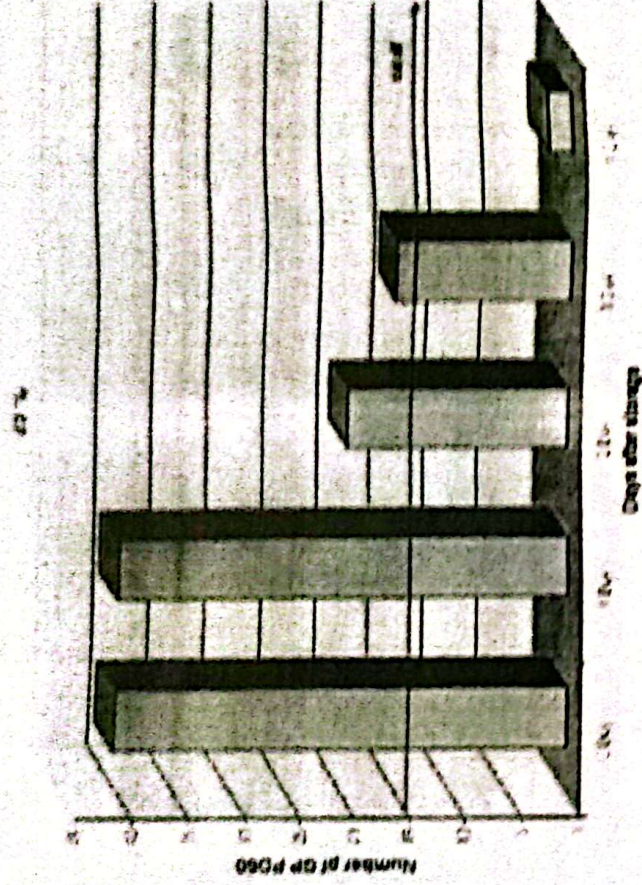
Table (1): Guinea pig protective dose<sub>50</sub> of oil FMD vaccine at different times of storage at 4°C

Month after storage	Dilution of vaccine					** G.P. PD <sub>50</sub> in log <sub>10</sub>	Number of G.P.PD <sub>50</sub>
	undiluted	1/4	1/16	1/64	1/256		
0	0/5*	0/5	1/5	3/5	5/5	5/5	40.7
3	0/5	0/5	1/5	3/5	5/5	5/5	40.7
6	0/5	0/5	2/5	3/5	5/5	5/5	31.6
12	0/5	1/5	2/5	4/5	5/5	5/5	20
15	0/5	1/5	2/5	4/5	5/5	5/5	20
18	0/5	1/5	2/5	5/5	5/5	5/5	15.8
21	1/5	1/5	3/5	4/5	5/5	5/5	10.47
24	2/5	4/5	5/5	5/5	ND	ND	1.58

\* Numbers of guinea pigs showed generalization over total number of challenged animals

\*\* Guinea pig protective dose<sub>50</sub> expressed in log<sub>10</sub>.  
ND: not determined





**Fig. (2):** Guinea pig protective dose<sub>50</sub> of FMD vaccine at different times of storage at 25°C

## DISCUSSION

For effective vaccination campaign, the vaccine should be stored and distributed under conditions which preserve the cold chain and take account of the shelf - life of the product (Doel, 1999). Guinea pigs have been used extensively as a model species for potency testing of FMD vaccine (Mowat and Rweyemamu 1997). The guinea pig test can be regarded as giving an indication of the potency of oil emulsion FMD vaccine. When guinea pig estimates of 15 PD<sub>50</sub> or more were obtained the vaccines generally protected all or almost all the animals in each group against challenge while estimates of 5PD<sub>50</sub> or less indicated that the vaccines protected fewer than five out of eight animals but if the GPPD<sub>50</sub> between less than

15PD<sub>50</sub> and more than 5PD<sub>50</sub> were obtained the proportion of animals protected varied from five of eight to eight of eight (Black et., al. 1985).

In table(1) revealed that the potency of oil FMD vaccine stored at 4°C was 40.7, 40.7, 31.6, 20, 20, 15.8, 10.47, 1.58 at 0, 3, 6, 12, 15, 18, 21, 24 months respectively. These results indicated that ISA-206 adjuvanted FMD vaccine were still fully potent after 3 months storage at 4 °C and were still potent for not less than 18 months after storage at 4°C .According to Black et., al. (1985) the ultimate shelf life of the tested ISA-206 adjuvanted FMD vaccine is not less than 18 months. These results were agreed with Bayramoglu and Burgut (1987)Tejstra et.,al. (1994) and Barnett et.,al.(1996) who concluded that the vaccines

keep their potency beyond the normal conservation period at 4°C and the PD<sub>50</sub> values of the vaccines and the titers of neutralizing antibody induced after storage 1 year were equal to the levels obtained shortly after formulation ,although the potency had markedly decreased after 2 years storage at 4°C .But our results were not agreed with Petzhold and Mohrdick (1979) who stated that FMD virus retained its immunogenic properties in vaccines for up to 14 months at 4°C.

In table (2) The vaccine bottle was left at room temperature, its guinea pig potency estimates was 40.7,40.7,20,15.8,1.99.at 0,1,2,3,4 day respectively. So, the shelf life of that vaccine was markedly decreased to not more than 3 days. These results were supported by Butchaiah et.,al.(1985)who stated that the FMD vaccines retain their potency after storage at 25 °C for at least two days.

## CONCLUSION

The ultimate shelf life of oil FMD vaccine is not less than 18 months while if the vaccine bottle is left at room temperature its shelf life will be markedly decreased to not more than 3 days.

SO, FMD vaccine must be properly shipped, stored and handled to avoid loss of their biological activity. Failure to adhere these requirements can lead to a loss of vaccine potency, resulting in an inadequate immune response of the vaccine.

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