



## Keeping quality of inactivated respiratory virus vaccines (Pneumo-5)

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### Abstract

In the keeping quality and the Efficacy of freshly locally prepared Bovine Respiratory virus vaccines (Pneumo-5) adjuvanted with either Aluminum Hydroxyl gel or montanoid oil , the serum neutralization test was used for humoral immunity evaluation using( potency sample value) as a parameter by studying the effect of different storage temperature on change on this value and its validity. it was found that the Gel adjuvanted pneumo-5 vaccine was valid for( 15 month, 2 week and 1week) in storage temperature 4-8°C( refrigerator) , 18-25°C( room temperature) and 37°C (incubator) respectively but we found that the Oil adjuvanted pneumo-5 vaccine was valid for ( 24 month, 3 week and 2 week) in storage temperature 4-8°C (refrigerator) , 18-25°C( room temperature) and 37°C( incubator) respectively after that the vaccine becomes invalid .

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### 1. INTRODUCTION

Bovine respiratory diseases (BRD) have an impact issue on the beef and dairy cattle industry, both for stocker and feedlot entities. Economic losses result from death, decreased performance of diseased cattle, lowered weight gain, increased cost of gain, reduced carcass value and treatment costs (Fulton et al., 2002). Pneumoenteritis problem in cattle and calves in Egypt is caused by viral agents include Bovine Viral Diarrhea virus (BVDV) with its two genotype ; genotype-1 and genotype-2 (Abdel-Fadil, 2007), Infectious Bovine Rhinotracheitis Virus (IBRV), Parainfluenza-3 Virus (PI-3V) and Bovine Respiratory Syncytial Virus (BRSV) (Samira *et al.*, 2001). Prevention and control of BRD is much more successful and economically feasible than treatment. An ideal processing protocol should include vaccination of the herd with safe, potent and

effective gel and oily adjuvanted inactivated Pneumo-5 vaccines to develop an adequate immune response contain the previous five viruses protection coat (Rasha et al., 2016). Locally prepared inactivated gel pneumo-3 vaccine containing three viruses (BVDV-1, IBRV and PI-3V) was first edition of locally prepared vaccine produce to face that syndrome in Egypt then Pneummo-4 vaccine was produced by adding(BRSV) to pneumo-3 composition .pneumo-5 developed by adding (BVDV-2) (Abdel-Fadil 2012).

The aim of this work was the studding of Keeping quality of pneumo-5 vaccines from Physical factor as Thermo stability of pneumo-5 vaccine at (4-8°C in refrigerator), ( 18-22°C in room temperature) and at (37°C in incubator) .

## 2. MATERIALS AND METHODS

### 1. *pneumo-5* Viruses in the prepared vaccines:

#### 1.1. *Bovine viral diarrhea virus genotype-1 (BVD-1)*

A local Egyptian strain (Iman strain) with a titer of  $10^{6.5}$  TCID<sub>50</sub>/ml.).

#### 1.2. *Bovine viral diarrhea virus genotype-2 (BVD-2)*

A cytopathic (strain 125) with a titer of  $10^{6.5}$  TCID<sub>50</sub>/ml .

Infectious Bovine Rhinotracheitis virus (IBRV): A local isolate of "Abou Hammad strain" with a titer of  $10^{7.5}$  TCID<sub>50</sub>/ml .

#### 1.3. *Parainfluenza-3 virus (PI-3V)*

Egyptian strain of PI-3 (Strain 45) with a titer of  $10^8$  TCID<sub>50</sub>/ml.

#### 1.4. *Bovine respiratory syncytial virus*

These viruses were supplied by Rinderpest Like Diseases Research Department, Veterinary Serum and Vaccine Research Institute Abbasia, Cairo (VSVRI) and used in vaccine preparation and SNT. And all viruses propagated on MDBK cell culture.

### 2. *Inactivation*

Bromo-ethyleneimine hydrobromide (BEI) was used for inactivation of the vaccinal viruses and . Sodium thiosulphate was used to stop the action of BEI according to Bahnemann (1975).

### 3. *Montanide Oil ISA 206*

It is a mineral oil based adjuvant a double emulsion . It was obtained from Seppic, Paris, France (2002)

### 4. *Aluminium hydroxide gel:*

It was used as stabilizer and adjuvant on the vaccine preparation. It was sterilized by autoclaving for 20 minutes at 121°C and pH was adjusted to 6.6 according to( Lei, 1985).

### 5. *Animals*

#### 5.1. *White Swiss mice*

20 Albino Swiss weaned mice of 18-21days of age were used for safety test of the prepared vaccine. They were obtained from Laboratory Animals Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

#### 5.2. *Calves*

A total of 64 susceptible healthy male native breed calves aged about 6 months were used to study the safety and potency of the prepared vaccine. These animals were found to be free from antibodies against the used viruses using SNT.

### 6. *Vaccine preparation*

The used viruses were propagated in MDBK cell line and the virus suspensions were harvested then subjected to two cycles of freezing and thawing and inactivated by 0.01M of binary ethyleneimine according to Zeidan et al. (1999). Sodium thiosulphate 2% of 20% was added to stop the action of BEI then divided into 2 part for preparation of the two different type of pneumo-5 vaccine.

#### 6.1 *preparation of oil adjuvanted pneumo-5 vaccine:*

Equal volumes of the inactivated virus fluid was mixed together and thoroughly mixed with montanide oil ISA 206 at a ratio 1:1 (vol/vol) according to (Barteling et al., 1990). The PH was adjusted to 7.5.

#### 6.1 *preparation of Gel adjuvanted pneumo-5 vaccine:*

Mix the other part of viral suspension with aluminium hydroxide gel (25%) and using of thiomersal 0.001% as a preservative according to Samira et al. (2001). Thiomersal was added as a vaccine preservative at final concentration of 0.001% to the both type of prepared vaccines and distributed in sterile bottles of 50 ml

### 7. *Quality control of the prepared vaccine.*

#### 7.1. *Sterility test*

It was performed in according to (Maha, 2012)

#### 7.2. *Safety tests*

In mice and In calves was adopted according to( maha , 2012)

#### 7.3. *Potency test*

Potency evaluation of the prepared polyvalent inactivated respiratory viral (pneumo-5) vaccines adjuvant with the both type Gel and Oil . and was carried out according to (Maha , 2012 )and (Rasha , et al.,2016).

8. Keeping quality of prepared vaccines: For measuring the efficacy evaluation of both

type of pneumo-5 vaccine, samples of the same batch of the combined inactivated vaccines were kept at various temperatures for different intervals as follows:

8.1 Effect of temperature at 4-8°C: Both type of stored vaccine Pneumo-5 was tested every month post storage till 16 months for Gel type and monthly till 26 months for Oil type post storage.

8.2 effect of temperature at room temperature (18-22°C): Both type of stored Pneumo-5 vaccines was tested every week post storage till one month post storage.

8.3 Effect of temperature at incubator (37°C): Both type of stored Pneumo-5 vaccines was tested every week post storage till one month post storage.

Both type of stored Pneumo-5 vaccines was tested every week post storage till one month post storage.

#### 9. Serum samples

Serum samples were collected from all calves on the first day of vaccination (0 day), the day of booster vaccination (14 days post vaccination) then every 2 week for Gel pneumo-5 Vaccine and every month for Oil pneumo-5 Vaccine to detect potency sample titre .Other keeping quality samples were collected at time of potency sample according to vaccine type . All serum samples were collected and inactivated at 56°C for 30 minutes in a water bath for inactivation of nonspecific substances, then stored at - 20°C until used in detection of specific neutralizing antibodies against all virus components of the prepared vaccine.

#### 10. Serum neutralization test

It was performed on MDBK cell line using the micro titer technique. The sera samples were expressed as neutralization index was carried out according to Fulton et al. (1995) , which protected 50% of the wells as calculated by Reed and Muench (1938).

### 3. Results

**Table 1.** Sterility test of the prepared both type Pneumo-5 vaccine .

Vaccine	Examined microorganism	Result
Gel	Aerobic bacteria	No colonies
	Anaerobic bacteria	No turbidity
	Fungus	No colonies
Oil	Aerobic bacteria	No colonies
	Anaerobic bacteria	No turbidity
	Fungus	No colonies

**Table 2.** Safety test of the prepared both type of Pneumo-5 vaccine.

	Animal observed			
	Sings	Mice		Calves
	Gel vaccine	Oil vaccine	Gel vaccine	Oil vaccine
local reaction	Negative	negative	negative	Negative
systemic reaction	Negative	negative	negative	Negative

mortalities	Negative	negative	negative	Negative
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**Table 3:** Mean serum neutralization indices in calves for gel adjuvanted prepared pneumo-5 vaccine viruses in weeks post vaccination(wpv) :

virus	0wp.v	2wp.v	4wp.v	6wp
BVD virus -1	0.0	0.2	1.2	1.5
BVD virus -2	∞	0.2	1.25	1.6
IBR virus	∞	0.0	1.4	1.8
PI-3 virus	∞	0.3	1.5	1.8
BRS virus	0.0	0.2	1.2	1.5
control	0.0	0.0	0.0	0.0

**Table 4:** Mean serum neutralization indices in calves for oil adjuvanted prepared pneumo-5 vaccine viruses in month post vaccination(mpv):

Virus	0mpv	1mpv	2mpv	3mpv
BVD virus -1	∞	0.5	1.2	1.5
BVD virus -2	∞	0.55	1.25	1.6
IBR virus	∞	0.6	1.4	1.8
PI-3 virus	∞	0.8	1.5	1.8
BRS virus	∞	0.7	1.2	1.5
control	∞	0.0	0.0	0.0

**Table (5) :** Heat stability along period storage of in months of storage of both type pneumo-5 vaccines at 4-8 C<sup>0</sup>.

period	2M*	4M	6M	8M	10M	12M	15M	16M	18M	20M	22M	24M	26M
vaccine													
Gel	st.*	st.	st.	st.	st.	st.	st.	unst.*	unst.	unst.	unst.	unst.	unst.
oil	st.	st.	st.	st.	st.	st.	st.	st.	st.	st.	st.	st.	unst.

M :month post storage

st. :stable (had potency sample titre nearly resembled the potency of 0 day storage sample).

unst. :unstable(had potency sample titre 80% or less of the potency of 0 day storage sample).

Table (6): Heat stability along period of storage in weeks of storage of both type pneumo-5 vaccines at different storage temperature.

period vaccine	1week	2week	3week	4week
Gel (18 -25 C <sup>0</sup> )	Stable	Stable	unstable	Unstable
Gel (37 C <sup>0</sup> )	Stable	Unstable	unstable	Unstable
Oil (18 -25 C <sup>0</sup> )	Stable	Stable	stable	Unstable
Oil ((37 C <sup>0</sup> )	stable	Stable	unstable	Unstable

week :weeks post storage

st. :stable (had potency sample titre nearly resembled the potency of 0 day storage sample).

unst. :unstable(had potency sample titre 80% or less of the potency of 0 day storage sample).

#### 4. Discussion

Respiratory disease occurs due to stress factors as bad environment, transportation, accumulation of ammonia and excessively high humidity in closed areas which lower the resistance of animal and enhance the multiplication of microorganisms (Bickert and Herdt, 1985). Infectious agents associated with bovine respiratory diseases include five viruses; BVD-1, BVD-2, IBR, PI-3 and BRS (Samira et al., 2001). Vaccination programs for breeding herds are integral parts of preventive health programs designed to lessen the effects of infectious respiratory diseases in cattle (Knezevic et al., 1990).

Currently the prepared vaccine showed complete absence of any bacterial, fungal or mycoplasma contamination on specific media for 15 days post inoculation. Moreover, the results of sterility test revealed that the prepared vaccines were also free from any infectious (Table 1). The obtained results supported safety tests when applied on mice and calves vaccinated with double times of vaccinal dose, where there was neither local nor systemic post vaccinal reaction and, there was no development of any clinical signs or

elevation of rectal temperature during the whole experiment period (Table 2). Such findings agreed with those obtained by Parker et al. (2009). Judgement of stability on this study by two methods , firstly physically ( no change in colour or vaccine homogeneity ) , secondly immunologically by Serum neutralization test which has been used for quantization of antibodies against vaccinal viruses. The test is sensitive, mostly specific and relatively simple to be performed according to Rossi and Kiessel (1971). The antibody titres were expressed as the log<sub>10</sub> of the inverse dilution which protected 50% of the tubes as calculated by Reed and Muench (1938). (Table 3,4)The results of the antibody titre expressed by neutralization index was indicated that vaccine was potent at 6 week post first inoculation dose incase of gel adjuvanted Pneumo-5 vaccine but three month post first inoculation in case of oily adjuvanted Pneumo-5 vaccine by using SNT that agree with ) (Maha , 2012)., (rasha et al., 2016). this result act as guide and model for judgment on immunological stability of other samples (the result of sample must around the result of model not less than 95% of the guide

result ,the unstable sample show 80% of the guide result ).

The potency evaluation of the thermo stability of prepared polyvalent viral vaccine in calves Pneumo-5 vaccine. That by inoculate every animal with first dose at 0 day then boosting after 2 weeks from each sample. (Tables 5,6) showed that the Gel adjuvanted Pneumo-5 vaccine gave 100% protection (the same potency result of freshly prepared vaccine) for storage the vaccine at (4-8°C) for 15 months; at (18-25°C) for 2 week and at (37°C) for 1 week ,While Gel adjuvanted Pneumo-5 vaccine gave 100% protection (the same potency result of freshly prepared vaccine) storage of the vaccine at (4-8°C) after 24 months; at (18-25°C) after 3 weeks and at (37°C) for 2 weeks. So it could be concluded that 4-8°C is the best temperature for storage of the inactivated pneumo-5 vaccine gel or oil adjuvanted .this agree with Mohamed Mostafa (1984) and Ehab El-Sayed et al ,(2012).

### 5. Conclusion:

The thermo stability of combined respiratory inactivated vaccine (Pneumo5) with its both type which kept in refrigerator at 4-8°C is valid to use for 15 months of storage for gel type and 24 month for oil adjuvanted type .And this is the suitable storage temperature for both type of Pneumo-5 vaccine These results are very useful for the farmers, veterinarians and their assistances who used this vaccine under different environmental conditions.

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