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AN INVESTIGATION OF THE POTENCY OF THE INFECTIOUS BRONCHITIS VIRUS IN DIFFERENT STABILIZING MEDIA AND AT VARIOUS TEMPERATURES

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ABSTRACT

The potency of the infectious bronchitis virus was studied in different stabilizing media and at various temperatures. Skimmed milk seems more suitable and the difference in titres between the vaccines kept for six months at -20°c and 4°c is practically negligible.

INTRODUCTION

Virus vaccines can be prepared in liquid or freeze dried forms. However, the distribution of the liquid form of the infectious bronchitis vaccine H-120 is not advised scientifically. Therefore, it is emphasized that the production and the distribution should be done in freeze dried form.

For this purpose, producers mix some sort of additive as a stabilizing medium to the vaccine. The general process is uniform all over, but the detail of production will be specialized by the producers.

For this reason, The production division of the Razi Institute decided to select those stabilizers which have the following properties; first, raw materials could be prepared easily, second, it preserved the vaccine property for a longer period of time.

The study in this field began from 1980. Several stabilizing media have been used with a specified concentration of the infectious bronchitis vaccine H-120 and titration was made. The process has been repeated three times in a period of two months and the results are summarized in Tables given below.

MATERIALS AND METHODS
CHARACTERISTICS OF THE STABILIZING MEDIA

In this process, the following stablizing media have been used:

1. Skimmed milk,
2. Sucrose, 5gr; Lactalbumin, 15gr, D.W. to 200 ml, } pH = 6.8
3. Lactose, 20 gr, D.W. to 200 ml, } pH = 5.5
4. Lactose Pepton Tris Buffer;

solution A { a – lactose, 2gr, b – pepton, 4gr, c – D.W. to 100 ml, } pH = 6.8

solution B { a – (Trishydroxymethyl) aminomethan, 2.42 gr/100, b – Hcl, 0.2 N, 16.2 ml, c – D.W. to 100 ml, } pH = 7.4

solution (A+B), pH = 6.8

5. Sucrose, 5 gr; N. Z. Case, 15 gr, D.W. to 200 ml, } pH = 7.1
6. Phosphate monopotassic and disodic,
   a – $PO_4HNa_2, 7H_2O$, 6.8 gr,
   b – PO_4H_2K, 1 gr,
   c – D.W. to 200 ml, } pH = 7.25
7. Vaccine without stabilizer.

All of the above stabilizers have been sterilized for 20 minutes at 110.°c

VACCINE PREPARATION AND PRESERVATION

A 10^{-2} dilution of the H-120 seed virus of Massachusetts type with 10^7 EID 50/ml was prepared. Then, 0.1 ml of this dilution was inoculated into the Allantoic cavity of 9 day old embryonated SPF eggs. The eggs were controlled after 24 hours. The initial dead embryos were discarded and the remainders were controlled again after 48 hours. The Allontoic fluid was collected and a sample was taken from this, which was freeze dried with each of the mentioned stabilizers. Titration was made for each of the vaccine prepared

according to Kaeber method. These samples were divided in three aliquots and kept at the temperatures of -70°c, -20°c, and 4°c for a period of six months. Then, titrations were made again. Table 1 shows the titre of the dried vaccine in different stabilizers after preparation. The titre of these samples kept for six months at -70°c, -20°c, and 4°c are also shown.

Table 1

stabilizer	vaccine titre after preparation	after 6 months kept at different temperatures		
		-70°c	-20°c	4°c
Skimmed milk	6.9*	6.5	6.3	6.1
Sucrose + Lactalbumin	6.6	6.1	5.9	5.9
Lactose	6.5	6.1	6.1	6.1
L.P.Tris Buffer	6.3	5.9	5.6	5.5
Sucrose + N.Z. Case	6.1	5.7	5.8	5.5
$PO_4HNa_2 + PO_4H_2K$	6.3	5.9	5.7	5.7
Vaccine without stabilizer	5.9	5.6	5.1	4.9

* Log_{10} EID 50

A short time after the preparation of the first batch, another batch from the original seed was taken, and the same process as before was followed. The results are shown in Table 2.

Table 2

stabilizer	vaccine titre after preparation	after 6 months kept at different temperatures		
		-70°c	-20°c	4°c
Skimmed milk	6.9	6.5	6.3	6.3
Sucrose + Lactalbumin	6.5	6.1	5.8	5.8
Lactose	6.3	5.8	5.7	5.6
L.P.Tris Buffer	6.1	5.8	5.6	5.4
Sucrose + N.Z.Case	6.3	5.8	5.8	5.5
$PO_4HNa_2 + PO_4H_2K$	6.3	5.9	5.6	5.6
Vaccine without stabilizer	6.1	5.5	5.1	4.8

Finally, after a short period from the preparation of the second batch, the third batch was prepared and the same process was carried out. The results are summarized in Table 3.

table 3

stabilizer	vaccine titre after preparation	after 6 months kept at different temperatures		
		-70°c	-20°c	4°c
Skimmed milk	6.9	6.5	6.1	5.9
Sucrose + Lactalbumin	6.6	6.1	5.7	5.8
Lactose	6.4	5.9	5.8	5.6
L.P.Tris Buffer	6.1	5.7	5.5	5.4
Sucrose + N.Z. Case	6.1	5.8	5.7	5.5
$PO_4HNa_2 + PO_4H_2K$	6.3	5.8	5.5	5.5
Vaccine without stabilizer	5.9	5.5	5.1	4.8

DISCUSSION AND CONCLUSION

If the results are assorted according to the temperature and their average are taken, the Tables, 4, 5, and 6, which indicate the EID 50/ml average, are obtained.

Table 4

stabilizer	4°c first trial	second trial	third trial	average titre
Skimmed milk	6.3	6.3	6.1	6.2
Sucrose + Lactalbumin	5.9	5.8	5.7	5.8
Lactose	6.1	5.7	5.8	5.8
L.P.Tris Buffer	5.6	5.6	5.5	5.5
Sucrose + N.Z.Case	5.8	5.8	5.7	5.7
$PO_4HNa_2 + PO_4H_2K$	5.7	5.6	5.5	5.6
Vaccine without stabilizer	5.1	5.1	5.1	5.1

Table 5

stabilizer	-20°c first trial	second trial	third trial	average titre
Skimmed milk	6.3	6.3	6.1	6.2
Sucrose + Lactalbumin	5.9	5.8	5.7	5.8
Lactose	6.1	5.7	5.8	5.8
L.P.Tris Buffer	5.6	5.6	5.5	5.5
Sucrose + N.Z.Case	5.8	5.8	5.7	5.7
$PO_4HNa_2 + PO_4H_2K$	5.7	5.6	5.5	5.6
Vaccine without stabilizer	5.1	5.1	5.1	5.1

Table 6

stabilizer	first trial	-70°c second trial	third trial	average titre
Skimmed milk	6.5	6.5	6.5	6.5
Sucrose + Lactalbumin	6.1	6.1	6.1	6.1
Lactose	6.1	5.8	5.9	5.9
L.P.Tris Buffer	5.9	5.8	5.7	5.8
Sucrose + N.Z.Case	5.7	5.8	5.8	5.8
$PO_4HNa_2 + PO_4H_2K$	5.9	5.9	5.8	5.8
Vaccine without stabilizer	5.6	5.5	5.5	5.5

The following results can be obtained from the Tables 4, 5, and 6:

1 – The skimmed milk has been shown to be superior to other stabilizers.

2 – Eventhough -70°c is scientifically more acceptable, it seems that the difference in titres at -20°c and 4°c is negligible, and 4°c is more economical and practical to keep or conserve them.

3 – As could be seen from the Tables, the titres of the vaccine at the time of preparation and six months later does not show any appreciable difference.

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