

## Comparative Assessment of Stabilizers Used for Freeze-Drying T<sub>1</sub>/44 Contagious Bovine Pleuropneumonia Vaccine (CBPPV) Produce in Nigeria

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**Abstract:** The effect of using 85% buffered sucrose solution plus gelatin (“S”) as stabilizer and a combination of buffered sucrose/lactalbuminhydrolysate solution in ratio 20:5% (w/w), respectively with gelatin (“L”) as stabilizer during freeze-drying process and under varying storage temperature conditions on CBPP vaccine was investigated. It was found that both stabilizers gave good freeze-dried preparation of T<sub>1</sub>/44 CBPP vaccines. During the process of freeze-drying, there was a total average loss of 4.73 cfu mL<sup>-1</sup> viable organisms in vaccine with stabilizing medium “L” plus gelatin and an average loss of 5.25 cfu mL<sup>-1</sup> viable organisms for vaccine with stabilizing medium “S” plus gelatin. The results favoured the choice of sucrose/lactalbuminhydrolysate plus gelatin stabilizer on viability of T<sub>1</sub>/44 *Mycoplasma mycoides* subsp. *mycoides* during freeze-drying process. Vaccine with stabilizing medium “L” plus gelatin stored at +4°C for 15 months maintained sufficient viable mycoplasma to protect cattle. The vaccine titre dropped from 1.5×10<sup>9</sup>-4.3×10<sup>7</sup> cfu mL<sup>-1</sup>, while vaccine with stabilizing medium “S” plus gelatin for the same period dropped from 3.1×10<sup>9</sup>-1.8×10<sup>7</sup> cfu mL<sup>-1</sup>. At room temperature of 25°C, the 2 types of vaccines maintained sufficient viable mycoplasma up to 4 months, but detrimental as the titre may drop suddenly when reconstituted for vaccination in the field. Storage of this vaccine at 37°C is not recommended for the same reason stated above.

**Key words:** Stabilizer, freeze-dried, Contagious Bovine Pleuro-Pneumonia Vaccine (CBPPV), assessment of stabilizers

### INTRODUCTION

Contagious Bovine Pleuropneumonia (CBPP) is a chronic and epidemic disease of cattle and affects cattle of all ages. In Nigeria, at the National Veterinary Research Institute Vom, vaccines against the disease are produce from live attenuated *Mycoplasma mycoides* subsp. *mycoides* T<sub>1</sub>/44. The earlier vaccines produced in Vom against the disease were broth culture of the organism. It looses its viability after short period of storage. In an effort to improve the keeping quality of these vaccines, further research work into the vaccine standardization was intensified leading to production of the freeze-dried form. Now the Institute produces freeze-dried form of the vaccine.

In order to achieve high quality freeze-dried vaccines, a supporting medium is normally added to the liquid vaccine to enhance formation of “plug” or “cake” (i.e. addition of stabilizer). Primarily, stabilizers provide vaccines during freeze-drying process support which allow moisture to escape easily. It also help maintain the vaccines effectiveness even when they are exposed to drastic changes in the environment such as temperature,

light, humidity etc. On the other hand, excessive use of stabilizer causes the freeze-dried vaccine “plug” to have a glass-like appearance and also prevent the escape of moisture. A good quality stabilizer prevents high loss of viable organisms in live vaccine during freeze-drying process and storage of the vaccine under ideal conditions for reasonable length of time. It does not constitute a source of hazard to the animal when the vaccine is finally administered.

Different stabilizer have been employed in production of freeze-dried Contagious Bovine Pleuro-Pneumonia Vaccine (CBPPV): mainly, the use of 0.5% molar glutamate (Priestly, 1961), embryonated egg fluid, 20% suspension of bovine brain and 20% inactivated bovine brain suspension (Gregory, 1967), peptone, Weybridge stabilizer for S<sub>19</sub> brucella vaccine (Fao, 1967), casitone, sucrose and monosodium glutamate (Hudson, 1921), skimmed milk (Orue and Doutre, 1971) and 11% tryptone (FAO, 1971). At earlier stages of CBPPV production in Vom laboratories, mistura desiccant was used (Karst, 1969). Now 2 types of stabilizer are in use. They are buffered sucrose solution with gelatin and buffered sucrose/lactalbuminhydrolysate and gelatin solution.

Both types of stabilizers support freeze-drying of CBPP vaccines and are readily reconstituted. This study was aimed to determine the supporting credibility of the 2 types of stabilizers used for freeze-drying CBPP vaccine T<sub>1</sub>/44. That is, the preferred advantages of using either stabilizer during freeze-drying process or the stability of the products on different storage temperatures.

## MATERIALS AND METHODS

**Preparation of buffered sucrose stabilizer (“S” stabilizer):** Eighty five percent of sucrose with buffer salts (potassium dihydrogen phosphate, (KH<sub>2</sub>P<sub>04</sub>) 0.12% and disodium hydrogen phosphate (Na<sub>2</sub>HP<sub>04</sub>) 0.77% were dissolved in warm de-ionised water. The pH of the solution was adjusted to 7.6, then distributed into 500 mL bottles and sterilized by tynderlization. They were then incubated at 37°C for 24 h to check for sterility and stored at +4°C ready for use.

**Preparation of buffered sucrose and lactalbumin-hydrolysate stabilizer (“L” stabilizer):** Sucrose and lactalbuminhydrolysate at 20% and 5% total concentration respectively with buffer salts (KH<sub>2</sub>P<sub>04</sub>, 0.12% and Na<sub>2</sub>HP<sub>04</sub>, 0.77%) were dissolved in warm de-ionised water. The pH was adjusted to 7.6 and then sterilized by filtration (using seitz filter with filter pad HP/EKS) into sterile 500 mL bottles. The filtered stabilizer were incubated at 37°C for 48 h and checked for sterility. The sterile preparations were stored at +4°C ready for use.

**Preparation of gelatin:** Twenty percent gelatin was dissolved in de-ionized water and sterilized by autoclaving at 15 lb for 30 min. The preparation was checked for sterility and stored at 37°C ready for use.

**Preparation of freeze-dried CBPP vaccine:** The *Mycoplasma mycoides* subsp *mycoides* T<sub>1</sub>/44 culture containing not less than 1 × 10<sup>9</sup> cfu mL<sup>-1</sup> viable organisms was pooled into sterile container. The culture constitutes 60% of the total composition of the vaccine and mixed with either stabilizer (“S” or “L”) at 20% plus 20% stock solution of prepared gelatin. The wet vaccine was mixed and dispensed into sterile vials at 2 mL amount and freeze-dried. Eight batches of vaccine per type of stabilizers were prepared in all. The vaccines were tested for vacuum by “spark test” (using ST<sub>4</sub>M spark testers).

**Titration of viable mycoplasma in the vaccine:** Before freeze-drying, samples of each type of wet vaccine (mixed culture of mycoplasma with stabilizers (“L” or “S” and gelatin)) were collected and titrated for viable counts

using standard method (Miles and Misra, 1938). After freeze-drying, another sets of vaccines per types of stabilizers were collected from each batch of vaccine and viable count performed on them. The difference in count of wet and immediately freeze-dried vaccines was taken as the loss of viable mycoplasma during freeze-drying process. Subsequent viable counts were performed on the freeze-dried vaccines after storage at different temperature conditions.

**Vaccine storage temperatures:** Freeze-dried vaccines from each batch of production were stored under three different temperature conditions (+4, 25 and 37°C). The three temperature conditions were chosen on assumption that these were likely temperature ranges (+4-37°C) vaccines may be exposed to after collection from the production laboratories. However, +4-8°C or lower temperature is the recommended storage temperature for this vaccine.

## RESULTS

**Viable counts of wet vaccines and counts immediately after freeze-drying:** The average viable organisms in wet vaccines with stabilizer “S” plus gelatin was 8.35 × 10<sup>9</sup> cfu mL<sup>-1</sup> and the average counts immediately after freeze-drying was 3.1 × 10<sup>9</sup> cfu mL<sup>-1</sup> (Table 1). The

Table 1: Viable counts of vaccines with stabilizer “S” plus gelatin before and after freeze-drying

Vaccine batches	Pre-freeze-drying viable counts (cfu mL <sup>-1</sup> )	Post-freeze-drying viable counts (cfu mL <sup>-1</sup> )	Total average loss in viable counts
1	8.0 × 10 <sup>9</sup>	2.7 × 10 <sup>9</sup>	5.3
2	6.8 × 10 <sup>9</sup>	1.2 × 10 <sup>9</sup>	5.6
3	8.9 × 10 <sup>9</sup>	3.6 × 10 <sup>9</sup>	5.3
4	8.6 × 10 <sup>9</sup>	3.4 × 10 <sup>9</sup>	5.2
5	7.9 × 10 <sup>9</sup>	2.7 × 10 <sup>9</sup>	5.2
6	8.5 × 10 <sup>9</sup>	3.2 × 10 <sup>9</sup>	5.3
7	9.4 × 10 <sup>9</sup>	4.2 × 10 <sup>9</sup>	5.2
8	8.7 × 10 <sup>9</sup>	3.73 × 10 <sup>9</sup>	4.97
Total average viable counts	8.35 × 10 <sup>9</sup>	3.09 × 10 <sup>9</sup>	5.25 cfu mL <sup>-1</sup>

Table 2: Viable counts of vaccine with stabilizer “L” plus gelatin before and after freeze-drying

Batch of vaccines investigated	Pre-freeze-drying viable counts (cfu mL <sup>-1</sup> )	Post-freeze-drying viable counts (cfu mL <sup>-1</sup> )	Total average loss in viable counts
1	5.9 × 10 <sup>9</sup>	1.4 × 10 <sup>9</sup>	4.5
2	6.5 × 10 <sup>9</sup>	1.4 × 10 <sup>9</sup>	5.1
3	5.8 × 10 <sup>9</sup>	1.0 × 10 <sup>9</sup>	4.8
4	5.9 × 10 <sup>9</sup>	2.2 × 10 <sup>9</sup>	3.7
5	7.5 × 10 <sup>9</sup>	1.6 × 10 <sup>9</sup>	5.9
6	5.9 × 10 <sup>9</sup>	5.4 × 10 <sup>9</sup>	4.6
7	5.6 × 10 <sup>9</sup>	1.0 × 10 <sup>9</sup>	4.6
8	6.8 × 10 <sup>9</sup>	2.1 × 10 <sup>9</sup>	4.7
Total average viable counts	6.25 × 10 <sup>9</sup> cfu mL <sup>-1</sup>	1.5 × 10 <sup>9</sup> cfu mL <sup>-1</sup>	4.73 cfu mL <sup>-1</sup>

Table 3: Viable counts of vaccine stored at +4°C

Months	Vaccine with stabilizing medium "L" plus gelatin (cfu mL <sup>-1</sup> )	Vaccines with stabilizing medium "S" plus gelatin (cfu mL <sup>-1</sup> )
1	1.5×10 <sup>9</sup>	3.1×10 <sup>9</sup>
2	1.3×10 <sup>9</sup>	2.6×10 <sup>9</sup>
3	1.2×10 <sup>9</sup>	2.3×10 <sup>9</sup>
4	1.2×10 <sup>9</sup>	19.×10 <sup>9</sup>
5	9.0×10 <sup>8</sup>	1.6×10 <sup>9</sup>
6	8.1×10 <sup>8</sup>	8.0×10 <sup>8</sup>
7	6.7×10 <sup>8</sup>	6.2×10 <sup>9</sup>
8	5.1×10 <sup>8</sup>	4.3×10 <sup>8</sup>
9	4.8×10 <sup>8</sup>	2.3×10 <sup>8</sup>
10	3.9×10 <sup>8</sup>	2.0×10 <sup>8</sup>
11	3.0×10 <sup>8</sup>	1.8×10 <sup>8</sup>
12	2.6×10 <sup>8</sup>	1.2×10 <sup>8</sup>
13	8.0×10 <sup>7</sup>	6.0×10 <sup>7</sup>
14	6.1×10 <sup>7</sup>	3.7×10 <sup>7</sup>
15	4.3×10 <sup>7</sup>	1.8×10 <sup>7</sup>
16	2.7×10 <sup>7</sup>	9.5×10 <sup>6</sup>

"L" = Stabilizing medium of sucrose plus lactalbuminhydrolysate, "S" = Stabilizing medium of sucrose

Table 4: Viable counts of vaccines stored at 25°C

Months	Vaccine with stabilizing medium "L" plus gelatin (cfu mL <sup>-1</sup> )	Vaccines with stabilizing medium "S" plus gelatin (cfu mL <sup>-1</sup> )
1	1.5×10 <sup>9</sup>	2.95×10 <sup>9</sup>
2	1.2×10 <sup>9</sup>	2.45×10 <sup>9</sup>
3	9.65×10 <sup>8</sup>	1.2×10 <sup>9</sup>
4	3.0×10 <sup>8</sup>	5.0×10 <sup>8</sup>
5	4.5×10 <sup>6</sup>	6.0×10 <sup>6</sup>
6	1.2×10 <sup>4</sup>	1.15×10 <sup>4</sup>

"L" = Stabilizing medium of sucrose plus lactalbuminhydrolysate, "S" = Stabilizing medium of sucrose

Table 5: Viable counts of vaccines stored at 37°C

Weeks	Vaccine with stabilizing medium "L" plus gelatin (cfu mL <sup>-1</sup> )	Vaccines with stabilizing medium "S" plus gelatin (cfu mL <sup>-1</sup> )
1	1.5×10 <sup>9</sup>	3.1×10 <sup>9</sup>
2	1.2×10 <sup>9</sup>	2.4×10 <sup>9</sup>
3	8.5×10 <sup>8</sup>	1.65×10 <sup>9</sup>
4	5.0×10 <sup>8</sup>	9.5×10 <sup>8</sup>
5	7.5×10 <sup>7</sup>	8.75×10 <sup>7</sup>
6	5.5×10 <sup>6</sup>	7.5×10 <sup>6</sup>
7	5.0×10 <sup>5</sup>	4.0×10 <sup>5</sup>
8	3.5×10 <sup>4</sup>	1.0×10 <sup>4</sup>

"L" = Stabilizing medium of sucrose plus lactalbuminhydrolysate, "S" = Stabilizing medium of sucrose

total average loss of viable organisms in this vaccine during freeze-drying process was 5.25 cfu mL<sup>-1</sup>. Vaccines with stabilizing medium "L" plus gelatin on the other hand, had an average count of 6.23×10<sup>9</sup> cfu mL<sup>-1</sup> organisms in wet vaccines, but dropped to 1.5×10<sup>9</sup> cfu mL<sup>-1</sup> after freeze-drying. The total average loss in viable organisms in this vaccine during freeze-drying process was 4.71 cfu mL<sup>-1</sup> (Table 2).

**Stability of vaccines stored at +4°C (refrigerator):** The vaccines with stabilizing medium "L" and gelatin fell from 1.5×10<sup>9</sup>-2.7×10<sup>9</sup> cfu mL<sup>-1</sup> after 16 months of storage at +4°C, while vaccines with stabilizing medium "S" and

gelatin fell from 3.1×10<sup>9</sup>-9.5×10<sup>6</sup> cfu mL<sup>-1</sup> for the same period (Table 3). The total loss of viable organisms in the 2 types of vaccines within 16 months of storage was 1.47×10<sup>2</sup> and 3.09×10<sup>3</sup> cfu mL<sup>-1</sup>, respectively.

**Stability of vaccines stored at +25°C (approximate room temperature):** Vaccines with stabilizing medium "L" with gelatin fell from 1.5×10<sup>9</sup>-1.2×10<sup>4</sup> cfu mL<sup>-1</sup> in 6 months, while vaccine with stabilizing medium "S" and gelatin fell from 3.1×10<sup>9</sup>-1.15×10<sup>4</sup> cfu mL<sup>-1</sup> within the same period (Table 4). The total loss in viable organisms during the storage period was 3.0×10<sup>4</sup> cfu mL<sup>-1</sup> for vaccines with stabilizing medium "L" with gelatin and 1.95×10<sup>5</sup> cfu mL<sup>-1</sup> for vaccines with stabilizing medium "S" with gelatin (Table 4).

**Stability of vaccines stored at 37°C (incubator):** The viable counts for vaccines stored at this temperature were performed at weekly intervals. Vaccines with stabilizing medium "L" with gelatin dropped from 1.5×10<sup>9</sup>-3.5×10<sup>4</sup> cfu mL<sup>-1</sup> in 8 weeks while that of vaccines with stabilizing medium "S" with gelatin dropped from 3.1×10<sup>9</sup>-1.0×10<sup>4</sup> cfu mL<sup>-1</sup> within the same period (Table 5).

## DISCUSSION

Few records exist on effects of stabilizers on freeze-dried CBPP vaccines. Priestly (1961) reported the possibility of recovering 100% of viable mycoplasma when using 0.5% molar glutamate stabilizer for freeze-drying CBPP vaccine (strain V5). However, not much was disclosed about effects of storage temperatures on this vaccine. Therefore, it is not possible to compare the present findings with the keeping quality of that vaccine. In this study, it was observed that the 2 types of stabilizers used for vaccine preparation gave good freeze-dried CBPP vaccine (T<sub>1</sub>/44). The large amount of sucrose used in stabilizer "S" may not be economical if large and continuous production is required. On the other hand, vaccines with stabilizing medium "L" gave good freeze-dried vaccine with less use of sucrose, except that lactalbuminhydrolysate was included as an additional additive. The 2 types of stabilizers used for vaccine production store well for more than 1 year at +4°C without significant drop in viable organisms.

## CONCLUSION

However, exposure to high temperatures (25 and 37°C) had adverse effects on these vaccines and is detrimental to these vaccines. The 2 types of stabilizers

may be use for production of CBPP vaccine since the vaccine titre did not drop more than 2 logs within one year of storage at +4°C (the recommended standard). However, vaccine with stabilizing medium “L” maintained viable mycoplasma better than stabilizer “S”, thus a better choice of stabilizer for CBPP production.

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