Laboratory study

Effects of thawing temperature and syringe size on cell integrity of cell-associated VAXXITEK® HVT+IBD frozen vaccine

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Abstract

Two questions on frozen cell-associated Marek’s vaccine preparation were raised: temperature of thawing of the frozen ampoules in water bath; recommended temperature by Merial is around + 27°C; does a higher temperature ensure a higher safety for cell integrity? Temperatures of + 30°C and + 33°C were tested as well as + 27°C used as reference, and size of the syringe used to pump out of the thawed ampoules the vaccine suspension to inject into the diluent bag; a large syringe with a volume of 5 mL may have more harmful effect on cell integrity than a smaller one containing 1 mL; question that was raised was about the impact of turbulences induced by the flow of thawed vaccine pushed out of two different sizes of syringes, through the same size of needle. All the mean percentages of cell mortality were comprised between 5% and 12% in this study. The overall trend was an increase during the time of cell mortality in most conditions of temperature either using 1-mL or 5-mL syringes. Effect of temperature of thawing when vaccine preparation was performed with a 1-mL syringe, was noticed for the lowest temperature of + 27°C with a trend to some advantage in recommending this temperature, as target temperature for smoother and more progressive thawing conditions. This was not confirmed with the vaccine prepared with the 5-mL syringe. Effect of choice of syringe equipped with the same needle gauge, whatever the temperature conditions, was noticed for the smallest volume of 1 mL with a trend to some advantage in recommending this volume, for less turbulent vaccine flow inside the needle.

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1. Introduction

Cell-associated (chicken embryo fibroblasts) Herpes Virus Turkey (HVT) vaccine has been used for chicken immunization by injection either in ovo or at day-old for more than 20 years. HVT vaccines alone are widely used to control Marek’s disease effects in broiler chickens and mainly condemnations. Rispens cell-associated vaccines may be added to HVT vaccines to produce bivalent HVT + Rispens vaccines that are widely used in future breeders for control of Marek’s disease. They are mostly injected to day-old chicks in the hatcheries. HVT vaccine may also be associated with SB-1 vaccine strain. vHVT13 vaccine VAXXITEK HVT+IBD is a vectored HVT vaccine used to control Infectious Bursal Disease (IBD) as well as control of certain forms of Marek’s disease, as HVT alone does.

Vaccine preparation has been identified as a crucial step in order to ensure immunization. Tests of the effects of mismanagement practices observed in hatcheries showed that very subtle deviations from the explicit directions accompanying Marek’s vaccines could cause dramatic losses of vaccine potency. Such mismanagement was found to result in 14 to 97% loss of vaccine titre [1]. Failures in hatchery vaccine preparation can lead to loss in plaque-forming units (PFU) of MD vaccine per chicken dose. Reconstituted vaccine samples in field conditions of breeder hatcheries where bivalent HVT + Rispens vaccines are injected showed in field conditions low PFU doses, as < 10 PFU when others had a titre equal to 10^3 PFU, which is the standard required. A study showed the usefulness to assess the PFU per chicken dose of reconstituted MD vaccine and vaccine ampoules to unravel true vaccine failures, which could result in disease outbreaks in the field [2].

Question of reconstituted vaccine quality has always been a concern and different techniques were developed in order to assess it. One accessible technique is the use of cell count and calculation of cell count decrease between a ready-to-use bag of vaccine suspension and the same vaccine suspension collected at the tip of the needles of the syringes used to prepare the vaccine suspensions. As cell count may not be correlated to protection induced by the vaccine, this technique has to be regarded as a tool used to validate hatchery practise and equipment [3].

Two questions on frozen cell-associated Marek’s vaccine preparation were raised:

- temperature of thawing of the frozen ampoules in water bath; recommended temperature by Merial is around +27°C; does a higher temperature ensure a higher safety for cell integrity? Temperatures of +30°C and +33°C was tested as well as +27°C used as reference,
- size of the syringe used to pump out of the thawed ampoules the vaccine suspension to inject into the diluent bag; a large syringe with a volume of 5 mL may have more harmful effect on cell integrity than a smaller one containing 1 mL; question that
was raised was about the impact of turbulences induced by the flow of thawed vaccine pushed out of two different sizes of syringes, through the same size of needle.

A study in laboratory conditions was performed in order to evaluate the influence of the thawing temperature on fibroblast mortality and to evaluate the influence of the syringe size used to prepare the vaccine suspensions.

2. Material and methods

2.1 Vaccine preparation

VAXXITEK HVT+IBD is a HVT cell-associated frozen vaccine formulated with the vHVT13 strain that provides also protection against IBD [4]. 1,000-dose ampoules were thawed in a water bath at three different temperatures: + 27°C, + 30°C and + 33°C then were reconstituted into the vaccine diluent. Two different syringes, 1 mL and 5 mL in volumes, were used to extract the thawed vaccine suspensions out of the ampoules from the former three temperature conditions. Needle attached to the syringes, was whatever the group of study, an 18G (1.2 x 40mm) one. 15 minutes after the reconstitution of the vaccine, 1 mL of Trypan Blue was added in each tube of vaccine sample.

2.2 Counting procedure

A coverglass was fixed on a Malassez plaque. Then a drop of the vaccine solution (25µl) was put between the coverglass and the Malassez plaque. Cells were examined under a microscope when they are settled. Cellular viability was evaluated through the percentage of mortality on 15 squares of a Malassez plaque. Cellular counts were expressed in number of cells whereas cellular mortality was expressed in percentage.

2.3 Experimental design

Effect of thawing temperature on cell counts was studied using two sizes of syringe in order to assess the effect of the turbulences induced by the vaccine flow into the two syringe sizes, 1 mL and 5 mL.

Six groups of study of the same vaccine preparation were constituted (Table 1). Three thawing temperatures were studied: + 27°C (Groups 1 & 2, for 1 mL and 5 mL syringes respectively), + 30°C (Groups 3 & 4, for 1 mL and 5 mL, respectively) and + 33°C (Groups 5 & 6, for 1 mL and 5 mL respectively) (Table 1).

Five samples were collected for cell counts out of the same vaccine container. They were carried out by optical microscopy, 15 minutes, one hour then two hours after reconstitution.

Total and dead cells were counted on 15 squares of a Malassez plaque. Dead cells were described as cells displaying a dark blue colour and/or presenting a
distorted or broken cell membrane. Percentage of cell mortality was determined for each sample and was considered as the main parameter of study.

2.4 Statistical analysis

Khi2 tests (alpha < 5%) were used to compare percentages of cell mortality between the Groups function of the syringe volume, either 1 mL or 5 mL, or function of temperature of vaccine thawing. Processed values were arithmetical means of percentages of the five repeats for each sample, named percentages of cell mortality.

3. Results

Effect of thawing temperature was evaluated by comparison of cell mortality percentages of + 27°C, + 30°C and + 33°C temperatures conditions, for 1 mL syringes and 5 mL syringes, respectively.

3.1 Effect of temperature of vaccine thawing on cell mortality percentages

- 5-mL syringe

A trend to increase of cell mortality during the two-hour long study was noticed for the + 27°C condition of temperature only with the 5-mL syringe. No significant difference was observed between the three temperature conditions, + 27°C, + 30°C and + 33°C right after vaccine reconstitution into the diluent, 15 minutes further to its preparation. A significantly higher percentage of mortality was observed for the + 33°C condition one hour after vaccine preparation. A significantly lower percentage of cell mortality was observed for the + 27°C condition two hours after vaccine preparation. A significantly lower percentage of cell mortality was observed for the + 27°C condition two hours after vaccine preparation (Figure 1). There was no clear difference between temperatures, except a trend to display the lowest percentage of mortality with the + 27°C condition of temperature for vaccine thawing after two hours, and a trend to display the highest mortality percentage, during one hour after vaccine preparation with the + 33°C condition.

- 1-mL syringe

A trend to increase of cell mortality during the two-hour long study was whatever the conditions of temperature noticed with the 1-mL syringe. A significantly higher percentage of cell mortality was observed for the + 33°C temperature condition right after vaccine reconstitution into the diluent, 15 minutes further to its preparation. A significantly higher percentage of mortality was observed for the + 33°C condition and significantly lower for the + 27°C condition, one hour after vaccine preparation. A significantly lower percentage of cell mortality was observed for the + 27°C condition two hours after vaccine preparation (Figure 1). There was no clear difference between temperatures, except a trend to display the lowest percentage of mortality with the + 27°C condition of temperature for vaccine thawing after two hours.
3.2 Comparison between 1-mL and 5-mL syringes on cell mortality percentages

- **Thawing temperature of + 27°C**

No significant difference was noticed between 1-mL and 5 mL syringe use for vaccine preparation right after vaccine reconstitution, 15 minutes further to its preparation and one hour after vaccine preparation. A significantly higher cell mortality percentage was observed when the vaccine was prepared with a 5-mL syringe two hours after vaccine preparation (Figure 3). There was no clear difference between 1-mL and 5mL syringe use for vaccine preparation, except a trend to display the highest percentage with the 5-mL syringe.

- **Thawing temperature of + 30°C**

No significant difference was noticed between 1-mL and 5 mL syringe use for vaccine preparation right after vaccine reconstitution, 15 minutes further to its preparation. A significantly higher cell mortality percentage was observed when the vaccine was prepared with a 5-mL syringe one hour after vaccine preparation. No significant difference was noticed between 1-mL and 5 mL syringe use for vaccine preparation two hours after vaccine preparation (Figure 4).

- **Thawing temperature of + 33°C**

A significantly higher cell mortality percentage was observed when the vaccine was prepared with a 1-mL syringe right after vaccine reconstitution, 15 minutes further to its preparation and one hour after vaccine preparation. No significant difference was noticed between 1-mL and 5 mL syringe use for vaccine preparation two hours after vaccine preparation (Figure 5). There was no clear difference between 1-mL and 5mL syringe use for vaccine preparation, except a trend to display the highest percentage with the 5-mL syringe.

4. Discussion

The interest of the selection of cell mortality as indicator of quality of Marek’s cell-associated vaccine preparation has been fully demonstrated, and recommended for years [5]. Cell counts results may always be given as relative, as compared to a reference, a temporal reference, time after reconstitution for the decay, and as compared to different conditions of preparation using the same batches of vaccine and diluent, to avoid misinterpretation of initial cell count results that may vary from batches to batches. Nevertheless, the final parameter that should be taken into account for final conclusions on conditions of cell-associated Marek’s vaccines should be virus titration [6].

All the mean percentages of cell mortality were comprised between
5% and 12% in this study. The overall trend was an increase during the time of cell mortality in most conditions of temperature either using 1-mL or 5-mL syringes.

5. Conclusion

Effect of temperature of thawing when vaccine preparation is performed with a 1-mL syringe, is noticed for the lowest temperature of +27°C with a trend to some advantage in recommending this temperature, as target temperature for smoother and more progressive thawing conditions. This is not confirmed with the vaccine prepared with the 5-mL syringe. Effect of choice of syringe equipped with the same needle gauge, whatever the temperature conditions, is noticed for the smallest volume of 1 mL with a trend to some advantage in recommending this volume, for less turbulent vaccine flow inside the needle.

### Table 1. Study groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Thawing temperature</th>
<th>Syringe volume</th>
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<tr>
<td>1</td>
<td>+ 27°C</td>
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<tr>
<td>2</td>
<td></td>
<td>5 mL</td>
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</tr>
<tr>
<td>4</td>
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<td>5 mL</td>
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<tr>
<td>5</td>
<td>+ 33°C</td>
<td>1 mL</td>
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<tr>
<td>6</td>
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<td>5 mL</td>
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### References

Figure 1. Percentages of cell mortality using 1-mL syringes

Red circle = significant difference (alpha risk < 5%)

Figure 2. Percentages of cell mortality using 5-mL syringes

Red circle = significant difference (alpha risk < 5%)

Figure 3. Percentages of cell mortality at +27°C

Red circle = significant difference (alpha risk < 5%)
Figure 4. Percentages of cell mortality at + 30°C

![Graph showing percentages of cell mortality at + 30°C.](image)

Red circle = significant difference (alpha risk < 5%)

Figure 5. Percentages of cell mortality at + 33°C

![Graph showing percentages of cell mortality at + 33°C.](image)

Red circle = significant difference (alpha risk < 5%)