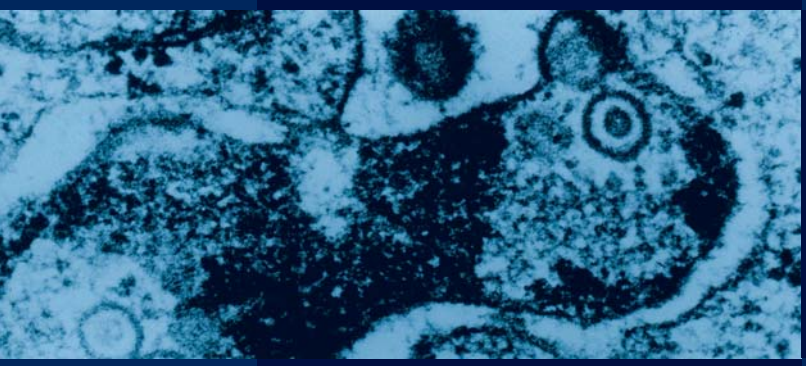

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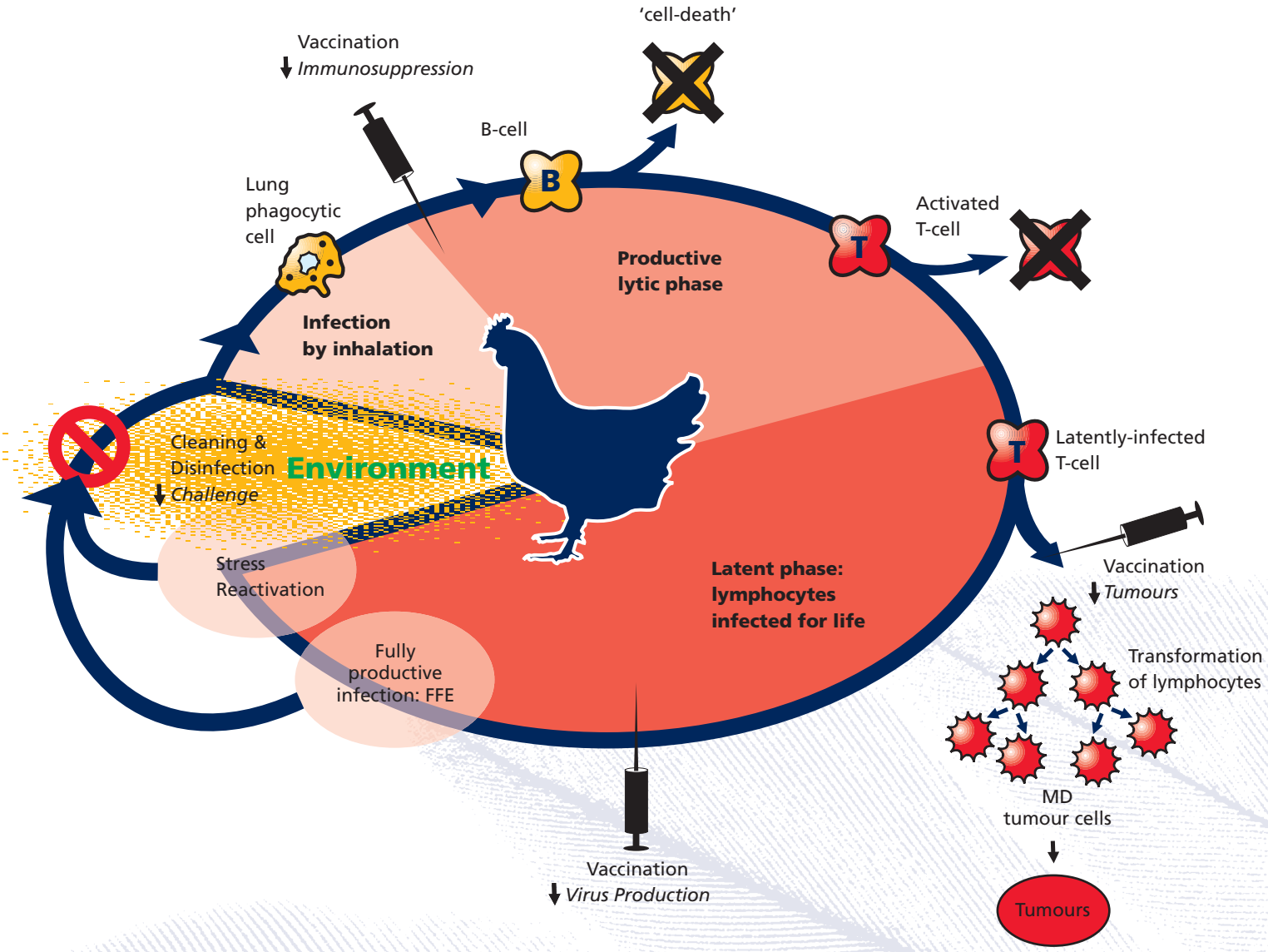
# Ross Tech

## MAREK'S DISEASE CONTROL IN PARENT STOCK

# Marek's Disease Control in Parent Stock




## STOP THE CYCLE

Adapted from "Marek's Disease: An Evolving Problem"
Edited by F. Davison and Venugopal Nair. 2004

# Marek's Disease Control

## KEY POINTS

*Marek's Disease (MD) is caused by a virus that is easily spread within and between flocks and the virus also survives well in the environment.*

- *Almost all poultry flocks are infected with Marek's Disease Virus (MDV), even in the absence of tumours, and represent a risk to young chicks.*
- *Vaccination does not stop infection but decreases tumour formation after infection.*
- *Direct and indirect contact between poultry and poultry premises by people, vehicles and equipment can spread the virus.*

*After vaccination chicks are not protected until the vaccine strain has multiplied in individual birds and viraemia has been established. Therefore chicks should not be exposed to MDV challenge for the first 14 days of life and ideally 28 days.*

- *Placing chicks on farms not cleaned and disinfected properly or on built up litter poses a MDV challenge.*
- *Mixed age and multi-age rearing farms represent a very high risk of spreading MDV and causing MD.*
- *Some people have "cured" MD problems by building isolated, very clean and very cleanable, biosecure rearing farms.*

*The development of a good immune system in the young chick is vital to MD protection. This requires the achievement of recommended early weights with good flock uniformity.*

- *Diets must be of good quality and contain the recommended protein and vitamins to ensure the proper development of the immune system.*
- *Other immuno-suppressive diseases must be properly controlled in the young chick – such as CAV, Gumboro, REV, Reo, mycotoxins and some other viruses.*
- *Where transport and other factors cause stress on the young chick all the above become even more important.*
- *Where revaccination is done this is often between 7 - 14 days and must be achieved with the minimum of stress to the chicks.*

*In the hatchery the route of vaccination is of less importance than the accuracy of vaccination. A single dose of a good quality vaccine will give perfectly adequate protection provided the other criteria above are also achieved.*

- *A combination of different MDV vaccines by different routes resulting in multiple doses to the day old chick will still not protect the young chick for the first few weeks, and in fact may exacerbate susceptibility due to the increased stress.*
- *Vaccination must follow good technique to prevent trauma and bacterial infections.*

## Control of Marek's challenge

Marek's Disease Virus (MDV) is not vertically transmitted. Horizontal transmission between poultry farms must be prevented to stop the spread of the infection. Vaccination does not stop the spread of infection but when effective decreases tumour formation after infection. MDV is in the feather and skin debris (dander) and therefore wherever one can see chicken dust the virus is potentially lurking.

**REMEMBER ALMOST ALL POULTRY FLOCKS REPRESENT A HIGH RISK OF TRANSMITTING MDV.**

In feather and skin dust MDV survives extraordinarily well. Chickens become infected by breathing in this contaminated dust. This dust must be completely removed from all vehicles and all buildings between transporting or housing birds. Thorough cleaning must include air inlets and outlets, curtains, all equipment, all contact airspaces (farm offices, feed stores, roof spaces, etc). During fumigation of a closed house the ventilation system should also be fumigated. For fumigation the seal should be at the entrance to the ventilation system not where the vents enters the house. The house surrounds must also be cleaned or the air inlets will suck in dust and it will also be walked into the house. Between poultry sites very strict control of people, vehicles and equipment is required to prevent the movement of MDV, this is absolutely vital for the first 4 weeks of the life of a flock. To achieve proper hygiene control on a multi-age site is very difficult if not impossible.

In some instances some producers have moved to purpose built highly biosecure rearing units, and in so doing have totally controlled MD.

## Vaccination

Effective application of the vaccine is a job for the hatchery. Currently the most effective vaccines are bivalent vaccines, i.e. Rispens (Serotype 1) and HVT (Serotype 3), combinations given at one day of age. There is no evidence that other bivalent or trivalent vaccines are any more effective than Rispens and cell associated HVT vaccination. Unfortunately Rispens vaccine is not available in some countries. This is sometimes on the assumption that it can cause encephalitis or that it reverts to virulence. In the course of one year Aviagen uses over 120 million doses of Rispens vaccine and has never had a problem with encephalitis. So-called "cell associated" vaccines are frozen MDV infected cells. Whole virions are not present and vaccine effectiveness relies on the cells being viable when they are thawed and administered, see *figure 1*. In the hatchery some operators measure cell viability using live/dead staining to assess potential vaccine potency.


***Figure 1:*** *Correct storage of the cell associated MD vaccine in liquid nitrogen is essential to the viability of the vaccine.*

***Table 1:*** *Below, shows vaccines available*

| VACCINE | CONTENTS | ADVANTAGES / DISADVANTAGES | COMMENT |
|---|---|---|---|
| Rispens | Serotype 1 | Rispens has been used successfully in many parts of the world. | Different Rispens CVI988 clones have different protection characteristics. |
| Rispens and HVT | Serotype 1 and 3 | Bivalent vaccines offer maximum protection. | These can be purchased in a combined form. |
| SB-1 vaccines | Serotype 2 | | Never used singly. |
| Freeze dried HVT | Serotype 3 for delivery | No cold chain needed. | May be neutralised by Maternal antibodies. |
| Cell associated HVT | Serotype 3 | Improved efficacy and perhaps not affected much by maternal antibodies. | Commonly used now *In ovo* vaccination of broilers. |

Vaccine dose may vary between hatcheries and in some instances are based on PFU titrations of vaccines. But the sensible choice for breeders is the use of a full dose. Some hatcheries do double vaccination of breeders. A single dose is given twice to the birds or two doses are given in a single injection. Other variations also occur. The options can be expensive and stressful on the chicks and investment is better made in giving a single dose accurately and efficiently to every chick. The best method of vaccinating chicks is to use the method in which the hatchery staff has the most training and expertise. The hatchery should have formal records and a training program for MDV vaccine handling and administration with regular retraining of the relevant staff (see appendix).

**Route of administration**

The most common route of administration is by subcutaneous vaccination in the neck, though intramuscular vaccination in the thigh is also practiced. There is some evidence that intramuscular vaccination may result in a slightly faster development of immunity; 8 days instead of 9 days. In all hatcheries during use the chick processing area and vaccination area become contaminated with bacteria including enterococcus, *E.coli* and staphylococci. These bacteria will contaminate vaccine needles and may cause bacterial infections and amyloidosis, when injected into leg muscles.

*In ovo* vaccination compared to vaccination at one day of age may reduce the labour cost and human error. But MDV vaccines are expensive and when given *In ovo* more than twice the number of doses are needed as vaccination is prior to sexing. For effective *In ovo* vaccination the timing of vaccination must be correct and advice is best sought from the equipment supplier and vaccine manufacturer.

**Revaccination**

Revaccination of birds with HVT or Rispens in the field between 1 and 3 weeks is commonly practised in areas with MD problems. It was popular in the past to use freeze dried HVT for this but now nearly all producers use cell associated vaccines which must be transported to the farm on dry ice or in liquid nitrogen and used immediately. Little research on revaccination has been done. Some people suggest that the mechanism of action may be as simple as the second vaccine working only in those chicks that did not receive the first vaccine correctly. If this were the case it would be more cost effective to improve vaccination in the hatchery. Revaccination has the added disadvantage of being expensive in terms of labour and interfering with the growth of the flock.

## HACCP of Marek's Vaccination

**Vaccine handling**

The delivery chain to the hatchery must be monitored. On delivery to the hatchery the vaccine must be covered with liquid nitrogen and the vaccines delivered should be identifiable in a way that allows subsequent identification of the vaccine to specific deliveries. This is important when serials (batch numbers) are delivered on more than one occasion as abuse in transit will not be able to be differentiated unless delivery is recorded. The serial number of the diluent should also be recorded and expiry date verified.

The storage of the MDV vaccine ampoules inverted allows the detection of thawing of the vaccine, see *figure 2.* During vaccine storage monitoring and topping up of the level of liquid nitrogen needs to be at least weekly. This can be done by measuring depth of liquid nitrogen or weighing the flask. Over time flasks become less efficient and will need topping up more frequently and eventually replaced. Safety equipment should be used when handling liquid nitrogen and ampoules. Liquid Nitrogen is best measured using a solid black plastic rod marked and dedicated to the particular flask. In some countries it is not possible to transport or store liquid nitrogen in hatcheries so vaccine has to be delivered daily on dry ice.

***Figure 2:*** *Vaccine vials should be stored and delivered inverted in liquid nitrogen. The vaccine vial on the left is normal with the frozen vaccine at the base. The vial on the right shows vaccine in the cap due to thawing and refreezing. This vaccine will be damaged and should not be used.*

Most diluents can be stored at room temperature and should be used at this temperature. In hot climates the diluent is sometimes put on ice. The diluent bag should be labelled with the time of vaccine make up, see figure 3. The size of diluent pack used should match the production plan so that vaccine will be used within the time frame indicated by the vaccine manufacturer. This should be no more than 1.5 hours.

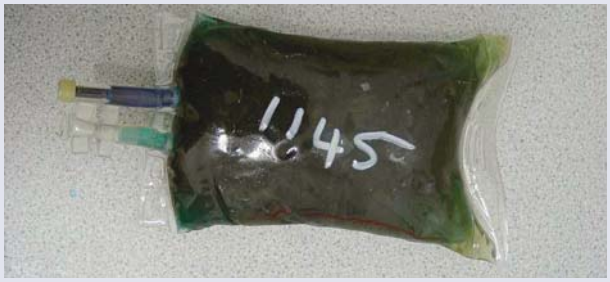



***Figure 3:*** *A bag of vaccine made up and ready to use. The vaccine bag also contains blue dye and the time of mixing is readily apparent. The vaccine must be used as soon as possible and at most within 2 hours.*

***The diluent is an excellent multiplication medium for bacteria; the introduction of even a very small number of bacteria during reconstitution of the vaccine may result in a significant bacterial challenge to the vaccinated chicks.***

***The vaccine is highly susceptible to killing by disinfectants and contact with these must be avoided.***

Vaccine should be handled and reconstituted in a special purpose built clean room, see *figure 4.*

***Figure 4:*** *This door is the restricted access to the clean room for making up MDV vaccine for administration. The room is under positive pressure with air filtration.*

This room should be like an operating theatre with no ledges for dust and no extraneous equipment stored there, see *figure 5*. The room should be well lit, cleaned and disinfected regularly and access controlled. To further enhance the prevention of bacterial contamination of the vaccine a laminar flow hood can be used for reconstituting the vaccine.

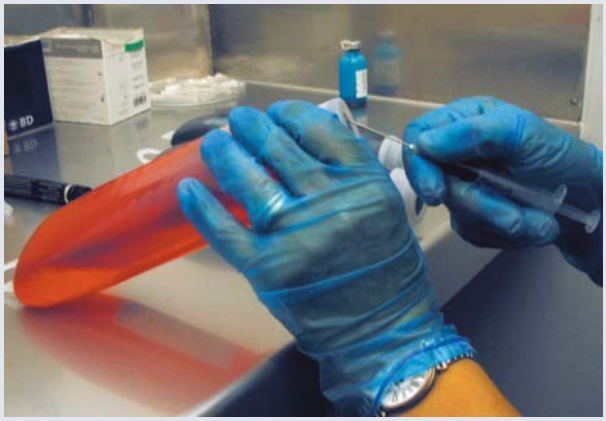


***Figure 5:*** *The clean room surfaces are all very clean and tidy, with minimal bacterial contamination.*

Clear instruction for vaccine handling should be readily available and used by the staff involved, see figure 6. The time from removal of the vaccine from the liquid nitrogen, or dry ice used to transport the ampoules, until addition to the special MDV vaccine diluent should not exceed manufactures specification, typically 2 minutes. This is a critical point in the reconstitution of the vaccine. The ampoules should be rapidly thawed by placing them in water between 30 and 35 degrees centigrade. Ampoules not to be thawed must be returned immediately to the liquid nitrogen. The vaccine should be mixed in the diluent. Nothing else should be added to the diluent unless you are sure that it will not harm the vaccine. Additives known **not** to harm the vaccine, when added at the recommended dose, are gentamycin and ceftiofur sterile powder. Additives known to harm the vaccine include enrofloxacin. The MDV vaccine should be the last thing added to the diluent.

It is now becoming popular to get diluent in plastic bags rather than glass bottles. In the future MDV vaccine ampoules may be replaced by plastic delivery systems. This has health and safety and disposal advantages. The introduction of premixed HVT and Rispens vaccine is a good recent innovation. Please record Vaccination Batch No. given to each individual Customer Order. *See appendix.*

Bacterial contamination of the vaccine can result in problems. Pseudomonas contamination will lead to massive mortalities in young chicks. Enterococcus contamination can cause amyloidosis of the joints. Staphylococcal contamination and other organisms can cause bacterial bone infections in young birds. Of course Salmonella contamination of the vaccine could cause Salmonella infection of the flock.

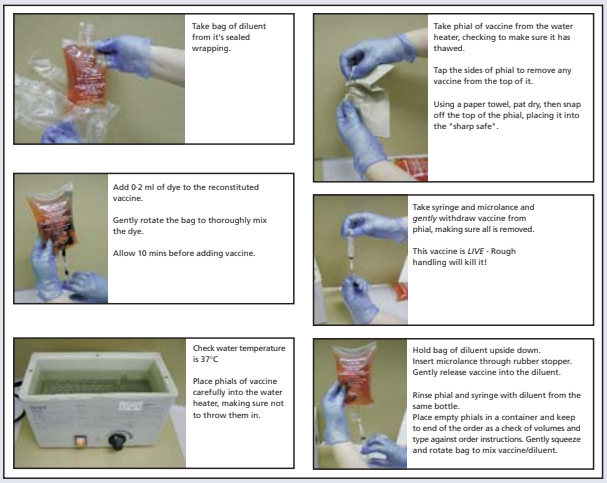



***Figure 6:*** *On the wall of the clean room for vaccine preparation there are clear laminated instructions and photos to assist in correct vaccine mixing and handling.*

The handling of the vaccine must be done in such a way as to prevent bacterial contamination of the vaccine. The water bath for thawing the vaccine should contain fresh clean water with no disinfectant (disinfectant may damage the vaccine). Quickly drying the outside of the thawed vial with paper towel will decrease the chance of liquid contamination during opening. Sterile disposable gloves should be worn during the vaccine make-up procedure.

The equipment used to make up the vaccine and administer the vaccine needs to be sterile but without disinfectant residues. The vaccine gun should be sterilized as a complete assembled unit. Reusable syringes should be cleaned with distilled water, placed in autoclave bags and then autoclaved in a vacuum autoclave with a proper drying cycle. Autoclave tape should be used and the date also written on it. Sterile syringes are then stored sealed until use. If prolonged storage is envisaged then double bagging should be used. It is certainly prudent to have spare sterile syringes stored in case they are required. Do not re-use disposable syringes as these are not designed to be sterilised with heat. During assembly care should be taken not to touch or let the "sterile" equipment touch surfaces. Using a surgical model of clean and dirty will help minimise bacterial contamination. Once something is touched it is dirty and anything else touching it becomes contaminated. Specific training in sterile technique must be given and should be revisited on a regular basis.

The vaccine should not be removed from the ampoule using narrow bore needles as this could damage the cells, 18g needles or wider bore must be used. Some manufactures consider it important to rinse the ampoules with the vaccine to wash more cells into the liquid before transferring the material to the diluent.

Assembling diluent bags and administration tubing needs to be done in a hygienic manner. Bags containing sterile equipment such as vaccine guns and administration tubing should not be opened till just before use. Do not touch sterile areas of equipment that will later be in contact with vaccine. When assembling administration tubing connect lose ends of tubing before it touches non-sterile objects. Hanging the bags or bottles during assembly can help stop inadvertent contamination.

Enumerating and identifying bacteria in vaccine samples can be used to monitor contamination. Typically 10 doses from the vaccine are taken before using the vaccine and then 10 more are taken at the end. If this is then cultured for bacteria it will allow the identification of contamination before or during use. The 10 vaccine doses are also used to check dosage volume delivery of each syringe. Most diluents contain *p*H indicators and bacterial growth should be suspected if the colour of the diluent changes before or during use. This can not be seen when dye is used, but this will only indicate very high levels of contamination.

Although hand vaccination of chicks is considered to be the most accurate method it has been largely replaced by machine vaccination. The rate of vaccination should not be more than 2000 chicks per hour per vaccinator operator. Above this rate quality is hard to maintain. Vaccinators should

receive special training on setting up the equipment and use of the equipment. During administration the vaccine should be agitated at least every 15 minutes to re-suspend the vaccine containing cells. Chick vaccinating needles should be of 20 gauge or wider bore and should be changed at the same time or more often than the diluent bags and giving sets.

Dye can be added to the diluent and this will allow assessment of the placement of vaccine in subcutaneous vaccination. This cannot be done with intramuscular vaccination. The dye can be seen under the skin of the bird for the next hour. Commonly the dye test can be used for training new vaccinators and as quality control routine, see *figure 7.* Typically a chick box from each vaccinator is checked every hour to assess vaccination. Surplus chicks, vaccinated and then killed, can also be used to train vaccinators and to accurately assess the efficiency of vaccination.

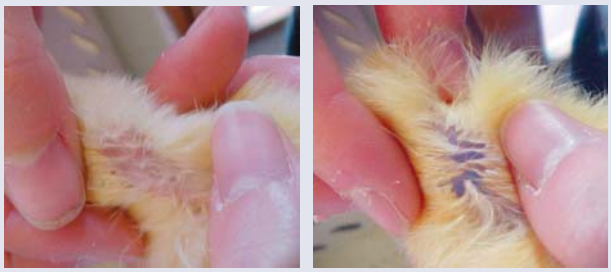


***Figure 7:*** *Immediately after vaccination squeezing the feathers on the back of the neck will show vaccine that has not been correctly injected under the skin. 100 chicks can be quickly assessed in this way. Incorrectly vaccinated chicks must be re-vaccinated.*

## Assessment of vaccine administration

The vaccine gun efficiency and the correct flow of vaccine through vaccine apparatus should be constantly monitored by the operator, see *figure 8.* Vaccine administration can be assessed by dye studies and volumes of vaccine used should be checked and recorded on a frequent basis. Further assessment is not readily or routinely available as it is highly specialised and very difficult to reliably perform. Options may include an assessment at four weeks of age by checking for viraemia. Viraemia from HVT may be a reliable assessment of the number of birds vaccinated effectively, as this virus does not spread readily between chickens. Serotype 1 viraemia is technically more difficult to perform starting with transportation of samples to the laboratory. Recently semi-quantitative PCR has been suggested as a possible means to check vaccine administration. These tests are currently advocated at about 2 to 4 weeks of age (post vaccination).

***Figure 8:*** *The use of a drip chamber during vaccination enables a visual assessment of the flow of vaccine during the use of the vaccine gun, but further accurate measuring of the volumes injected is required.*

Assessment of the titre of vaccine in the ampoule can be done by specialised laboratories. At the hatchery live/dead staining evaluation of vaccine is undertaken by some companies. Recent studies have demonstrated that the live cell concentration after reconstitution and after passage through the needle can vary depending on type of vaccination equipment. Rupturing of cells during turbulent flow may occur with some machines or rough hand vaccination or inappropriate needle sizes – this can reduce vaccine potency by 30%.

***Table 2***

| | |
|---|---|
| Do not reuse disposable equipment and maintain reusable equipment or vaccine administration. | This is false economy. |
| Use full dose of vaccine for Parent stock . | Follow the manufacturer's guidelines. |
| Do not rely on vaccination alone or delay challenge. | Biosecurity to decrease or delay challenge will help limit MD. |
| Do not try to vaccinate too many birds per hour. | Take enough time to do the job properly. Pay attention to detail. |

## Investigation of problems

Gross post mortem analysis of tumour site frequency in dead and cull birds is a useful diagnostic criterion. A common misconception is that with MD there will always be gross pathological changes observable in the nerves. This is not present in every case. Histopathological examination by a competent histopathologist familiar with avian tumours is usually the end of most field investigations on the affected birds. The differential diagnosis should always include ALV and REV induced tumours and chronic inflammatory conditions.

Further field problem investigations involve the isolation of viruses. Frequently vaccine strain and field strains of MDV will be isolated. Virulence characterisation in challenge and vaccine protection studies may be required to fully understand the problem. Demonstration of expanded repeat regions in the MDV genome by PCR is characteristic of vaccine strains.

Hatchery records should be checked to see if there are epidemiological links between outbreaks that could be due to vaccine batch/delivery failure. As most MD outbreaks are in birds over 5 months of age vaccine retention samples should be kept for at least 12 months.

Immunosuppression can be a cause of vaccination failure. Stressors like high stocking density even for short periods of time can prevent an effective immunisation from vaccination. Although the mechanism of action of MDV vaccination is not known the deleterious effect of stress on this mechanism is clear. *See Table 2*

## Paper trail

The identification of which birds received which delivery of MDV vaccine is important. Documentation of the proper handling of the vaccine is also important including matching usage to chick output. This reconciliation of vaccine stock used and chick throughput should be done at the end of each workday.

**DELIVERY**

**Vaccine Storage**

**CHECK ON DELIVERY**

- Check that the vaccine still in the bottom of inverted ampoules
- Are the ampoules covered with liquid nitrogen
- Identify the delivery for traceability (colour code)
- Store retention sample (2 vials) for 12 months in liquid nitrogen
- Send the vaccine back if it does not meet standards previously agreed with the supplier
- Monitor liquid nitrogen levels
- Record actions and operator

**Thawing**

- Check type of vaccine
- Move quickly into water at 30 - 35°C
- Maximum time from water to diluent 90 seconds
- Use sterile technique and gloves
- Don't rupture cells – use correct needle size
- Record serial and delivery information to birds to be vaccinated and operator

**Reconstitution**

- Use sterile technique and gloves
- Dry outside of vial before opening
- Don't rupture cells – use correct needle size
- Label bag with time of reconstitution

**Vaccination**

Equipment

- Use reconstituted vaccine within 1.5 hours
- Clean equipment and sterilise in autoclave bags
- Check dosage
- Check sterility at start and finish of vaccination
- Check vaccination by counting dye in 100 chicks within 15 minutes of vaccination
- Agitate the bags/bottles every 15 minutes to stop sedimentation
- Record time of bag on and off vaccinator
- Balance stock inventory and record operator

# Appendix

## Documentation

1) Vaccine stock control
2) Vaccine administration record
3) CCPs recording
4) Video recording of vaccine reconstitution
5) Training records

## 1. Vaccine balance for Wondervac Rispens vaccine

| Date | Serial | Delivery ID | Delivered | Used | Balance | Sign off |
|---|---|---|---|---|---|---|
| | | | | | 500 | Chris |
| 11/11/01 | MS2213 | 001 | | 30 | 470 | Chris |
| 11/11/01 | MS2213 | 002 | 1000 | | 1470 | Ch |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |

## 2. Vaccine balance for Wondervac HVT vaccine

| Date | Serial | Delivery ID | Delivered | Used | Balance | Sign off |
|---|---|---|---|---|---|---|
| | | | | | 1500 | Chris |
| 11/11/01 | AT2213 | Blue01 | | 30 | 1470 | Chris |
| 11/11/01 | AT2213 | Blue02 | 1000 | | 2470 | Ch |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |

## 3. Liquid $N_2$ levels/Weight

Level in Bulk vaccine store to be kept between 50 and 60 cm.

| Date | Height | Sign off |
|---|---|---|
| 11/11/01 | 55cm | Chris |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

## Marek's vaccine recording

| Date | Customer | Number of birds | Number of vials used | Vaccine brand and name | Delivery number | Serial number | Diluent ID | Dossage & additives | Sign off |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| | | | | | | | | | |
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| | | | | | | | | | |

## Marek's Training recording - Mr Andrew McVaccinator

| | Initial Training dates and trainer | Assesed as competent date and assessor | Re-assessment date and assessor | Re-assessment date and assessor | Comments - further training needs, etc |
|---|---|---|---|---|---|
| Vaccine Delivery | | | | | |
| Vaccine Storage | | | | | |
| Sterile Technique | | | | | |
| Vaccine Reconstitution | | | | | |
| Vaccine gun cleaning and sterilization | | | | | |
| Vaccine equipment assembly | | | | | |
| Vaccine administration | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

*Re-assessment should be carried out weekly after initial training and gradually extended but should never exceed 3 months*

This information comes to you from the Technical Team of Aviagen. Although it is considered to be the best information available at the present time, the effect of using it cannot be guaranteed as performance can be affected substantially by many factors including flock management, health status, climatic conditions, etc.

Every attempt has been made to ensure the accuracy and relevance of the information presented. However, Aviagen accepts no liability for the consequences of using the information for the management of flocks. Data presented in this Ross Tech should not therefore be regarded as specifications but illustrate potential performance.

Aviagen™

For further information on the range of technical literature available please ask your local Technical Service Manager or contact our Marketing Department at:


Newbridge
Midlothian
EH28 8SZ
Scotland
UK
**tel:** +44 (0) 131 333 1056
**fax:** +44 (0) 131 333 3296
**email** infoworldwide@aviagen.com

Cummings Research Park
5015 Bradford Drive
Huntsville
Alabama 35805
USA
**tel:** +1 256 890 3800
**fax:** +1 256 890 3919
**email** info@aviagen.com

**website** www.aviagen.com