Wherever possible, today’s large poultry operations prefer to use mass application techniques for vaccination with live vaccines.

Even though these techniques involve application to thousands of birds at one time, the goal is the same as for individual bird vaccination: deliver a minimum of one dose of vaccine to the immune system of each bird. Not only does the dose of vaccine have to be adequate, but it must be alive when it reaches the birds in order to replicate and cause an immune response.

Under practical conditions our aim is to vaccinate the highest possible proportion of birds in a flock. This prevents the proliferation of a field pathogen on a farm and thus minimises the effects of a particular disease. The actual proportion of birds which need to be effectively vaccinated will vary according to the infectious agent involved, the current disease situation and the type of vaccine.

Mass application through the water offers the advantages of lower labour costs, minimal bird stress and stimulation of good mucosal immunity. Its main disadvantages are inconsistencies of vaccine dosage depending on water consumption, and the potential for some birds to receive no vaccine at all. Incomplete coverage of the flocks can result in post vaccinal reaction and/or ‘heating up’ of vaccine virus due to repeated bird to bird transmission.

Although vaccination via drinking water would seem to be the least labour intensive it is certainly not the simplest and fastest method if we want to do it correctly. There are quite a few precautions that need to be taken for the vaccination to be effective and reliable.
How live vaccines work

All live virus vaccines must reproduce themselves within the host before they can stimulate a protective immunity. They do this primarily by attaching themselves to target cells within the host and then causing the cells to make identical virus particles. This is the phase that results in the vaccination reaction or ‘take’, which accompanies the use of live virus vaccines. The replication of the vaccine virus prompts the host to produce circulating and local protective antibodies against the particular virus. It follows therefore that three things are critical in this sequence of events:

1) the virus must still be alive when it reaches the host.
2) sufficient live virus particles must reach target cells to stimulate a protective response on the part of the host.
3) each member of the flock must receive sufficient live virus particles since further spread of virus within the flock cannot generally be relied upon to stimulate uniform protection.

Live vaccine viruses are quite stable so long as they are in freeze dried form and stored in the fridge. Once they are in diluent they begin to die after a while. Normally the farm’s water is the diluent for the vaccine. How suitable a diluent this is depends on several factors (see later).

It follows that everything possible must be done to keep as much of the virus contained in the vaccine alive and to ensure that all birds have a chance to receive enough vaccine. Live virus vaccines are particularly susceptible to

1. Ultraviolet light: avoid exposure to sunlight
2. Heat: avoid prolonged exposure to high temperatures (e.g. vaccine vials left by a window). The drinking water should be cool i.e. 15-20°C.
3. Heavy metals: don’t use utensils or drinking water containing these. Use plastic buckets or containers for vaccine reconstitution.
4. Chlorine: Do not administer vaccines in drinking water containing chlorine (if you can smell it or taste it, the level is definitely very high)
5. Disinfectants and detergents: make sure that all utensils are free from traces of these.
6. Organic matter (litter, feed etc): make sure that all utensils and drinkers are clean.
7. Moisture: don’t use the contents of damaged vials. Also don’t break the seals on the vaccine vials until you are ready to reconstitute the vaccine.

The major concerns in water vaccination are those of prevention of inactivation and proper distribution of the live vaccine.

Inactivation of the vaccine virus can be prevented by 1. rapid administration and by 2. protection of the virus particles from inactivating agents which may be present in the water and water lines.

Rapid administration

Submersible pumps (Figure 1) can be connected to both bell drinker and nipple line systems. They ‘push’ the vaccinated water through the lines very quickly helping distribution of the vaccine evenly throughout the whole shed.

We must ensure that each bird has enough drinker space to drink enough of the vaccinated water at the same time. Nipple drinkers are associated with less water wastage and also less social competition for drinking sites when, after the withdrawal period, birds are allowed to drink water containing vaccine.

In the case of bell drinkers, if the existing numbers are not enough to ensure adequate drinking space for all birds, (and therefore prevent uniform vaccine uptake) additional drinkers (e.g. plastic trays) should be installed for the period of vaccination. For broiler breeders 100 birds per bell drinker is adequate in most circumstances. Layer type birds may need one cup/12 birds, one nipple per 8 birds, one bell drinker per 50 birds or 2.5 cm (one inch) of trough per bird.

If the drinker system is based on bell drinkers and the header tank cannot be used it is essential to have enough manpower on the farm to carry vaccinated water to each drinker as quickly as possible to prevent birds fighting for water and spilling vaccine. (Figure 2)
Protection from inactivation

Emphasis is always placed on proper handling, storing and mixing of the vaccine but all these efforts can be quickly spoiled by an improperly maintained water system. *Sanitising products* in the water such as chlorine, ammonia compounds or acidifiers like citric acid; residual sediment in the water lines and in the filters will inactivate the vaccine virus. The *sediment* in the water system can bind with or destroy vaccine viruses. Failure to protect the vaccine may lead to partial protection only against the disease.

It is advisable to check the quality of the drinking water on a regular basis. Too high or too low pH can have a negative effect on the efficacy of the vaccine virus. In addition a *chlorine level as low as one ppm* or contamination with heavy metals for example may *inactivate the vaccine virus*. Oxygenated water has a strong antimicrobial effect therefore the oxygenating system should be switched off at least 48 hours before vaccination.

**Use of skimmed milk in water**

To neutralise the detrimental effect of chlorine or other sanitizers in the water adding 2.0 g of *skimmed milk powder* (Figure 4) per litre of water or 2.0 (8 pints) litres of *skimmed milk* (Figure 3) per 100 litres (22 gallons) of water is recommended. Once the milk or milk powder is added to the water it should be left to stand for 15-20 minutes before using it for reconstituting and administering the vaccine.

Skimmed milk powder can form lumps when mixed with water and be difficult to dissolve. To overcome this problem, warm water at about 60°C can be poured onto the milk powder and stirred thoroughly with a whisk.

The reconstituted milk then can be be added to the drinking water. The *milk proteins in the water bind chlorine and metallic ions*, helping the survival of the vaccine virus in the water.
Filters
Filters must be removed or bypassed before vaccination. Slime and dirt build up on filters can concentrate undesirable disinfectants, minerals etc. on the surface of the filter, which may inactivate the vaccine virus. (Figures 5)

Dirt in bell drinkers
If litter gets into the drinkers it is usually a mixture of litter and faeces. This ‘cocktail’ can change the pH of the water in the drinkers and can adversely affect the vaccine virus. If the flock is under medication, residues of drugs or their metabolites in the faeces can also change the pH conditions. (Figures 6 & 7)

Shavings or other litter material can act as weight in the bell type drinkers and keep the valves closed preventing vaccinated water flowing down into the drinkers.

Dirt in the drinkers can also absorb the vaccine onto its surface adhering it to the bottom or side of the drinkers. In other words a certain proportion of the vaccine will remain on the drinker and not enter the birds. This will lead to a non-uniform immune response and poor flock protection. It is always advisable to scrub clean the drinkers on the morning of vaccination but use no sanitizer or disinfectant. (Figure 8)
Distribution of vaccine

As well as problems surrounding the quality of the drinking water there are various problems connected with the extensive range of drinking water installations available. For example there are some systems which are difficult to drain and retain substantial volumes of chlorinated water in their ‘dead spaces’.

If drinker lines are not drained before vaccination the residual water in them can not only neutralise vaccine virus but slow down its distribution as well. In such a case, birds at the top of the shed may consume enough vaccinated water whilst the ones at the bottom of the shed may receive no vaccine at all. It is therefore essential to allow birds to drink only after the drinker lines have been drained and then primed with vaccinated water.

Problems with cage systems

One of the difficulties associated with vaccination of caged pullet flocks is over-consumption of the vaccine solution by birds at the inlet end of the water line. Birds further down the water line may not receive vaccine solution, which results in uneven vaccination of the flock.

This can be a particularly acute problem when the flock is excessively thirsty at the time of vaccination or when the vaccine volume is insufficient. It was observed using Hi-Light dye tablets in a caged pullet house that the vaccine solution did not even reach the end of the cage battery by the time all the vaccinated water had disappeared from the header tank. A remedy to this problem might be to close the water system for the night, let the birds drink out all the water from the lines and prime the lines with vaccinated water before lights come up in the morning. This way all the birds have equal chance to take the vaccine as long as the header tank contains enough water for the duration of vaccination.

Depending upon the water system design, water lines of different cage batteries may not fill at the same time. (Figure 9) The result of this unequal filling rate is that exposure time to vaccine solution is not equal throughout the house, leading again to uneven vaccination. Each house needs to be evaluated individually to determine the best approach towards vaccination.

In other trials with dye tablets it was found that houses charging water lines with vaccine averaged 98% in tongue staining with dye, while houses not charging water lines averaged 61%. (See details of dye tablets later)
Further factors affecting outcome of water vaccination

**Age related problems**
Vaccinating birds younger than three weeks old needs to be done with extra care because the intake of water before that age can be irregular.

**Water consumption**
Water consumption is influenced by breed, type of feed, ambient temperature, length of water withdrawal time, lighting programme and type of drinker system. We can get some information from tables in books and brochures but more accurate information can be obtained by using water meters or checking water levels in the header tanks. (Figures 10 & 11)

If in doubt a 'trial' vaccination can be done a day or two before the real one to establish the volume of water consumed within a two hour period, at the same time as planned vaccination. In this way we shall be able to find out how many litres or gallons of water are required for the vaccination. In addition we will also be able to detect and rectify any flaws in delivery of the vaccine.

**The amount of water needed for vaccination**
This depends principally upon the age of the birds. Mixing the vaccine in an adequate amount of water is essential in gaining uniform and desired immune response. If too much water is used, birds do not consume their portion of the vaccine within the allotted time, yielding weak and inconsistent titres. If too little water is used more dominant birds or the ones nearest the drinker system over-consume leading to uneven uptake and non-uniform immune response among birds in the same house.

As a rule of thumb for 1,000 broilers, 1,000 doses of vaccine should be dissolved in as many litres of water as the age of the birds in days. This is the minimum volume and it can be doubled if climatic or other reasons justify it.

For layer pullets at an ambient temperature of 21°C the following volumes can be used as guidelines for drinking water vaccination

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Water per 1000 birds</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
</tr>
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<td>5</td>
<td>8</td>
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<td>12</td>
<td>12</td>
</tr>
<tr>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

As a guide for broiler breeders the following data can be used for vaccination at 21°C ambient temperature.

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Water per 1000 birds</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3</td>
<td>5.5</td>
</tr>
<tr>
<td>4-6</td>
<td>6.5</td>
</tr>
<tr>
<td>7-10</td>
<td>10</td>
</tr>
<tr>
<td>10-15</td>
<td>13</td>
</tr>
</tbody>
</table>
For birds on lighting programmes e.g. layer pullets or broiler breeders, vaccine may be given after a feeding, drinking and water withholding period or with the first water in the morning. This eliminates the need for a withholding period. The choice of method depends on management practices on a given farm.

**Water withdrawal time.**
As a general guideline **all vaccinated water should be consumed within two - three hours.** To facilitate this, water has to be withheld from the birds for a period of time prior to vaccination. The length of time depends on several factors such as the type and age of birds, and climatic conditions.

Trial results with food dye in the water suggest that the **ideal length of water-withdrawal for vaccination of broilers is 1-1½ hours.** This time can be used as a guideline for other type of poultry too. Ambient temperature must be carefully considered when establishing water withdrawal times. If birds are made excessively thirsty they will fight for the water leading to uneven uptake and spillage of vaccine. *(Figures 12 & 13)*

**Timing of vaccination**
Vaccination **early in the morning** is recommended since this is the time when birds will exhibit peak activity and therefore peak water consumption.

**Exposure time to vaccinated water**
Two factors must be taken into consideration when determining the length of exposure time required to successfully water-vaccinate a flock. First, increased exposure time to the vaccine solution gives more birds the opportunity to drink. On the other hand the time should be limited since the titre of reconstituted vaccines generally starts to decline after two hours. Stability in water is not the same for all vaccines and depends on the type of vaccine virus being administered. The titre of an IB vaccine for example declines faster after two hours than that of an IBD (Gumboro) vaccine.

Virus stability in water should be considered when developing water vaccination protocols.
Evaluation vaccination using a blue dye
A water soluble blue dye (Figure 14 & 15) is available to evaluate and monitor water vaccination technique.

This blue dye has the ability to mark birds having consumed vaccine solution by temporarily staining their tongues. It has been observed that the intensity of tongue staining varies depending on the amount of vaccine solution consumed. The intensity of tongue staining is related to the protection offered by vaccination.

Newcastle Disease challenge of vaccinated birds having different degrees of tongue staining has demonstrated that birds with more intensely stained tongues were better protected than those with lightly stained tongues. The dye can also be used in trial vaccinations to evaluate vaccination technique or to check the water system.

If you want to monitor whether vaccinated water has reached all the drinkers one Hi-Light tablet per 200 litres (45 gallons) is sufficient. If you want to estimate the rate of vaccine uptake of a flock, one tablet per 20-30 litres is needed. To have a reasonably accurate picture about success or failure of vaccination, pick up 50 birds from each corner of the shed and look at their tongue and crop. If vaccination has been done correctly, at least 90% of the tongues and crops will be coloured blue.
Dead Space
Depending on the type of drinker system up to 10% of the vaccinated water can remain in the dead spaces of the system and remain unavailable to the birds. In certain types of header tank there is residual water under the level of the outlet pipe. This means there is always some vaccine left in the tank once vaccination is finished. It is important that extra vaccine is added to allow for this. The manifold of the water system can also contain substantial residual water. Residual chlorinated water in the dead space of nipple lines (Figure 19) can be detrimental to the vaccine virus, therefore the system must be drained completely before allowing vaccinated water to enter the drinker lines. (Figure 18)

Utensils
Only clean, dedicated utensils must be used for vaccination. It is false economy to use the same bucket or measuring jug for preparing foot baths, weed killers and vaccine stock solution! Residues of disinfectants or other chemicals are capable of killing vaccine virus at a very low level. (Figure 20)
Preparation of vaccine vials
Vaccine vials should be prepared in an office or a dedicated room on a clean bench or desk, free from residues of sanitizers or disinfectants. If these conditions cannot be met, covering the required surface with a clean paper tissue or plastic bag is adequate. (Figures 21 & 22)

The floor of the entrance area of a shed is not acceptable for vaccine preparation because of the dirt and disinfectant residues with which vaccine vials can come into contact. (Figure 23)

Methods of administration
The four common methods of delivering vaccines through water are by
- directly pouring vaccine solutions into drinkers,
- using overhead gravity flow tanks,
- directly pumping vaccine solution from a mixing tank (power flush)
- or delivery through a proportioner.

As delivery systems vary from farm to farm we are only able to give general advice on practical steps of vaccination (see page 11) and each farm has to develop its farm adapted SOP (Standard Operating Procedure) for vaccine administration.
### One or two days before vaccination

**Preparation**

- Keep vaccine refrigerated.
- Observe expiry date, batch number and type of vaccine and record them. Have you got the right type for this vaccination?
- Read manufacturers recommendations on the insert label.
- Check the health status of birds and only vaccinate healthy birds.
- Make sure there are no sanitizers or acidifiers in the water system. Remove all medications, acidifiers and sanitizers at least 48 hours before vaccinations.
- Calculate dead space in the system and include extra vaccine for it.
- If you use header tanks for vaccination clean them if needed and check water levels.
- Record water meter readings for the time period you intend to vaccinate.
- If in doubt do a trial vaccination using dye or milk as an indicator.
- Prepare clean utensils (measuring jug, stirrer, bucket, watering can, scale etc.).
- Wash troughs or bell drinkers with clear water without any disinfectant or sanitizers.
- Test run submersible pumps if you intend to use them.
- If you do not use header tank, fill up plastic bins/containers with the required amount of water the evening before vaccination.
- Ensure adequate amount of skimmed milk powder or liquid skimmed milk are available.
- If you use a proportioner, a separate proportioner should be used for vaccination only.
- Plan timing and other details to avoid failure on “D-day”.

### Day of vaccination

**Vaccination of broilers on nipple system with header tank**

- Turn off the main tap to the drinker lines, let the birds ‘drink the lines dry’ and then raise the drinkers. Fill up the header tank with the volume of water required for two-three hours. Shut down water supply to the header tank. If header tanks contain too much water for the vaccination drain the tanks till the required volume is reached.
- Mix the milk into the water in the header tank to achieve a 2% solution (two litres of milk into 98 litres of water).
- Dim the light and drain the water lines to get rid of residual chlorinated water in the system.
- Prepare the right type and amount of vaccine for vaccination on a clean surface (not on the floor) free of residues of sanitizers or disinfectants.
- Using a jug or a bucket of milky water, mix the vaccine into it by opening the vials under the water. Rinse the vials of vaccine several times to ensure there is no vaccine left in the vials.
- Pour the vaccine into the header tank and mix it thoroughly.
- Prime the drinker lines with vaccinated water and let the milk-stained water reach the far end of each line. Preparation of the lines in this way ensures that birds at the end of the water line have the same chance to drink the vaccine as birds at the front of the lines.
- Turn off the end-valve of lines, lower the drinkers to bird level.
- Increase light intensity and activate feeders.
- Walk along the drinkers and encourage birds to go to the feeders and drinkers. Check drinkers for any blockage or leakage.
- Ensure the main tap of the water system is reopened just before the header tank runs dry to prevent air locks.
- Rinse utensils used for preparation and administration with plenty of water. Do not use any sanitizer or disinfectant for this job!
- Dispose of vaccine vials following waste disposal guidelines.
- Once utensils are dry store them in sealed plastic bags in a clean area of a farm.
The steps and principles of this vaccination method can be adapted for any drinker system. It requires careful planning and testing to establish a farm specific vaccination protocol that can be adhered to by existing and future farm staff.

Record keeping

Complete the vaccination record form with name and batch number of vaccine(s) used and keep the record available for inspection.

REMINDER

The success or failure of vaccination depends on the administration of vaccines. We must keep in mind however, that we cannot achieve 100% protection by vaccination only. Massive numbers of infectious agents are sometimes capable of breaking through the immunity of a flock like floods break through dams. This is why it is so important to implement bio-security and hygiene measures to minimise entry and spread of pathogens to and within a farm so reducing infection pressure.