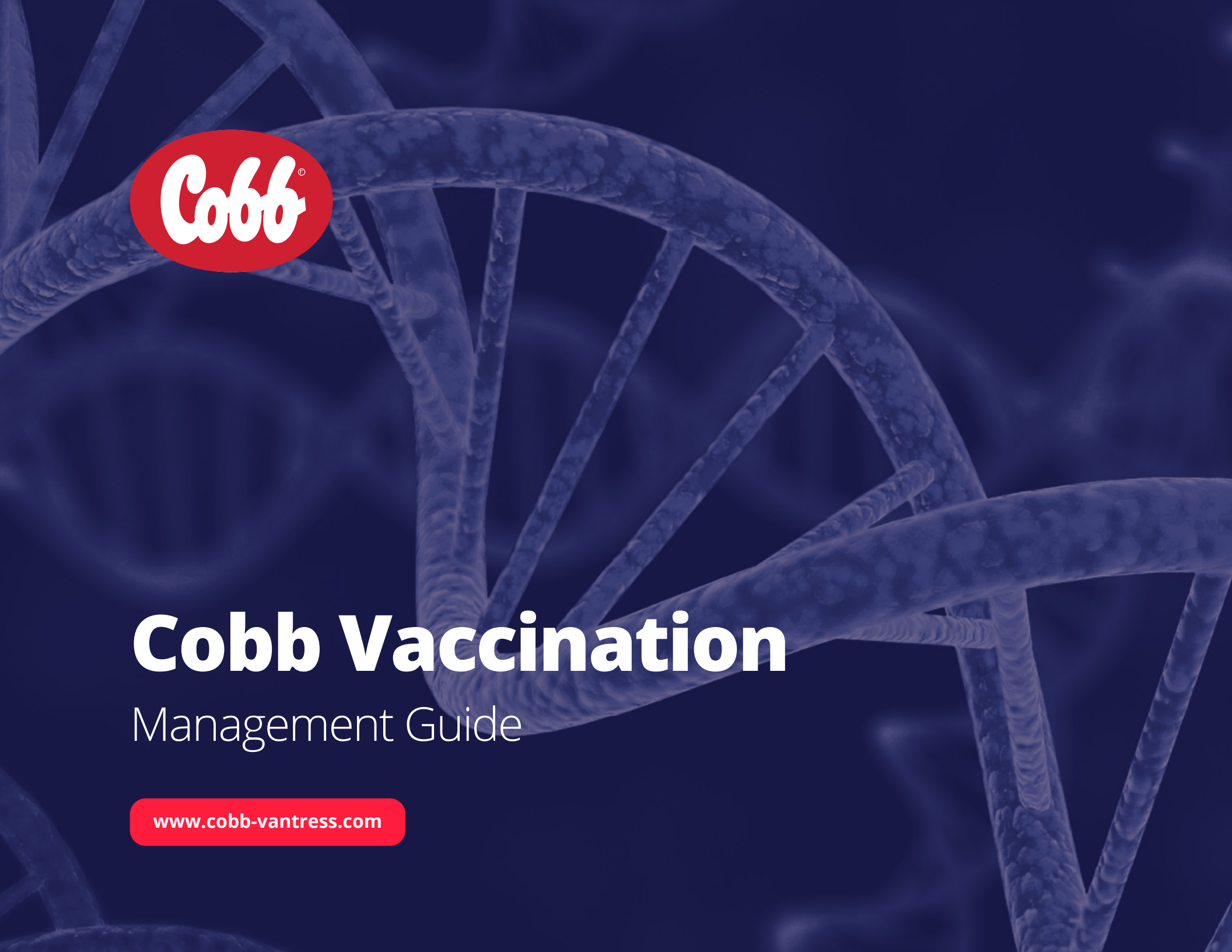


Cobb Vaccination

Management Guide

www.cobb-vantress.com

Introduction

1

This guide is designed to help field personnel in the proper use and administration of poultry vaccines. It is intended as a practical field reference to offer standard operating procedures to improve the delivery and efficacy of vaccines in the hatchery and at the farm in order to optimize flock performance and immunity.

For more information on proper vaccination procedures, please consult your Cobb Technical Service Representative for any questions and assistance.

1.1 Why Do We Vaccinate?

Correct vaccination is an essential part of a good poultry management program and vital to the success of any poultry operation. Effective preventive procedures such as immunization and biosecurity protect hundreds of millions of birds worldwide from many contagious and deadly diseases and have resulted in improved flock health and production efficiency.

Immunization cannot be a substitute for poor biosecurity and sanitation. Thus, vaccination programs may not protect birds that are under stress or raised in unhygienic conditions.

The primary objective of immunizing any poultry flock is to reduce the level of clinical disease and to promote optimal performance. The use of certain vaccines in chicken flocks (i.e. *Salmonella* vaccines), can be useful to reduce disease in the poultry flock and may also have a positive impact on human health by reducing the risk of human infection through food consumption.

For breeders, we also want to accomplish some additional goals:

- ✓ Protect the bird (as a pullet or hen) against specific diseases
- ✓ Protect the progeny of the hen against vertical transmission of disease
- ✓ Provide passive immunity to progeny

Animal Welfare Tips

Look for this Cobb Cares symbol throughout the guide that highlights the Animal Welfare Tips and important aspects of management to improve poultry welfare outcomes during vaccination procedures.

*Cobb Vaccination Management guide is available online under **Resources > Management Guides***

1.2 How Do Vaccines Work?

Poultry vaccines are biological products that induce an immune response to specific disease causing agents. Depending on the vaccine, they can be administered in various ways which are discussed in this guide.

Depending on the type of antigen in the vaccine, the bird's immune system will react, creating a "memory" response of antibodies and immune cells. The more a bird is exposed to the same antigen, the greater the antibody response and resulting protection. This is the reason why many flocks are vaccinated multiple times for the same disease – to maximize the immune system's response.

1.3 Vaccines and Vaccination

Vaccines for poultry come in three general forms: Modified or Attenuated (Live), Inactivated (Killed), and Recombinants. Live vaccines are strains that are naturally or genetically modified milder forms of field strains. Inactivated vaccines are whole viruses or bacteria that have been killed during production and formulated into a deliverable product. Recombinant vaccines, known also as vector vaccines, are made by using live viruses or bacteria as a vector to transport the gene coding for the protective antigen of a second infectious agent for which immunity is desired.

Various Vectors

The main viral vectors used for the development of recombinant vaccines are Herpes Virus of Turkey (HVT) and the Poxvirus among others. These viruses have genomes that are large enough to accept large inserts.

Examples of recombinant vaccines are:

- ✓ HVT expressing Newcastle Disease virus protein
- ✓ HVT expressing Avian Laryngotracheitis virus protein
- ✓ HVT expressing Infectious Bursal Disease virus protein
- ✓ HVT expressing two inserts (Infectious Bursal Disease and
- ✓ Newcastle Disease)
- ✓ Fowl pox virus expressing Avian Influenza virus protein
- ✓ Fowl pox virus expressing Newcastle Disease virus protein
- ✓ Fowl pox virus expressing Infectious Laryngotracheitis virus protein

Recent research has shown differences in replication within the recombinant HVT (rHVT) products, and therefore, it is very critical to add the Rispens vaccine strain when long-life birds such as breeders and commercial layers are vaccinated with rHVT products.

There is also a clear interference between rHVT vaccines and conventional HVT strains. Therefore, no chick should receive both products as the interference will lead to poor vaccine replication and may affect the expression of the insert.

Table 1 Comparison of Live, Inactivated and rHVT Vaccines

Aspect of the Vaccine	Live	Inactivated	rHVT
Safe	Yes	Yes	Yes
Economical	Yes	Expensive	Varies
Mass Application	Yes	No	No
Rapid Onset of Immunity	Yes	No	No
Duration of Immunity	Short	Long	Intermediate
Combination of Antigens Available	Yes	Yes	Yes
Maternal Antibody Interference	Yes	Low	No
In-Ovo Application	Some	No	Yes

1.4 Vaccine Handling and Storage

For All Vaccines:

- ✓ Vaccines should arrive with cool packs in a well-insulated box.
- ✓ If vaccines arrive hot, call manufacturer or distributor.
- ✓ Storage temperatures should be 2 to 7°C (35 to 45°F).
- ✓ Avoid freezing, extreme heating and intense light.

For Live Vaccines:

- ✓ Transport to farm in coolers with ice packs to keep temperature constant.
- ✓ Mix with diluent (reconstitute) just before application.
- ✓ Use vaccine within 45 minutes after dilution for Marek's vaccine and up to 2 hours for Infectious Bursal Disease and Newcastle Disease.

For Inactivated Vaccines:

- ✓ Always follow the manufacturer's instructions regarding preparation and delivery of any vaccine.
- ✓ Inactivated vaccines are especially susceptible to temperature extremes or poor handling. These products are typically in an oil emulsion and mishandling these products can result in disruption of the emulsion, known as a broken emulsion.
- ✓ Pre-warm oil emulsion vaccines at room temperature for 12 to 24 hours or using a warm water bath (do not exceed 37.7°C (100°F) for more than 5 hours). Pre-warming the vaccine reduces the viscosity of the mineral oil, making the administration easier and reducing any intense local reactions.

1.4 Vaccine Handling and Storage (cont.)

For Inactivated Vaccines (cont.):

- ✓ Gently agitate bottles thoroughly prior to use. If the vaccine still has separate layers after agitating, test to see if the emulsion is broken, by shaking the bottle vigorously for 2 minutes. Let the bottle rest for 5 minutes. If separation persists, do not use that bottle of the vaccine and contact the manufacturer (See Figure 1).
- ✓ Do not leave bottles in direct sunlight during transport to farm.

Figure 1. Varying presentations of inactivated vaccines and which are safe to use:

1	2	3	4	5
Normal	**Normal**	**Normal**	**Broken Emulsion**	**Broken Emulsion**
Uniformly milky white	Significant settling has occurred	Slight settling has occurred	Bottom layer is black or dark brown	Plus, a thin layer of black or dark brown
	Vaccine has two layers	Vaccine has two layers	Vaccine has three layers	Vaccine has three layers
✓	✓	✓	✗	✗
Ok to use	**Ok to use**	**Ok to use**	**Do Not Use!**	**Do Not Use!**

Hatchery Vaccination

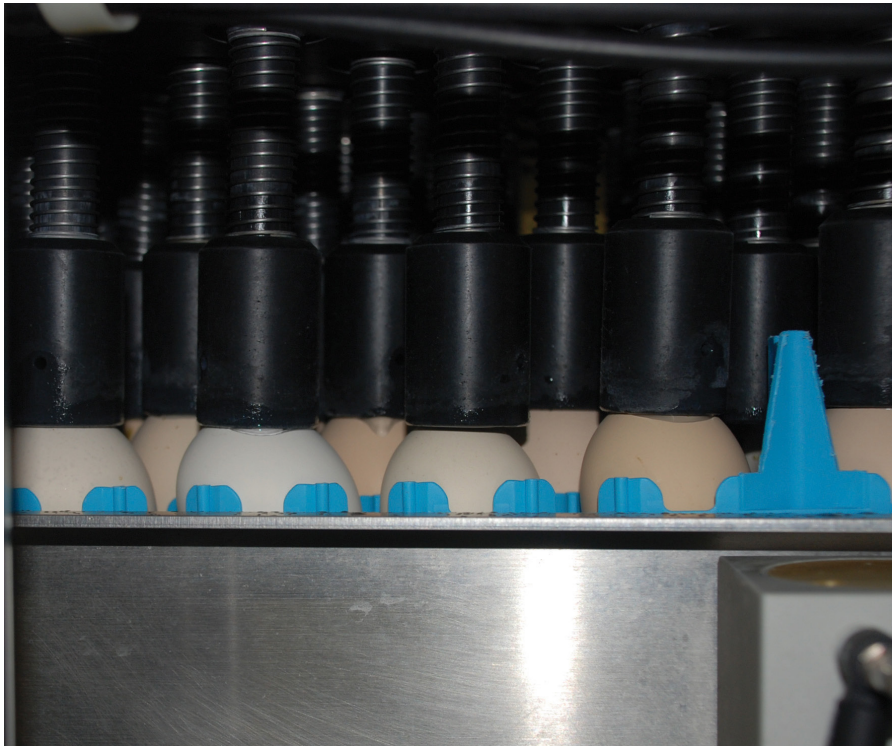
2

The hatcheries are a place where many chicks can be vaccinated conveniently and effectively. For this reason, an increased number of vaccinations are being given at this point.

2.1 *In-Ovo* Vaccination

One technology that has increased hatchery vaccination in recent years is ***in-ovo*** vaccination. ***In-ovo*** vaccination, most commonly using Marek's vaccines, is performed at the time when the hatching eggs are transferred from the setter to the hatcher. The process and technique used to administer vaccines *in-ovo* is critical as the delivery must be made to precise locations within the egg and with the highest hygiene levels possible. For optimal performance, vaccine inoculation must be done between 18 and 19 days of incubation either via the amniotic or the intraembryonic route.

Providing certain criteria are met, including timing and site of vaccine application, vaccine mixing, machine sanitization, and hatchery management specifications, the *in-ovo* vaccination has proven to be an efficacious and convenient method of vaccination. In the last few years, *in-ovo* technology has been extended for other vaccines, including live and recombinant vaccines, and efforts to extend it for other viral, bacterial and coccidiosis vaccines are in progress.



2.2 Marek's Vaccine Preparation

Use only a clean and sanitized room for the preparation and reconstitution of the vaccine. Preferably, the vaccine should be prepared in a room located away from chick rooms to prevent possible chick dust contamination and to limit any unnecessary people entering or exiting the area while vaccine is being prepared.

Only designated and trained personnel should perform vaccine reconstitution. Before and after every batch of vaccine is reconstituted, the work area should be cleaned and sanitized.

- ✓ All additives (dye, antibiotics, etc.) must be added to the diluent at least 5 minutes prior to adding vaccine. For each additive, use a sterile syringe.
  - *Add the vaccine dye to the diluent*
  - *Add antibiotic, only when prescribed by a veterinarian, at the recommended dose*
- ✓ Record all additives on the diluent bag.
- ✓ Prepare a clean water bath with a chlorine disinfectant (final concentration of 200 ppm). Set water temperature to 27°C (80°F).
- ✓ Remove the vials to be reconstituted from the liquid nitrogen tank.
- ✓ Place the vials in the prepared water bath 27°C (80°F) and allow to thaw (approximately 70 to 90 seconds depending on the dose).
- ✓ Once thawed, remove the vials from the water bath and dry using a clean paper towel.
- ✓ Spray or wipe the vials with 70% alcohol. Then, break the cap off the vial, taking care not to touch the vial openings (top and bottom).
- ✓ Wipe the port of the diluent bag with a 70% alcohol wipe before withdrawal of the diluent. Using a sterile 20 ml syringe with 18-gauge needle, draw approximately 10 ml of prepared diluent (containing additives) from the diluent bag. This will act as a buffer for the vaccine.
- ✓ Gently tap the top of the vial to ensure all the vaccine is in the bottom of the vial. Use an ampule opener or clean paper towel to avoid injury when opening the ampule. Using the pre-prepared syringe containing 10 ml of the diluent and additives, gently draw the vaccine from all the vials (approximately 3 seconds per vial), and gently insert into the diluent bag (approximately 3 seconds per vial used). Take care not to withdraw or expel the vaccine too quickly with the syringe as this can cause damage to the vaccine due to excessive force on the cells which can reduce the potency of the vaccine.

2.2 Marek's Vaccine Preparation (cont.)

- ✓ Gently massage and invert the diluent bag to ensure the vaccine is mixed thoroughly (do not shake vigorously).
- ✓ Rinse the vials to recover the maximum amount of vaccine possible. For this procedure, use the same syringe and gently draw up 5 to 10 ml of the solution (diluent, vaccine, additives) from the diluent bag. Gently insert the diluent in the empty vial until the liquid level is up to the neck of the vial. Gently withdraw all the liquid back into the syringe. Repeat this for all the vials used and then return the contents of the syringe to the diluent bag.
- ✓ Swirl and invert the diluent bag again to ensure the vaccine is mixed thoroughly (do not shake vigorously).
- ✓ Never force vaccine through a syringe, as this will break down vaccine cells, and dramatically reduce the potency of the vaccine.
- ✓ Record the contents and the time when the vaccine was prepared on the diluent bag.
- ✓ Discard all used needles, syringes, and vials in an appropriate waste receptacle.

2.3 Marek's Vaccine Storage

Marek's Disease vaccine is a very unique vaccine in that they are live viruses that are cell associated and kept frozen in liquid nitrogen. The vaccines must be carefully thawed and mixed prior to administration in any form. The nitrogen tanks must also be properly maintained to ensure the vaccines stay at a constant temperature.

2.4 Nitrogen Storage Tank Maintenance

- ✓ **Always wear safety glasses and insulated gloves while handling vaccine from liquid nitrogen containers and measuring the level of nitrogen in the tank.**
- ✓ Avoid moving the storage tank abruptly and avoid falls or bumps, which might break the internal walls and/or neck tube, resulting in loss of tank vacuum and/or total loss of liquid nitrogen.
- ✓ The tank must be stored in a cool place, away from direct sunlight and any other heat sources.
- ✓ The tank lid must be properly placed (see photo on right).

Proper nitrogen tank lid placement (completely closed)

2.4 Nitrogen Storage Tank Maintenance (cont.)

- ✓ After using all the vaccine ampules, do not allow all the nitrogen to evaporate from the tank. Putting liquid nitrogen in an empty tank can cause damage and tank failure.
- ✓ Handle the tank with both hands, keeping it in an upright position. Do not lift the tank with one hand.
- ✓ Vaccine ampules must always be submersed in the liquid nitrogen.
- ✓ The level of liquid nitrogen must never be under 30 cm (11.8 in), as measured with a suitable ruler. The nitrogen level must be checked daily.
- ✓ Use Personal Protective Equipment (PPE) when measuring the level of nitrogen.

2.5 Subcutaneous (SC) or Intramuscular (IM) Injection at Day of Hatch

Day-old vaccination is generally accomplished by giving 0.2 to 0.5 ml of vaccine subcutaneously under the skin at the back of the neck or intramuscularly in the leg. The automatic vaccination machines used in many parts of the world generally are designed for neck injection. A skilled operator can vaccinate about 1600 to 2000 chicks per hour. A dye is frequently mixed with the vaccine to allow visualization of the vaccine after the injection. Needles should be changed several times during the day. Burred or bent needles must be replaced immediately.

Automated vaccinator checklist:

- ✓ Put on safety goggles and insulating gloves.
- ✓ Calibrate all vaccinators before vaccination for accuracy.
- ✓ Verify the position of the needles.
- ✓ Have an adequate supply of new sterile needles.
- ✓ Check all vaccinators for dose accuracy.
- ✓ Check the pneumatic pressure.
- ✓ Evaluate the hygiene status of the machine.
- ✓ Use a new needle with the bevel up towards the neck of the chick.
- ✓ Verify that the vaccine diluent has the correct color (not yellow, not purple) and that it is not cloudy or has any kind of sediment or foreign particles.
- ✓ Verify that the vaccine vials to be used have not been thawed. Many hatcheries invert the vaccine vials to leave the frozen product on top. If the vaccine is thawed inverted, the vaccine will flow to the cap of the vial and become visible.

2.5 Subcutaneous (SC) or Intramuscular (IM) Injection at Day of Hatch (cont.)

Vaccine administration:

- ✓ Begin the vaccination process with properly sanitized equipment.
- ✓ Test the system before chicks are vaccinated.
- ✓ The amount of vaccine delivered is usually 0.2 to 0.5 ml.
- ✓ Needles must be replaced with new needles at least every 1000 chicks.
- ✓ Once reconstituted, the vaccine should be used completely within 30 to 45 minutes. Should the vaccination personnel need to stop or interrupt the procedure at any time, document the interruption.
- ✓ A chick sample may be taken per vaccinator to verify the quality of vaccination. Because dye has been added to the vaccine, one can look for evidence of dye in the subcutaneous (SC) tissue. Count the number of chicks with SC dye for every 100 chicks sampled and determine the percent of chicks missed. Correct any problems immediately. The inspection must be done within 15 minutes post vaccination or the dye will no longer be visible under the skin.
- ✓ Determine any percentage of chicks with visible blood, which would be an indication of the needles being mal-positioned, burred or blunt, or of too much pressure being applied.
- ✓ Verify that the machine remains properly calibrated and consistently delivers the prescribed volume of vaccine.
- ✓ Verify that the prescribed air pressure is correct (most machines operate with 75 PSI, or 5.2 Bars). Excess pressure will hurt the chicks and may promote leakage of vaccine or break down the cells in the vaccine. Insufficient air pressure may result in reduced doses of vaccine.

Post Vaccination

- ✓ Ensure proper cleaning, sanitation, sterilization and maintenance of the vaccination equipment at the end of the day.
- ✓ Discard all unused vaccine, including vaccine left over from personnel breaks and any excess vaccine remaining after the completion of the hatch day.

Animal Welfare Tips

When handling chicks for day-old vaccination, each operator should carefully pick up the individual chick and support the body weight while vaccinating. Chicks should never be held solely by the head or neck.

Animal Welfare Tips

After vaccination, each operator should conduct a chick quality assessment. Any chicks that are bleeding post-vaccination should be removed from the box and evaluated. The operator should also check the needle to verify if it needs to be replaced.

A quality assurance staff member in the hatchery should also check boxes from each operator on a regular basis to ensure that the vaccination is correct, and no visible blood is present. We recommend using a log sheet to note the quality findings for each hatch day.

2.6 Spray Vaccination in the Hatchery

In many areas, chicks are vaccinated with live vaccines using a spray cabinet that administers a defined amount of water-based vaccine to each box of chicks. The droplet size is carefully controlled, and vaccination can be visualized on the chicks as either moisture or dye. This method is typically used for respiratory vaccines (IBV, NDV) and live coccidiosis vaccines.

Important points for spraying respiratory vaccines in the hatchery:

- ✓ Although the volume of vaccine delivered for most respiratory vaccines is about 7 ml per box, it is important to check with the specific vaccine manufacturer regarding the vaccine volume per box for their product.
- ✓ The water volume will change in respect to the vaccine type and spray equipment used.
- ✓ Run an empty chick box through the spray cabinet to check for uniform spraying side to side and end to end.
- ✓ A particle size of 100 to 300 microns in diameter is ideal for spray vaccination in the hatchery. Smaller droplets will move with air currents and will not settle evenly over the chicks.
- ✓ The water used for vaccine reconstitution should be fresh, cool distilled water. Warm water may have a negative impact on vaccine viability and cold water will chill the chicks. Water should be no cooler than 16°C (60°F) and no warmer than 27°C (80°F).
- ✓ Items to monitor include the air pressure, nozzle spray pattern, volume delivered per nozzle in every actuation, orientation of the nozzles, belt speed, and height of the chick box.

Important points for coccidiosis vaccination by spray cabinet:

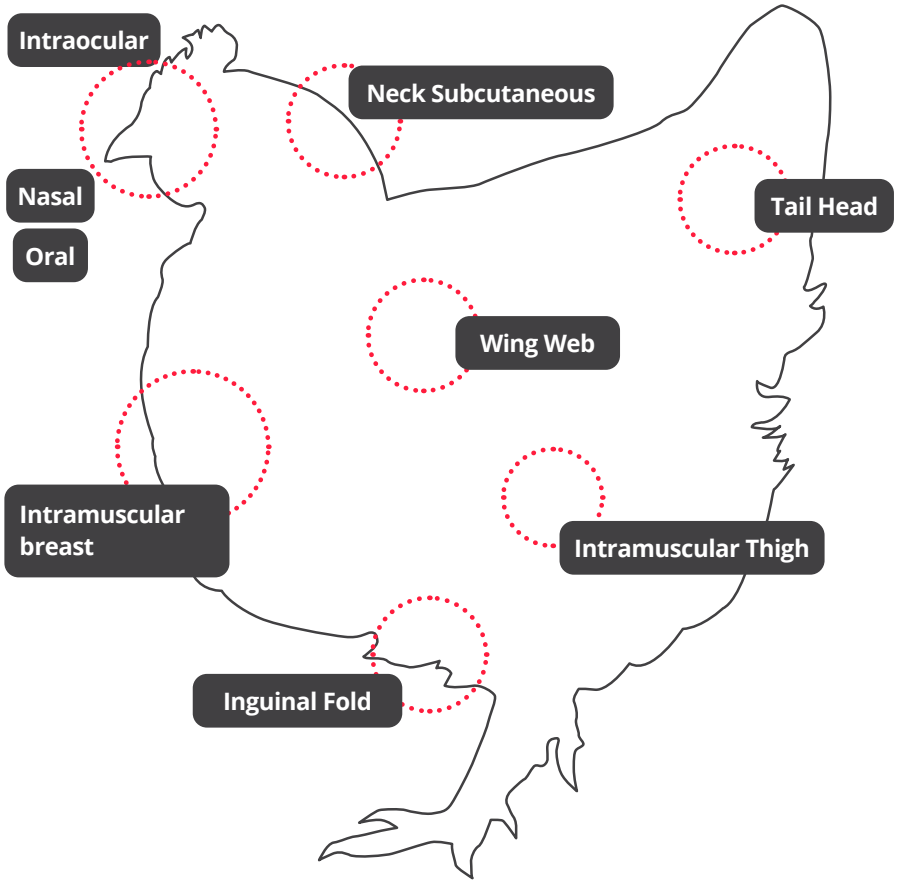
- ✓ Coccidiosis vaccines must be stirred or agitated gently and continuously to ensure that the oocysts stay in suspension. If oocysts are allowed to settle to the bottom, significant variation will occur in the actual oocyst dose delivered.
- ✓ Coccidiosis vaccines are generally delivered with a fan pattern while respiratory vaccines are usually sprayed with a cone-shaped pattern.
- ✓ Coccidiosis vaccines utilize a larger droplet size and the volume of vaccine delivered is approximately 21 ml per box.
- ✓ The reconstituted vaccine is dyed in order to stimulate preening post-vaccination, distribution and consumption of the vaccine.
- ✓ After vaccination chick boxes should be placed in a warm, draft-free area with bright lighting for at least 30 minutes to stimulate chick activity and vaccine consumption by preening. The use of lighting will encourage chicks to be active, will limit huddling, and will promote better future health and welfare for the flock due to optimal vaccine consumption.
- ✓ Some coccidial vaccines are formulated as a gel drop sprayer. Follow the manufacturer's recommendations for operation and use of these formulations.

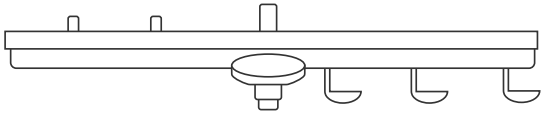
cares **Animal Welfare Tips**

Before vaccination: Place boxes on the conveyor gently so that chicks will be well-distributed in the box prior to spray vaccination. This will help ensure more uniform vaccination for all chicks in the box.

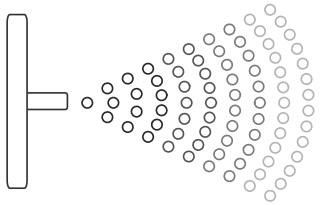
During vaccination: Chicks will naturally crouch down due to the noise and the moisture from the vaccine. After exiting the spray cabinet, chicks should be active and should immediately begin preening. Ensure chicks are placed in a warm, draft-free area.

Sites of vaccinations on a chicken





Through Water Line



Spray

3

Field Vaccination

There are various ways to mass apply vaccinations to poultry in production housing situations. In some cases, the emphasis is on effective application with the lowest labor costs. In areas where labor is inexpensive and readily available, application strategies that maximize the immune response can be selected. Disease challenges in the production area will also factor into the type of vaccine application best suited for that area.

The techniques to deliver vaccines can be used across all types of poultry production. Accommodations are needed for the type of housing (floor, slats, cages), water systems (open, closed, hand), equipment available (backpack sprayer, handheld sprayer, etc.) and age of the birds.

3.1 Spray Vaccination with Backpack Sprayer System

Backpack sprayers have become a popular method to mass administer live respiratory vaccines, especially to broilers. Several manufacturers are available, and modifications can be made to agricultural sprayers to accomplish this technique as well. Follow the manufacturer's instructions for the equipment used. Handheld sprayers are also available for smaller housing situations.

Personnel

- ✓ Always use at least two people to vaccinate. Broilers may require up to three people for proper vaccination. A designated vaccination crew is preferred.
- ✓ The flock service technician should be present for guidance if possible when a flock is vaccinated.

Equipment

- ✓ Three backpack sprayers (one sprayer for each side of the house and a third to go through the middle).
- ✓ Vaccine storage - Insulated cooler with ice or cold packs.
- ✓ Distilled water for mixing.
- ✓ Gloves, mask and safety glasses.

Before Vaccination

- ✓ Spray 3.8 liters (1 gallon) of rinse water through the backpack sprayer.
- ✓ Observe spray particle size and pattern. The particle size for young chicks should be 80 to 120 microns and for older birds, between 60 to 80 microns.
- ✓ The sprayer must be used for vaccination only (never for pesticides, herbicides, or disinfectants).
- ✓ Wear gloves, mask and safety glasses during preparation and vaccine administration.

3.1 Spray Vaccination with Backpack Sprayer System (cont.)

Vaccine Mixing

- ✓ Mix the vaccine on the farm, just prior to vaccinating each house.
- ✓ Use clean, non-chlorinated water or water that has had vaccine stabilizer added. Distilled water is ideal. Water should be no cooler than 16°C (60°F) and no warmer than 27°C (80°F). Error should be towards the cool side as water too warm can damage the vaccine.
- ✓ Pour enough water into the sprayer tanks to allow the vaccination team to walk the length of the house twice SLOWLY without running out of vaccine. (minimum 1.25 liters per 10m, or 1 gallon per 100 feet).
- ✓ Dissolve the vaccine in the vaccine bottle using distilled water, and then add the vaccine to the water in the sprayer tank. Rinse the vaccine bottle thoroughly, otherwise up to 15% of the vaccine may be lost.
- ✓ Shake the tanks on the sprayer to allow the vaccine to be mixed thoroughly.
- ✓ **IMPORTANT:** mix only enough vaccine to vaccinate one house.
- ✓ For quality control records, note the serial number, expiration date for the vaccine used, the date and time of vaccination, the location (farm and house number), and the names of the staff members involved in the vaccination process.

House Preparation

- ✓ Minimize ventilation if possible.
- ✓ Dim the lights as low as possible to keep the birds calm during vaccination.
- ✓ Raise brooders (if possible).
- ✓ During hot weather, vaccinate very early in the morning.

Vaccine Administration

- ✓ Walk slowly. Start at one end of the house and make two complete passes through the house.
- ✓ One person should walk ahead of the vaccinators to part the birds and to keep the birds from piling against the back wall.
- ✓ Each vaccinator sprays one side of the house.
- ✓ Direct the nozzle 1 m (3.3 feet) above the birds' heads.
- ✓ Keep a constant pressure of 65 to 75 PSI (4.5 to 5.2 Bars).

Post Vaccination

- ✓ Properly dispose of all empty vaccine vials, water jugs, etc.
- ✓ After vaccination is completed be sure to restore proper ventilation by setting fans to previous settings.
- ✓ Restore lighting to previous intensity.

Sprayer Maintenance

- ✓ Fully charge batteries prior to use.
- ✓ Change batteries after spraying 114 liters (30 gallons) of liquid or when the sprayer has been sitting unused for an extended time.
- ✓ Thoroughly rinse the tank with 3.8 liters (1 gallon) of distilled water at the end of each day or if changing vaccines.
- ✓ Remove and clean or replace the filter as needed.
- ✓ Clean the outside of the sprayer using a damp cloth and a mild detergent.
- ✓ Rinse the tank and pump thoroughly by spraying distilled water through the sprayer after using a bleach solution. Use a final rinse of isopropyl alcohol and spray before emptying and storing.
- ✓ Store the sprayer upside down in an area where it will not be exposed to temperature extremes.
- ✓ Periodically check all hoses and connections for signs of wear. Replace as needed.

Animal Welfare Tips

Before and during vaccination, one person should walk ahead of the vaccinators so that the flock will naturally move apart and to keep the birds from piling against the back wall. This division of the flock will help ensure a more uniform vaccination of the birds and will reduce stress for the flock during the process.

After vaccination the farmer or service technician should walk through the house to adjust lighting and equipment if needed and to verify that the bird behavior and distribution have returned to normal.

3.2 Water Vaccination

Utilizing the drinking water systems in poultry housing is a common method to administer live vaccines. Birds must be water restricted for approximately one to two hours to ensure all birds are ready to drink once the vaccine is administered.

Water consumption is an important variable to calculate so that the correct amount of water can be used to mix with the vaccine. For houses with water meters, the consumption rate is easily obtained. Without a water meter, the information in Table 2 shows water consumption for broilers at different ages (estimation provided by Dr. Tom Tabler, Mississippi State University Extension Service Department).

When medicators are available in the house, a practice run using only water two days before vaccination will verify the amount of water needed. When using a water pump, it is assumed that the amount of water to be used for vaccination should be approximately 30% of the daily intake.

Table 2 gives general guidelines on broiler water consumption based on research conducted by Mississippi State University Extension Service on 12 consecutive broiler flocks.

Table 2 Water Consumption/1000 birds/day

Broiler Age (Days)	Minimum Usage		Maximum Usage		Average Usage	
	gallons	liters	gallons	liters	gallons	liters
7	13.3	50	19.4	73	16.0	61
14	28.4	108	37.9	143	32.8	124
21	38.7	146	56.1	212	46.2	175
28	49.1	186	71.7	271	60.0	227
35	59.1	224	85.5	324	72.6	275
42	66.2	251	96.4	365	82.3	312
49	67.2	254	97.7	370	85.9	325
54	76.5	290	98.8	374	87.5	331

3.2 Water Vaccination

Before Vaccination

- ✓ Always administer the oral vaccine on the morning the birds are fed (for pullets on feed schedules).
- ✓ All medication, disinfectants and chlorine must be removed from the drinking water 48 hours before vaccination.
- ✓ Water withdrawal prior to vaccine administration:
  - *30 to 60 minutes in hot climates*
  - *60 to 90 minutes in cool climates*
- ✓ Always administer the vaccine in the water early in the morning.
- ✓ Enough drinker space is required to allow free access to the vaccine solution.

Vaccine Preparation

- ✓ The use of a vaccine stabilizer or skim milk powder to the water 20 to 30 minutes prior to adding the vaccine is recommended as a stabilizer. Add the skim milk powder at a ratio of 500 g / 200 L (1 lb / 50 gal).
- ✓ Open the vaccine vial by removing the aluminum seal and the rubber stopper. With water that will be used in the vaccination, fill the vial approximately 2/3 full. Close the vial with the rubber stopper and gently agitate in order to reconstitute the lyophilized vaccine. Rinse the vaccine vials several times to remove all the vaccine.
- ✓ Use a graduated plastic bucket or prepare the vaccine directly in the water tank.

- ✓ If using a proportioner, calculate the average water consumption from the last 4 days, in order to obtain the amount of water used by the proportioner. Calculate 30% of the volume of water used by the proportioner to prepare the vaccine in the bucket.
- ✓ Following the manufacturer's directions, add the vaccine stabilizer to the tank. Then, add the stock solution that contains the reconstituted vaccine. When the vaccine is administered with a proportioner, also add a dye. Stir and mix using a plastic stick or other clean utensil.

Vaccine Administration

- ✓ Pour the reconstituted vaccine into the drinkers or open the valve of the water tank or the proportioner. For water tank and water pump specific information on priming and distribution see following sections (3.2.1 and 3.2.2, respectively).
- ✓ Walk through the house to check if the birds are all drinking water. If using hand drinkers, redistribute drinkers if necessary.
- ✓ Note that the birds must drink all the vaccine solution within two hours, but not less than 1 hour.

Post Vaccination

- ✓ Record all vaccine information and any problems that may have occurred with the birds or the vaccination process. This information may be important for the evaluation of the results.
- ✓ All medication, disinfectants and chlorine must be suspended from the drinking water until 24 hours after vaccination.

3.2.1 Using Water Tanks

Before Vaccination

- ✓ 48 hours prior to vaccination, close the water chlorinating system and remove the chlorine tablets from the proportioner. Water tanks must be clean and free of biofilm.
- ✓ Determine the number of birds that drink water from a water tank in the house and calculate the number of vaccine vials to be used in the water tank.
- ✓ The volume of clean water to be used for vaccination will be approximately 30% of the average daily volume of water consumed.

Vaccine Mixing

- ✓ Following the manufacturer's instructions, first mix the vaccine stabilizer into the water. Water should be no cooler than 16°C (60°F) and no warmer than 27°C (80°F).
- ✓ Add the vaccine vials and blue dye according to the calculation.

Vaccine Administration

- ✓ Open the water tank valve to allow the birds to consume the vaccine.
- ✓ After the vaccine is consumed, open the water flow normally.

3.2.2 Using a Pump System

A water pump can be used to drive the vaccine into the water lines. Water pump vaccination requires a closed water system (nipple drinker lines).

Before Vaccination

- ✓ Flush the drinker lines with fresh water to eliminate unwanted residues.
- ✓ Raise the drinking lines to prevent the birds from drinking 1 to 2 hours prior to vaccination.

Vaccine Mixing

- ✓ Calculate the amount of water needed so the vaccine is consumed in 80 to 90 minutes. This amount should be approximately 30% of the daily water intake. If the water restriction period was excessive, the birds will be thirsty and consume the vaccine too quickly. In this case, every bird may not have the opportunity to receive a dose of vaccine.
- ✓ Mix vaccine into a container or containers large enough to hold the required volume of mixed vaccine. The set up typically allows for fittings that enable the containers to be connected to the drinker lines.

Vaccine Administration

- ✓ Once the vaccine, vaccine stabilizer and vaccine dye (usually blue in color) are mixed in the large container, the vaccine is pumped into the drinker lines with the assistance of a water pump.
- ✓ The end of the drinker line is open to improve flow.
- ✓ One staff member must observe the water coming out of the end of the drinker lines until the blue solution (the vaccine) is visible. When the dye is seen, close the end of the drinker lines.
- ✓ Lower the drinker lines to allow the chickens to consume the vaccine.
- ✓ Alternate the drums of mixed vaccine until all the vaccine doses are consumed.
- ✓ Walk through the house at least 2 to 3 times while the vaccine is being consumed in order to stimulate consumption by all birds in the house.

3.3 Intraocular (Eye Drop) or Nasal Drop Vaccination

Vaccine Mixing

- ✓ Confirm that the vaccine to be administered by eye drop is approved and manufactured for eye drop application. Serious issues may occur if the wrong vaccines are dropped into the eye.
- ✓ Open the vaccine vial and the diluent bottle, removing the aluminum seals and the rubber stopper. At the time of vaccine reconstitution, the diluent's temperature must be between 2 to 8° C (36 to 45° F).
- ✓ Open the diluent bottle and, using a syringe, remove 3 ml of diluent and inject it into the lyophilized vaccine vial. Some vaccines come with a special adapter to mix diluent and vaccine – in this case, connect the adaptor on the diluent bottle to the vial of lyophilized vaccine.
- ✓ Rinse the vaccine vials several times with diluent in order to remove any residues.
- ✓ Slowly shake the diluent bottle with the already reconstituted vaccine, without shaking vigorously.
- ✓ Attach the dosing/eye drop nozzle onto the diluent bottle.

Vaccine Administration

- ✓ The vaccination will only be considered successful if the drop (0.03 ml) is placed into the opened eye or nasal cavity and absorbed. For this to occur, it is important to wait a few seconds after administering the drop, before releasing the bird.
- ✓ If the drop is not totally absorbed, a new drop should be administered.
- ✓ To prevent the contents of the vaccine vial from getting warm against the hands of the vaccinator, divide the contents of the reconstituted vaccine into two or three empty vials, and alternate their use while keeping the others in a cooler with ice or cool packs.

Post Vaccination

- ✓ Check the number of doses used versus the number of birds vaccinated. Record all information regarding the vaccination as well as any problems that may occur with the birds or the vaccination process.

Cobb cares **Animal Welfare Tips**

For correct administration of the eye drop vaccine, the vaccinator may use his/her free hand to gently restrain the head of the bird. He or she can then rest the side of the other hand behind the bird's eye and then carefully tilt the tip of the bottle towards the eye. This should result in correct placement of the drop into the eye with minimal distress for the bird. The tip of the bottle should never touch the eye.

3.4 Wing Web Vaccination

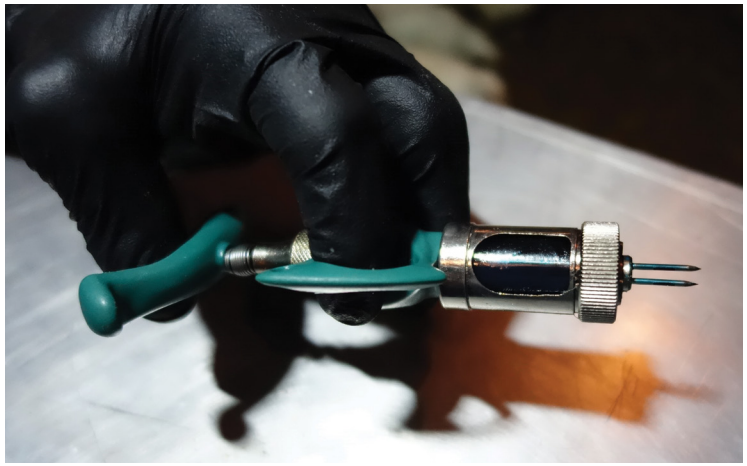
This method is commonly used for Fowl Pox, Avian Encephalomyelitis, Chicken Anemia and Live Fowl Cholera.

Vaccine Preparation

- ✓ The preparation of this vaccine is similar to that of the eye drop vaccine.The vaccine is lyophilized and must be reconstituted in the same manner as other vaccines.
- ✓ Only use the specific diluent which comes packaged with the vaccine. Shake the vaccine vial carefully, turning the vial from one side to the other without tapping.

Vaccine Administration

- ✓ Administer the vaccine in the center of the wing web, using a two-pronged needle applicator or other wing web applicator (Grant inoculator or others).



A small area of dye and no blood should be visible after vaccination

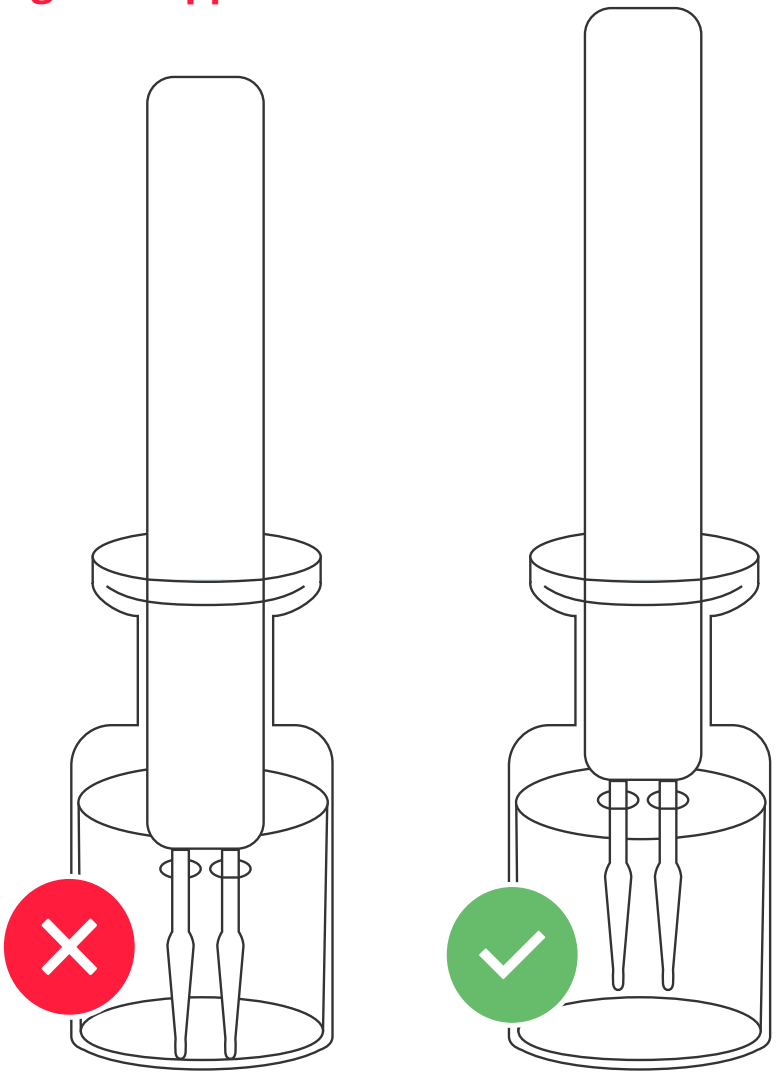
Vaccine Administration

- ✓ Dip the two-pronged applicator into the diluted vaccine and pierce the web on the underside of the wing, avoiding feathers, blood vessels and bones.
- ✓ Change needles every 500 to 1,000 birds.
- ✓ If the wing vein is punctured during the application, immediately change the needle and repeat the vaccination.

Post Vaccination

- ✓ Used needles can be disinfected and used again if they remain sharp and have not been used on more than 1,000 birds.
- ✓ 7 to 10 days after vaccination, check for "vaccinal takes". Check at least 50 birds per house. Please refer to Vaccination Quality Control section for examples (Section 4).

Wing Web Applicators



Take care not to dip wing web applicators too deep into vaccines. This wastes vaccines and dulls needles.

Animal Welfare Tips

Before vaccination: the vaccinator should gently lift the wing to clearly expose the wing web area and to visualize the placement of the needle applicator.

During vaccination: the handler should securely hold the bird to optimize human safety and bird welfare.

After vaccination: the vaccinator should see a small area of blue dye in the wing web and no blood. The vaccination crew supervisor should regularly check birds throughout the process to verify the location of the dye and correct placement of the vaccine in the wing web.

3.5 Injectable (Inactivated) Vaccines

Injectable vaccines must be manually injected into each bird using an 18 gauge needle that is 0.635cm (¼ in) in length. There are two major injection methods in avian species to allow suitable vaccination:

Intramuscular (IM)
Into the muscle

Subcutaneous (SC)
Under the skin

Table 3
Vaccination Injection Sites

Subcutaneous	Intramuscular
Neck	Breast
Inguinal Fold	Thigh
	Leg
	Wing
	Tail Head

In order to utilize these methods, several sites are available for each type of injection – see Table 3. Research has shown that all common injection sites can give satisfactory results if done properly. When selecting the injection site, consideration should be given to ease of application, reaction at the injection site and human safety. Comparisons should be made to decide which injection site gives the best result in an individual operation.

Crew Safety

Accidental human injection with oil emulsion products poses a serious danger. If this occurs, immediate medical attention should be administered to the injured person. If these products are injected into the hands, fingers or body, they can alter circulation leading to severe injury. Immediate treatment will involve removing the oil emulsified product to improve healing in the affected area. This should be done by a qualified medical professional.

Proper injection technique and bird handling will prevent human injection. Bird handlers have an important responsibility to present the birds for injection at the proper angle for the chosen site of injection. If the syringe operator has to struggle to reach the site of injection, the chance for misapplication and accidental injection is much higher.

Injectable Vaccines Before Vaccination

- ✓ Gently agitate the vaccine container before and during the vaccination process to homogenize the contents.
- ✓ Pre-warm oil emulsion vaccines at room temperature for 12 to 24 hours or using a warm water bath (do not exceed 37.7°C (100°F) for more than 5 hours). Pre-warming the vaccine reduces the viscosity of the mineral oil, making the administration easier and reducing any intense local reactions.

Vaccine Administration

- ✓ Prime tubing and gun to avoid “dry” injection.
- ✓ Administer the vaccine by using only the labeled dose at the chosen site of injection.
- ✓ Needles should be replaced every 500 to 1,000 birds.
- ✓ Make sure that there is no air in the tube when the vaccine is administered.

Post Vaccination

- ✓ Record vaccine information and any problems that may have occurred regarding the birds or the vaccination process.
- ✓ After vaccination, all needles, syringes and plastic tubes must be washed prior to sterilization and disinfection. Needles may be used again if they remain sharp and have not been used on more than 1,000 birds.
- ✓ Sterilize all equipment that was used in the vaccination, using an autoclave, alcohol or boiling water.

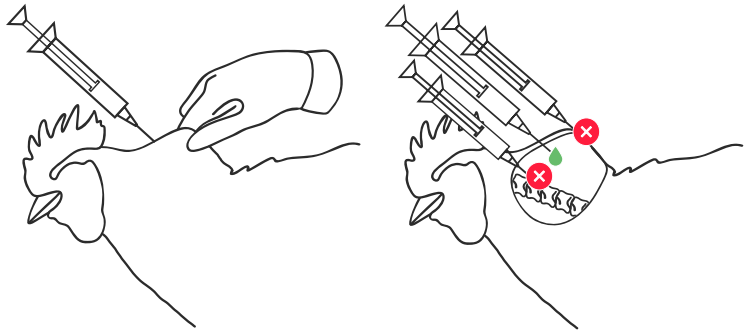
3.6 Instructions for Each Site of Administration

Neck

The skin on the back of the neck should be lifted to create a pocket between the skin and neck muscles. Insert the needle through the skin into this pocket with the needle pointing toward the bird's body. The site of injection should be the middle to lower neck region on the dorsal mid line of the neck. There will be resistance as the needle passes through the skin followed by free movement into the SC space. If this difference is not noticed or is followed by resistance again, the needle may be in the skin, the neck muscle or the spinal cord. Avoid injecting vaccine into the neck muscles, intradermally or too close to the head. Once the needle is in the SC space, a full dose of vaccine is injected before retraction. Early retraction of the needle will result in birds receiving a partial dose.

In the photo above, a yellow needle guard is being used to help prevent accidental injection into the spine and spinal cord.

Neck Vaccination



Breast

Vaccine is injected into the superficial pectoral muscle about 3 to 5 cm (1 to 1.5 in) lateral to the keel bone, depending on the age of the bird. The needle should be directed caudally at a 45° angle to the body. This will help avoid injecting the vaccine through the muscle and into the body cavity.

Leg

When using the leg muscle for vaccination, the injection should be made in the lateral side of the gastrocnemius muscle mid-way between the stifle joint and the body. The needle should be directed towards the head (proximally). Avoid major vessels, nerves, joints and the bone.

Wing Muscle

The wing muscle (medial side of the biceps) can be used as an alternative IM site. The injection should be made into the large muscle group on the underside of the wing with the needle pointed toward the body. Avoid major vessels and bone.

Tail Head

This injection is made into the underside of the tail head. The needle is directed to the side of the tail bone and toward the head (cranially). Care should be taken not to withdraw the needle too quickly, which can lead to leakage of vaccine out of the injection site.

Inguinal Fold

Vaccine is injected into the pocket created by skin connecting the abdomen and the thigh. This SC space is large and creates less of an issue with spent hen processing as compared to IM injections. Research in commercial layers has shown good immune responses following inguinal vaccination. However, the same research shows a more drastic decrease in titers over time with this technique. Therefore, make sure titers are routinely evaluated overtime to ensure that good titer levels are maintained in the flock.

Injection into the inguinal fold can provide a good immune response.

The wing muscle can be used as an alternative injection site.

Circled areas are ideal injection locations in the tail head.

Vaccination Quality Control

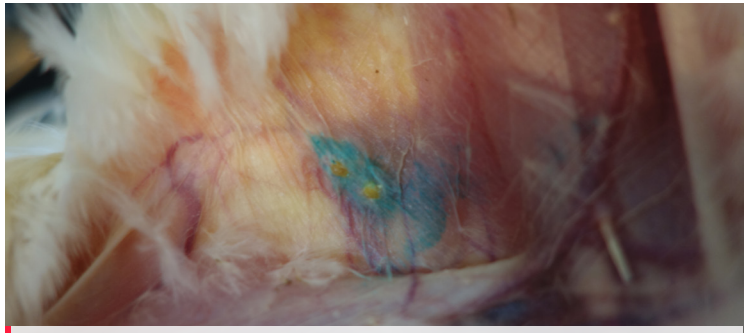
4

The best vaccination program can only be achieved with proper administration and monitoring to ensure the population is well protected. Within poultry flocks, several quality control strategies can be implemented to maximize vaccine administration.

1. Vaccination crews should be randomly inspected by the veterinarian to examine their application techniques – for all administration methods. This is especially important for more valuable birds such as breeder pullets, where injection errors can impact future growth and egg production.
2. Designate one crew member for quality control to evaluate 50 to 100 birds during the vaccination sessions for wet feathers, hemorrhages, or other signs of improper application of vaccines.
3. Necropsy of cull birds or mis-sexed birds can allow immediate evaluation of vaccination techniques of injectable vaccines.
4. Vaccine use must be carefully recorded throughout the vaccination procedures – number of doses used, vaccine lot and serial numbers, and number of birds vaccinated. Comparing the doses of birds vaccinated will allow for easy determination of dosage errors or missed birds.
5. Dyes can be added to both live and killed vaccines to visualize the vaccine at the time of administration by the vaccinator or immediately after vaccination for quality control checks in these areas:
    - **a.** On the tongue or in the crop following water administration
    - **b**. Mouth and tongue following eye drop
    - **c.** Under the skin after SC injection

Blue dyed vaccine under the skin of a vaccinated pullet.

6. Another method for verifying the quality of intraocular vaccination is to use a paper lining on the litter where the birds are released. If the drop 'rolls off' the eye, it will fall onto the paper, which will then be stained by the dyed diluent. If this happens, the vaccination is incomplete, leading to inconsistent titers and susceptibility to disease challenges.
7. For wing web vaccination, "takes" can be observed 7 to 10 days following administration. Select and examine 50 to 100 birds chosen randomly throughout the house.
8. Use a table similar to the example below to record your observations from the vaccination.



An acceptable vaccine reaction showing the presence of the two nodules following Fowl Pox vaccination via wing web.

Table 4 Example Table to Record Wing-Web Vaccine Efficacy Assessment

	House 1	House 2	House 3
Good: Presence of two nodules			
Medium: Presence of one nodule			
Poor: Absence of nodules			
Total Birds in the House, Total Birds Checked			
% of Good			
% of Medium			
% of Poor			

4.1 Errors When Using Injectable Vaccines

SC neck injection is a safe method of vaccination; however, improper technique can cause harm to the birds. The following misapplications can have serious consequences:

1. Vaccine is placed into the skin layer (intradermal). The area will develop into a hard lump and/or scab that may rupture, which birds will peck at causing bleeding and possible mortality. This will provide poor immunity.

2. Vaccine is injected into the neck muscle (intramuscular). The muscles of the neck are very small and the immune reaction to the vaccine creates inflammation and pressure. The damaged muscle heals by forming scar tissue. This scar tissue can lead to birds with twisted necks, resulting in poor performance.

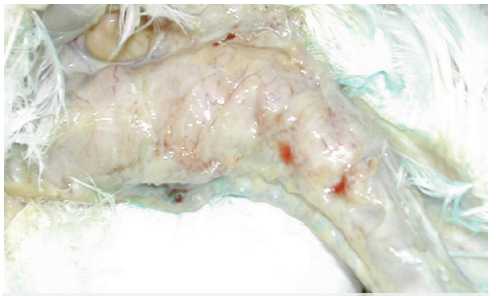
3. Vaccine is injected into the bone or spinal cord. If the needle is inserted too deeply, it will pass through the neck muscles and vaccine can be injected into the spinal cord. Birds usually die within a few minutes after injection into the spine.

4. Vaccine is injected too close to the head. This can cause swollen heads which can impair feed consumption and vision. Injecting too low results in swelling over the back. Flock mates may peck at these noticeable swellings causing further issues.

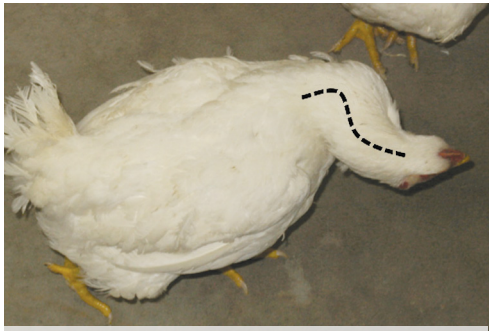
5. Vaccine is injected into the side of the neck (not on the mid-line of the neck). In this case, large vessels and soft tissue of the neck may be damaged. The needle can damage blood vessels causing SC bleeding. The thymus gland lies below the skin on both sides of the neck. Vaccine injected into the thymus leads to swelling with an eventual necrosis of the surrounding tissue.

6. Vaccine is injected through the side of the neck. If the needle passes through both layers of skin, the vaccine will likely be deposited outside the bird and will wet the feathers on the opposite side of the neck. The bird will not develop a proper immune (good) response.

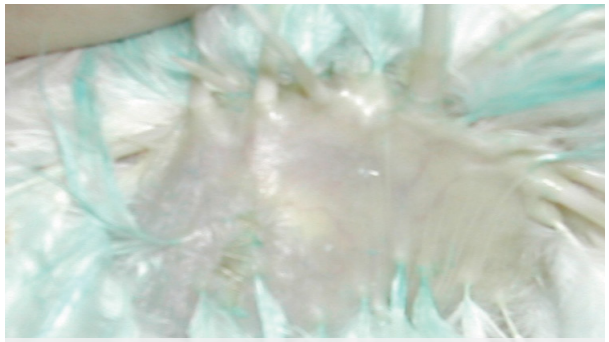
Subcutaneous bleeding from puncture of the blood vessels



Neck muscles of breeder pullet improperly injected causing muscle inflammation



Bird with crooked neck follwing SC injections that were administered too deeply into the neck muscle



Dyed vaccine showing excessive coloration of feathers – bird potentially received only a partial dose or no vaccination

Large mass in area of thymus – birds were injected off mid-line

Bird with swollen head following SC injection that was too high on the neck

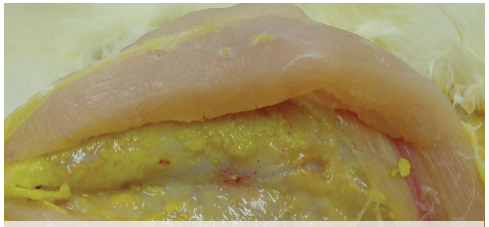
Intramuscular (IM) leg, thigh or wing injection

Intramuscular leg, thigh or wing can also be utilized as sites of administration. The wing is a very small target and misapplication can easily occur. The leg is often selected as the site of injection for cage-reared layer pullets as the leg can be easily accessed with minimal stress to the pullets. When vaccinating in the leg or thigh, post-vaccination stress should be minimized since moving the pullets may exacerbate the vaccine reaction and cause leg swelling. The use of a concentrated vaccine (0.25 ml per dose) will also minimize the reaction when injecting into small muscle locations.

Excessive reaction to injection in the leg

Intramuscular (IM) breast injection

Intramuscular (IM) breast injection is an easier technique with increased accuracy but can have problems. Adverse lesions, in the form of granulomas, may remain in the muscle and be found at spent fowl processing. Vaccinators may insert the needle closer to the end of the breast, entering the abdomen or internal organs, resulting in the formation of abscesses in organs or adhesions to the abdominal wall. Certain injectable vaccines can create excessive reactions when injected into the muscle, leading to birds refusing feed for a few days. Carefully evaluate the products you plan to inject into the muscle – those containing inactivated bacteria tend to be more reactive in this manner.



Reaction within the breast muscle to Pasteurella bacterin Injection

4.2 Monitoring the Vaccination Program

The objectives of using inactivated products include longer duration of immunity in long-lived birds and hyper-stimulation of antibodies to improve passive transfer of maternal antibodies to progeny. Therefore, the production of immunity in the hen and progeny may be directly impacted by vaccination quality.

The most common serological test used to monitor flock immunity and the success of the vaccination program is ELISA (Enzyme Linked Immuno Sorbent Assay). A variety of kits are available for numerous antigens through several commercial companies. The results are also quantitative for most antigens – giving Mean Titers, Geometric Mean Titers (GMT) and Coefficient of Variation (%CV) in the results. The desire in breeder hens is to achieve high GMT's and low %CV for the common antigens - IBDV, NDV, IBV and Reovirus.

Reference: **www.biocheck.com; www.idexx.com**

New ELISA technology allows the detection of antibodies to expressed insertions after administration of recombinant vaccines (example: rHVT-IBD; rHVT-ND). This detection method not only allows measurement of the immune response to a specific insert but also helps build the database for any producer using such recombinant products.

Reference: **www.id-vet.com**

Poor vaccine administration can raise the %CV and lower GMT of flocks sampled. This may be explained by higher numbers of non-vaccinated birds, vaccine leakage, poor quality or expired vaccine, or improper location of injection. The duration of titer levels can also be impacted by improper vaccination as titers diminish quickly in birds that receive a partial dose of vaccine.

Other additional serological tests can be used to evaluate vaccine administration – virus neutralization (VN) will show the level of neutralizing or protective antibodies. Hemagglutination inhibition (HI) can be used for ND, paramyxovirus - type 3, avian influenza, and *Mycoplasma gallisepticum*.

Table 5 gives examples of common antigens tested several weeks after vaccination with a specific ELISA kit. These are typical ranges – the normal values for your specific operation and vaccine program should be obtained through routine monitoring.

Table 5

Indications of Titers to Expect with IDEXX ELISA Kits

Test	Type	Mean ELISA Titer	Weeks Post-Vaccination	Mean ELISA Titer of Day-Old-Chicks
AEV	Live	3,000-4,000	5 - 8	-
IBV	Live	1,000-4,000	3 - 5	-
	Inactivated	5,000-6,000	5 - 8	2,000-6,000
IBD	Live	1,000-4,000	3 - 5	-
	Inactivated	4,000-15,000	5 - 8	3,000-7,000
NDV	Live	1,000-4,000	3 - 5	-
	Inactivated	10,000-32,000	5 - 8	5,000-10,000
REO	Live	2,000-4,000	3 - 5	-

IDEXX Ten Principles of Serology Interpretation

- ✓ Use rapid, convenient, sensitive, specific, and economic serological methods.
- ✓ Create and organize an in-house database.
- ✓ Compare your database against your own region.
- ✓ Consider seasonal changes in serological data.
- ✓ Know the power and limitations of each laboratory assay.
- ✓ Avoid risky single time-point evaluations.
- ✓ Comply with state, federal, and official regulations, especially when exporting hatching eggs or day-old poultry from vaccinated flocks.
- ✓ Consider pathogen strains, variants, or serotypes circulating locally.
- ✓ Know your field situation and disease epidemiology when interpreting serology.
- ✓ Know how and when to interpret serology qualitatively and quantitatively.

Abbreviations

HVT - Herpes Virus of Turkeys

rHVT - Recombinant Herpes Virus of Turkeys

NDV - Newcastle Disease Virus

IBD - Infectious Bursal Disease

IBDV -Infectious Bursal Disease Virus

SC - Subcutaneous

IM - Intramuscular

ELISA -Enzyme Linked Immunosorbent Assay

GMT - Geometric Mean Titers

CV - Coefficient of Variation

VN - Virus Neutralization

HI - Hemmaglutin Inhibition

AEV - Avian Encephalomyelitis Virus

REO - Reovirus

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