



Cobb Vaccination

Management Guide



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ANIMAL WELFARE TIPS

Look for animal welfare tips throughout the guide that highlight important aspects of management to improve poultry welfare outcomes during vaccination procedures.



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Introduction

This guide is designed to help field personnel in the correct 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 vaccination procedures, please consult your Cobb Technical Service Representative.



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 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, some additional goals include:

- » 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

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, typically the greater the antibody response and resulting protection. For this reason, many flocks are vaccinated multiple times for the same disease.

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.

Vaccine Vectors

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

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.

Examples of recombinant vaccines include:

- » 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

Table 1 Comparison of Live, Inactivated and Recombinant Vaccines:

Aspect of Vaccine	Live	Inactivated	Recombinant
Safe	Yes	Yes	Yes
Economical	Yes	Expensive	Varies
Mass application	Yes	No	No
Rapid onset of immunity	Yes	No	No
Immunity duration	Short	Long	Intermediate
Multivalent 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 extreme heating and intense light.

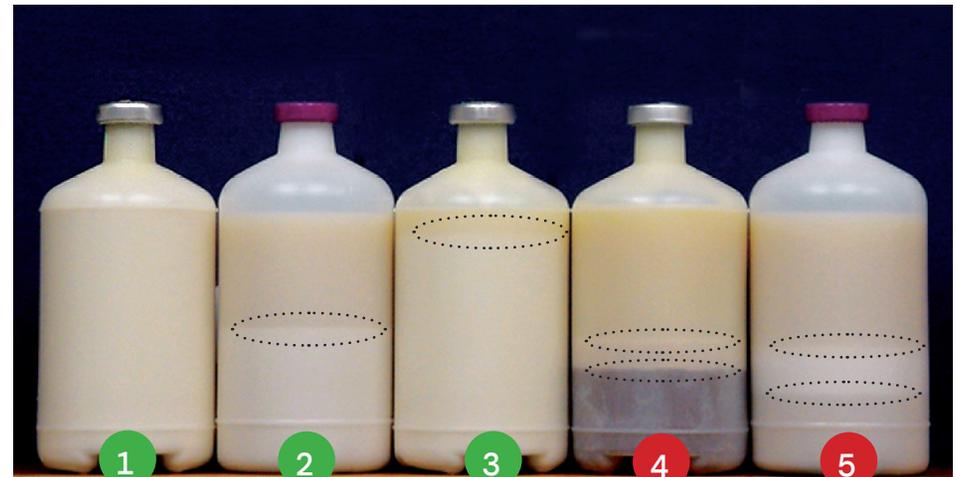
For Live Vaccines:

- » 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 32.2 °C (90 °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.
- » 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 15 to 20 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. Normal	2. Normal	3. Normal	4. Broken Emulsion	5. Broken Emulsion
Uniform color; milky white	Significant settling has occurred	Slight settling has occurred	Bottom layer is dark brown or black	Bottom layer
1 layer	2 layers	2 layers	3 layers	3 layers
Ok to use	Ok to use	Ok to use	Do not use!	Do not use!

Hatchery Vaccination

In the hatchery, 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

At the hatchery, there are two options for vaccine timing; in-ovo (embryonic developmental day 18 to 19,) or the day of hatch. To inoculate embryos at transfer, live or recombinant vaccines are injected through the air cell of the egg. The efficiency of the in-ovo vaccination process has improved throughout the years with the introduction of machines to inject a whole tray of eggs simultaneously. Eggs must be oriented correctly in the trays with the air cell facing up. Malposition eggs will not be vaccinated correctly resulting in lack of vaccine efficacy or even embryonic death.

In-ovo vaccination is performed at the time when the hatching eggs are transferred from the incubator 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.

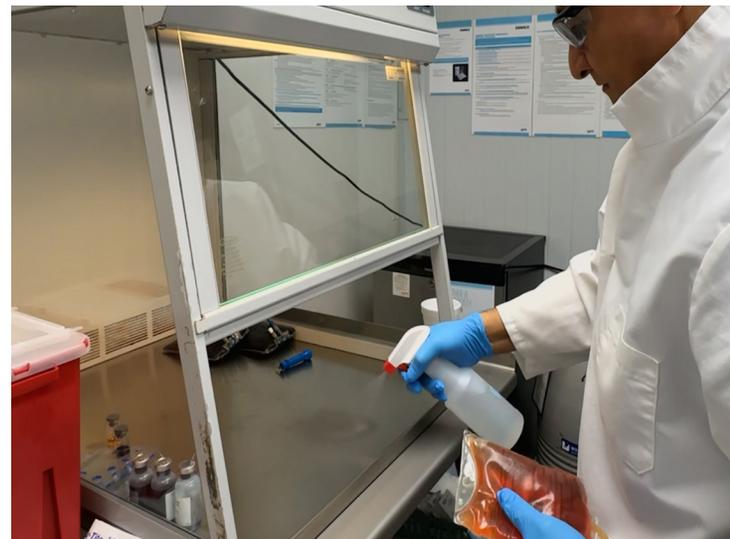
Provided certain criteria are met, including timing and site of vaccine application, vaccine mixing, machine sanitization, and hatchery management specifications, the in-ovo vaccination has proven to be an efficacious and convenient method of vaccination. In the last few years, in-ovo technology has been extended for other vaccines 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 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.

1. 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 vaccine dye to the diluent, then add antibiotic (only when prescribed by a licensed veterinarian and at the recommended dose).
2. Record all additives on the diluent bag.
3. Prepare a clean water bath with a chlorine disinfectant (final concentration of 200 ppm). Set water temperature to 27 °C (80 °F).
4. Remove the vials to be reconstituted from the liquid nitrogen tank.
5. 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).
6. Once thawed, remove the vials from the water bath and dry using a clean paper towel.
7. 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).
8. 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.
9. 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. Agitate by gently tapping the top of the bag while injecting the vaccine into the bag.



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 placed correctly (see photo on right).
- » 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.

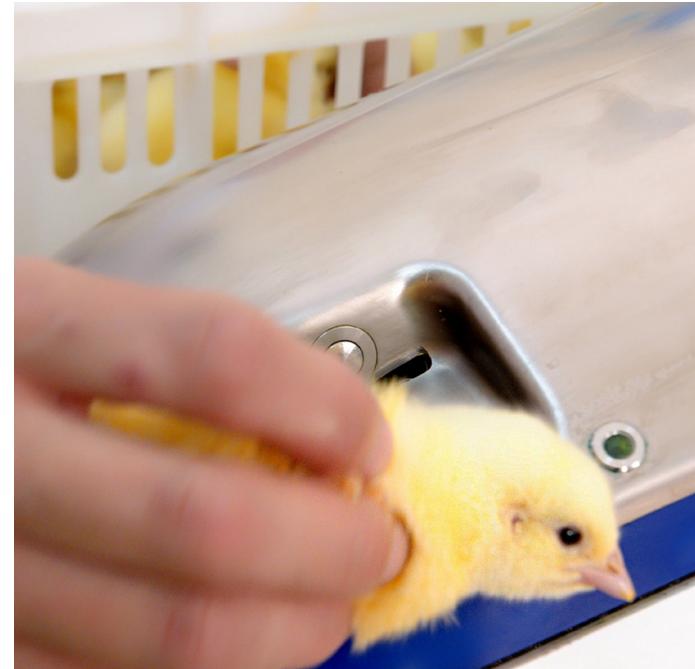


2.5 Subcutaneous (SC) or Intramuscular (IM) Injection at Day of Hatch

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:

- » Use personal protection equipment
- » 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, evidence of dye in the subcutaneous (SC) tissue is visible. 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 indicates that needles are mal-positioned, burred or blunt, or excessive pressure.
- » Verify that the machine remains calibrated and consistently delivers the prescribed volume of vaccine.
- » Verify that the air pressure is correct (most machines operate with 50 to 75 PSI, or 3.4 to 5.2 Bars). Excess pressure will hurt the chicks and may promote leakage of vaccine or damage the cells in the vaccine. Insufficient air pressure may reduce 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. 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 regularly 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 to 21 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, height of the chick box, and trigger mechanism.

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 use 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. Using lights will encourage chick activity, limit huddling, and promote health and welfare for the flock.

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.

2.7 Gel Vaccination in the Hatchery

Superabsorbent hydrogels are materials that can absorb large quantities of water and retain their shape. Any oral vaccine, competitive exclusion (CE) products, and some coccidiosis vaccines can be administered using a gel format. When dyed, the gel drop allows visual assessment for vaccinators and promotes chick consumption by preening other hatch mates. The use of a dye in the vaccine also allows visual confirmation of consumption. Gel vaccines offer a few advantages over liquid spray vaccines. Gel holds the dense particles like oocysts in suspension more effectively than liquids. Sprays tend to drift in the air, while gel droplets are heavier and less likely to float, reducing waste. Gel applications are usually less stressful compared to spray vaccinations. Liquid sprays can startle and cool chicks rapidly.

Important points for spraying respiratory vaccines in the hatchery:

- » Some vaccine gels require adding gel powder to the distilled water and some products are available as premade gels. The water or the premade gel should be cool or at room temperature with a temperature range of 13oC (55oF) to no warmer than 27oC (80oF).
- » Before adding the vaccine, most gel products will require adding and mixing a dye (if not already dyed) or other additives such as a buffering agent with the gel.
- » Gel products are usually mixed with a hand held motored device using an attached mixing blade (similar to a portable drill with a paint or mortar mixing blade).
- » After the mixing of all additives, the vaccine and/or the CE oral products can be added and mixed together with the same hand held mixing device.
- » The mixed vaccine gel can be added to the gel application device to deliver to the chicks.
- » As with any device, check the delivery application by running a few empty chick boxes with paper through the application device to evaluate the gel application pattern.
- » After vaccination chick boxes should be placed in a warm, draft-free area with bright lighting for at least 10 to 15 minutes to allow consumption by preening of the drops. With good lighting the chicks readily consume the vaccine in a few minutes. Begin visual assessment and recording of vaccine consumption soon after vaccinating. The gel drops are rapidly consumed and the dye staining the mouth may fade rapidly.



Photos courtesy of Dr. László Kőrösi, originally published in "Infectious bronchitis hatchery vaccination: comparison between traditional spray administration and a newly developed gel delivery system in field conditions." *Veterinary Sciences* 8.8 (2021): 145.

Field Vaccination

There are various ways to mass apply vaccinations to poultry in production housing. 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. Considerations include 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 offer products, and modifications can be made to agricultural sprayers. Follow the manufacturer's instructions for the equipment used. Handheld sprayers are also available for smaller housing.

Personnel

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

Equipment

- » Two to three backpack sprayers (one sprayer for each side of the house and, for large houses, 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 and mix only enough vaccine to vaccinate one house.
- » Use clean, non-chlorinated water or water that has had vaccine stabilizer added. Distilled water is ideal. Water should be between 16 °C (60 °F) and 27 °C (80 °F). Err towards the cool side as hot water 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 10 meters, 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.
- » Shake the tanks on the sprayer to mix the vaccine thoroughly.
- » For quality control records, note the vaccine expiration date and serial number, date and time of vaccination, location (farm and house number), and staff members.

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 keep them from piling against the back wall.
- » Each vaccinator sprays one side of the house (a third in the middle for large houses).
- » 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 ventilation by setting fans to previous settings.
- » Restore lighting to previous intensity.



3.1 Spray Vaccination with Backpack Sprayer System (cont.)

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 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

Using the drinking water systems in poultry housing is a common method to administer live vaccines. Prior to vaccination, 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. Water consumption per 1000 birds each day.

Broiler Age (Days)	Minimum Consumption		Maximum Consumption		Average Consumption	
	liters	gallons	liters	gallons	liters	gallons
7	50	13.3	73	19.4	61	16.0
14	108	28.4	143	37.9	124	32.8
21	146	38.7	212	56.1	175	46.2
28	189	49.1	271	71.7	227	60.0
35	224	59.1	324	85.5	275	72.6
42	251	66.2	365	96.4	312	82.3
49	254	67.2	370	97.7	325	85.9
54	290	76.5	374	98.8	331	87.5

3.2 Water Vaccination (cont.)

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.
- » Always administer the vaccine early in the morning.
- » Enough drinker space is required to allow access to the vaccine solution.
- » For hand drinkers, withdrawal water 30 to 60 minutes in hot climates and 60 to 90 minutes in cool climates prior to vaccine administration. If the water restriction period is 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.
- » To help ensure that the birds drink the water lines dry, turn water off approximately two hours before the lights are turned off the day before the vaccination. Drinker lines should be raised the morning of the vaccination and high enough that birds cannot reach the nipples.
- » Always transport the vaccine in an insulated cooler with ice or cold packs. The vaccine should be maintained at a temperature range of 2°C (35°F) to 7°C (45°F) during storage and transport to the farm.

Vaccine Preparation

- » It is recommended to add a vaccine stabilizer or skim milk powder to the water 20 to 30 minutes prior to adding the vaccine. Add the skim milk powder at a ratio of 500 g / 200 L (1lb / 50 gal).
- » Prepare the stock solution in a graduated plastic bucket prefilled with distilled water. If distilled water is not available, add a vaccine stabilizer before adding the vaccine.
- » Use enough water for the vaccine stock solution so that the solution is consumed between 1.5 and 2 hours after the drinker lines are lowered.
- » If using a proportioner, calculate the average water consumption from the last 4 days, 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 (if necessary) and dye to the bucket.
- » Open the vaccine vial and fill the vial 2/3 full with distilled or stabilized water and gently rotate the vaccine vials to dissolve all the vaccine. Water should be between 16 °C (60 °F) and 27 °C (80 °F). Err towards the cool side as very warm water can damage the vaccine. Close the vial with the rubber stopper and gently agitate to reconstitute the vaccine. Do not aggressively shake the vials which can damage the vaccine.
- » Add the reconstituted vaccine to the mixture. Rinse the vaccine vials several times to remove all the vaccine. Stir and mix well with a clean utensil.

3.2 Water Vaccination (cont.)

Vaccine Administration

- » Pour the reconstituted vaccine into the drinkers. For water tank, water pump or medicator and proportioner specific information on priming and distribution see following sections (3.2.1, 3.2.2, and 3.2.3, respectively).
- » If using hand drinkers, redistribute drinkers if necessary.
- » Walk through the house at least 2 to 3 times to check if the birds are drinking and to encourage all birds to drink.
- » The birds should 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 evaluating the results.
- » Check 25 birds from each pen or house for evidence of dye in the mouth (see photo). Over 95 % of birds evaluated should have stained mouths.
- » All medication, disinfectants and chlorine must be suspended from the drinking water for 24 hours after vaccination.



3.2.1 Using Water Tanks

Before Vaccination

- » Determine the number of birds per water tank in the house and calculate the number of vaccine vials required.
- » The volume of distilled or stabilized water used for vaccination will be approximately 30 % of the average daily volume of water consumed.

Vaccine Preparation

- » Prepare the vaccine following manufacturer's instructions and guidelines in section 3.2. Add the prepared vaccine to the water tank.

Vaccine Administration

- » Open the valve of the tank with the vaccine to allow the birds to consume the vaccine.
- » After the vaccine is consumed, close the valve to the tank(s) with the vaccine and open the valve of the tank(s) with "normal" unvaccinated water.



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). A hose is connected to the water line and the vaccine is pumped from a container(s) through the hose into the water line.

Before Vaccination

- » Flush the drinker lines with fresh water to eliminate residues.

Vaccine Preparation

- » Calculate the amount of water needed so the vaccine is consumed in about 90 minutes. This amount should be approximately 30% of the daily water intake.
- » Prepare the vaccine following manufacturer's instructions and guidelines in section 3.2. Mix vaccine into a container or containers large enough to hold the required volume of water.

Vaccine Administration

- » Put the end of the hose that is connected to the water line into the container with the vaccine. Start the pump to inject the water lines with the vaccine.
- » Open the end of the drinker line to improve flow. One staff member must observe the water coming out of the end of the drinker line until the dyed solution (the vaccine) is visible (see photos). When the dye is visible, close the end of the drinker line.
- » Lower the drinker lines to allow the chickens to consume the vaccine.
- » If more than one container was necessary to mix the volume of vaccine required, alternate the containers of mixed vaccine until all the vaccine doses are consumed.



3.2.3 Using a Medicator or Proportioner

A medicator or a proportioner can be used to deliver vaccine through the drinker lines to the birds. Medicators and proportioners are connected inline to the drinking system.

Before Vaccination

- » Flush the drinker lines with fresh water to eliminate residues.

Vaccine Preparation

- » Calculate the amount of water needed so the vaccine is consumed in about 90 minutes. This amount should be approximately 30% of the daily water intake.
- » Prepare the vaccine following manufacturer's instructions and guidelines in section 3.2. Mix vaccine into a container or containers large enough to hold the required volume of mixed vaccine.
- » Prime the medicator with the vaccine solution. Bypass or remove water filters during vaccination.

Vaccine Administration

- » Open the end of the drinker line. One staff member must observe the water coming out of the end of the drinker line until the dye solution (the vaccine) is visible (see photos page 20). When the dye is visible, close the end of the drinker line.
- » Lower the drinker lines to allow the chickens to consume the vaccine.
- » Every 15 to 20 minutes, check the vaccine solution to ensure the pump is operating correctly and that the vaccine is still flowing through the drinker lines.



3.3 Intraocular (Eye Drop) or Nasal Drop Vaccination

Vaccine Preparation

- » Confirm that the vaccine is approved and manufactured for eye drop application. Serious issues may occur if the wrong vaccines are dropped into the eye. Therefore, designate one person to mix the eyedrop vaccine.
- » Open the vaccine vial and the diluent bottle. Reconstitute the vaccine with diluent that is 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 adaptor 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.



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). 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. Do not touch the bottom of the vaccine container when dipping wing web applicators. This can dull the needles and waste vaccine.
- » Change needles every 500 to 1,000 birds. Monitor for signs of dulling or burr formation.

Post Vaccination

- » 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).



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 methods to inject chickens; intramuscular (IM; into the muscle) and subcutaneous (SC; under the skin).

Several sites are available for each method of injection (see table below). Research has shown that all common injection sites can give satisfactory results if done correctly. When selecting the injection site, consider ease of application, reaction at the injection site, and human safety. Compare 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.

Correct injection technique and bird handling will prevent human injection. Bird handlers have an important responsibility to present the birds for injection at the correct angle for the 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.

Injection sites for each injection method	
Intramuscular (IM; into the muscle)	Subcutaneous (SC; under the skin)
Breast	Neck
Wing	Inguinal fold
Tail Head	
Thigh	
Leg	

3.5 Injectable (Inactivated) Vaccines (cont.)

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 in a warm water bath (do not exceed 32.2 °C (90 °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

- » Make sure that there is no air in the tube when the vaccine is administered by priming the tubing and gun to prevent “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.

Post Vaccination

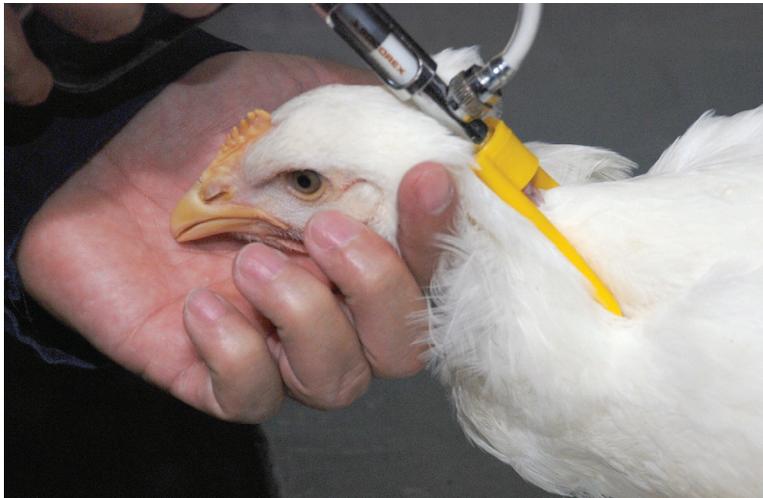
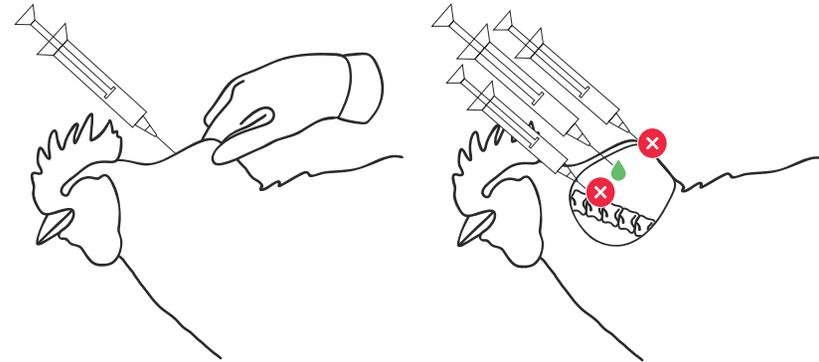
- » Record vaccine information and any problems that may have occurred regarding the birds or the vaccination process.
- » After vaccination, all syringes and plastic tubes must be washed prior to sterilization and disinfection.
- » Sterilize all equipment used in the vaccination, using an autoclave, 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, a yellow needle guard is used to help prevent accidental injection into the spine and spinal cord.

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 towards the rear or vent (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.

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.



Circled areas are ideal injection locations in the tail head.

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. Routinely evaluate titers over time to ensure that good titer levels are maintained in the flock.



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

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, joints, nerves, and bones.



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

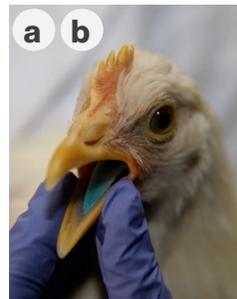
Vaccination Quality Control

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.

▶ 4.1 Vaccination Quality Control

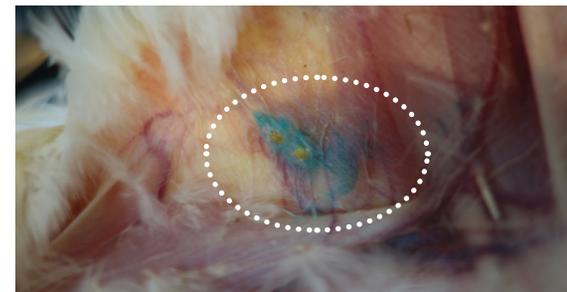
- » Vaccination crews should be randomly inspected by the veterinarian to examine their application techniques. This is especially important for breeders, where injection errors can impact future growth and egg production.
- » Designate one crew member for quality control to evaluate 50 to 100 birds during the vaccination sessions for wet feathers, hemorrhages, or other signs of improper application of vaccines.
- » Necropsy of cull birds or mis-sexed birds can allow immediate evaluation of vaccination techniques of injectable vaccines.
- » 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.
- » 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
- d. On the skin of the wing web



4.1 Vaccination Quality Control (cont.)

- » 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.
- » 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.
- » Use a table similar to the example below to record your observations from the vaccination.



An acceptable vaccine reaction indicated by the presence of 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	House 4	House 5	House 6	House 7
Total number of birds in the house							
Total number of birds examined							
Number of good (presence of two nodules)							
Number of medium (presence of one nodule)							
Number of poor (Absence of nodules)							
% Good (Number of Good ÷ Total Examined) *100							
% Medium (Number of Medium ÷ Total Examined) *100							
% Poor (Number of Poor ÷ Total Examined) *100							

▶ 4.2 Errors Using Injectable Vaccines

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

A) **Vaccine is placed into the skin layer (intra-dermal).** 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.

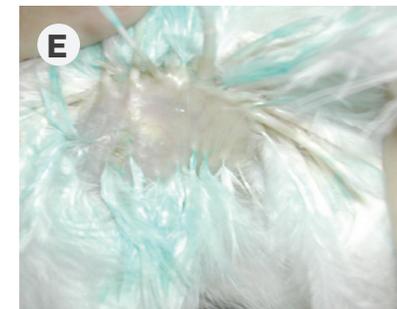
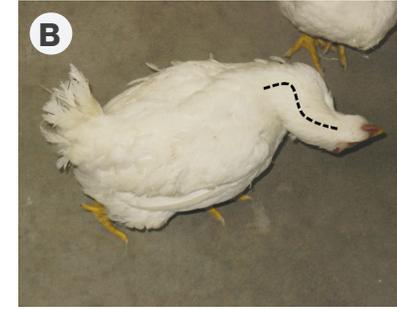
B) **Vaccine is injected into the neck muscle (intramuscular).** The muscles of the neck are very small and the immune reaction to the vaccine creates inflammation and pressure. The damaged muscle heals and scar tissue forms. This scar tissue can cause birds with twisted or crooked necks, resulting in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

C) **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.

D) **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.

E) **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.

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.



4.2 Errors Using Injectable Vaccines (cont.)

Intramuscular (IM) leg, thigh or wing injection

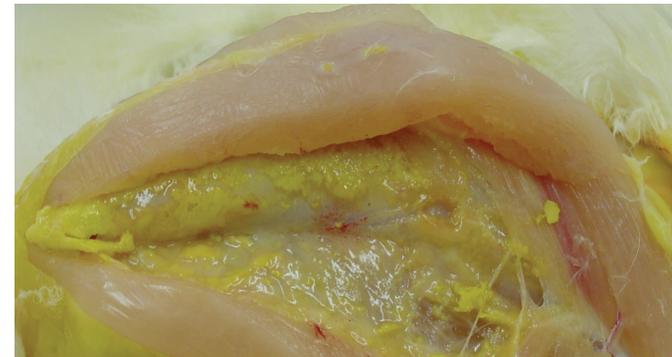
Intramuscular leg, thigh or wing can also be used as sites of injection. The wing is a very small target and misapplication can easily occur. The leg is often selected as the site of injection for cage-reared layer pullets as the leg can be easily accessed with minimal stress to the pullets. When vaccinating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



Excessive reaction to a leg injection.

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 in the breast muscle to a Pasteurella bacterin injection.

4.3 Monitoring the Vaccination Program

The objectives of using inactivated products include long 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 Enzyme Linked Immuno Sorbent Assay (ELISA). A variety of kits are available for antigens through commercial companies. The results are also quantitative for most antigens – giving Mean Titers, Geometric Mean Titers (GMT) and Coefficient of Variation (%CV) as metrics. The desire in breeder hens is to achieve high GMT's and low %CV for the common antigens - IBDV, NDV, IBV and Reovirus.

ELISA technology allows the detection of antibodies to expressed insertions after administration of recombinant vaccines. This detection method measures the immune response to a specific insert while also building the database for any producer using such recombinant products.

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 incorrect location of injection. The duration of titer levels can also be impacted by incorrect 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.





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