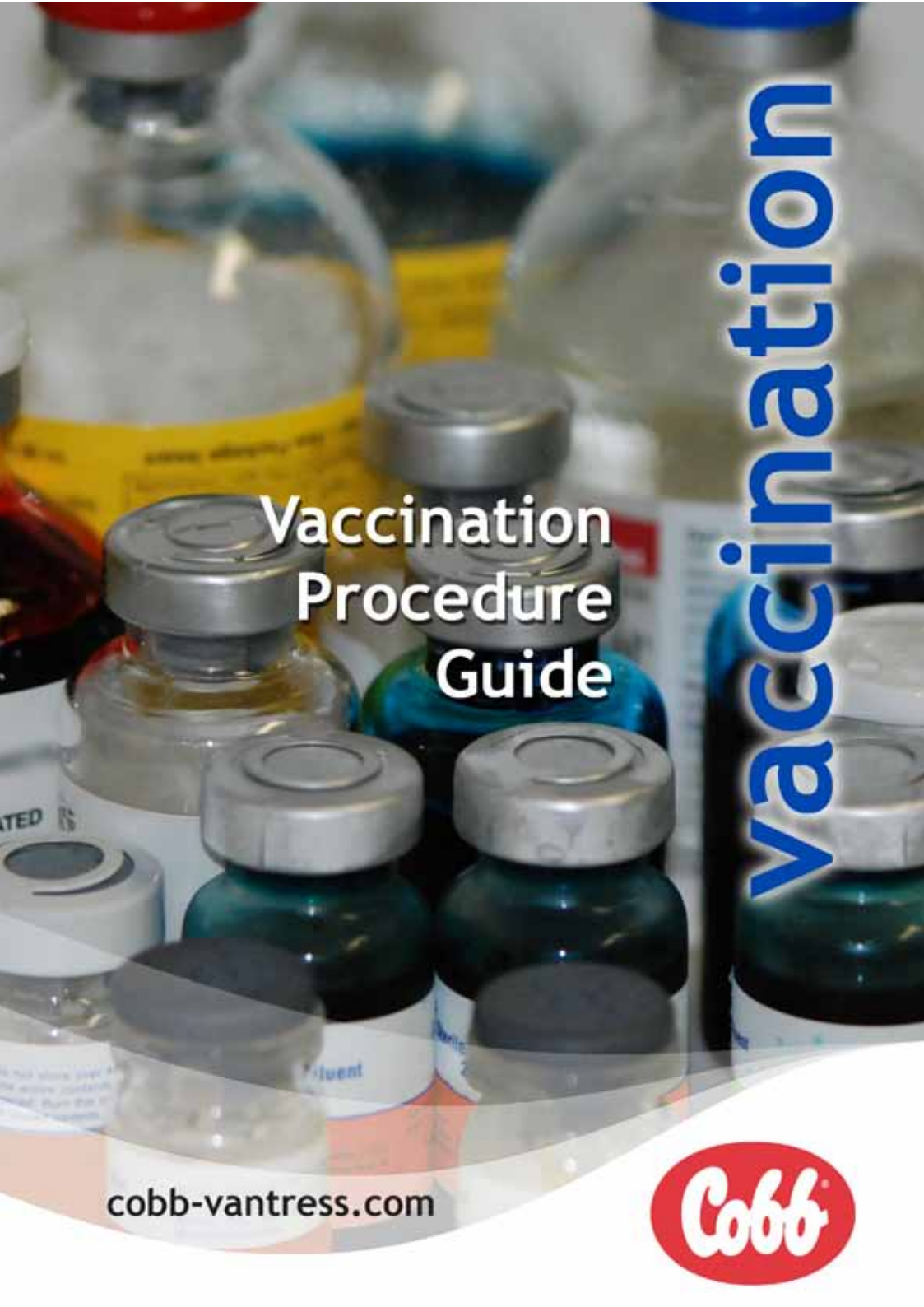

Vaccination Procedure Guide

vaccination

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Cobb

Vaccination Procedure Guide

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Produced by Cobb-Vantress World Technical Support Veterinary Services with the purpose of helping worldwide producers with proper vaccination procedures for poultry.

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1. INTRODUCTION

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

1.1 WHY DO WE VACCINATE

Proper vaccination is an essential part of a good poultry management program and for the success of any poultry operation. Effective preventive procedures such as immunization protect hundreds of millions of poultry 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 totally protect birds that are under stress or in unhygienic conditions.

The primary objective of immunizing any poultry flock is to reduce the level of clinical disease and to promote optimal performance. Certain vaccines may also have an impact on human health (i.e. Salmonella vaccines).

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

A. Protect the bird (as a pullet and hen) against specific diseases.
B. Protect the progeny of the hen against vertical transmission of disease.
C. Provide passive immunity to progeny.

1.2 HOW DO VACCINES WORK?

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

Depending on the type of antigen in the vaccine, the birds' 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 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 inactivated during production and formulated into an injectable form. Recombinant vaccines are made by using live virus or bacteria as a vector to transport the gene coding for the protective antigen of a second infectious agent for which immunity is desired.

Live and inactivated vaccines are compared in Table 1 below:

TABLE 1

Aspect of the vaccine	Live	Killed
Safe	Yes	Yes
Economical	Yes	More expensive
Mass application	Yes	Inject each bird
Rapid onset of immunity	Yes	No (2 weeks)
Duration of immunity	No	Yes
Combination of antigens available	Yes	Yes
Maternal antibody interference	Yes	Low

1.4 VACCINE HANDLING AND STORAGE

For all vaccines:

- Vaccine should arrive with cool packs in a well-insulated box
- If vaccine arrives hot, call manufacturer or distributor.
- Storage temperature = 35-45º F (2-8º C).
- Avoid freezing, extreme heating and intense light.

For Live Vaccines:

- Transport to farm in cooler with ice packs to keep temperature constant.
- Mix with diluent (reconstitute) just before application.

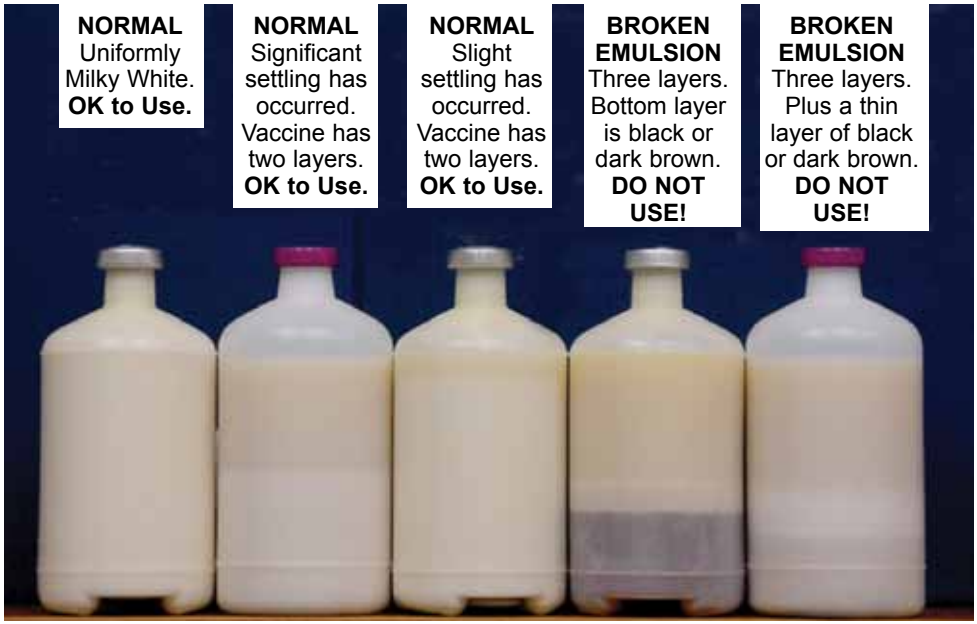
For Inactivated Vaccines:

- Remove 24 hours prior to vaccinating so that the product can warm to room temperature. Also, can use warm water bath–do not exceed 100º F for more than 5 hours .
- Do not leave bottles in direct sunlight during transport to farm.
- Gently agitate bottles thoroughly prior to use.

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.

- To test the product to see if the emulsion is broken – shake the bottle vigorously for 2 minutes. Let bottle rest for 5 minutes. If separation persists, do not use that bottle of the vaccine and contact the manufacturer.

This image shows varying presentations of killed vaccines and which are safe to use:



2. HATCHERY VACCINATION

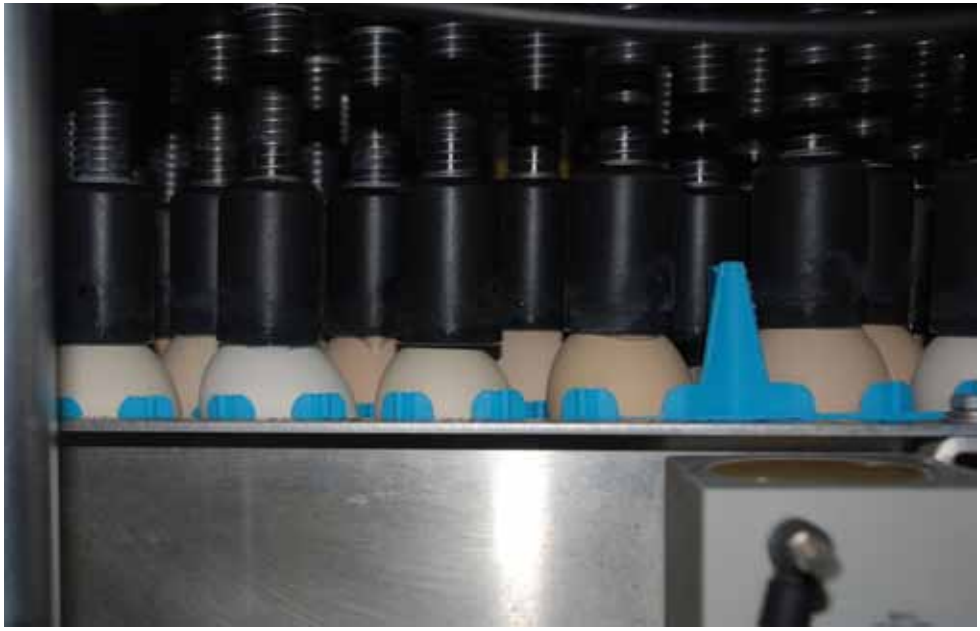
The hatchery is a place where a large number of chicks can be vaccinated conveniently and effectively. For this reason, an increased number of vaccinations are being given at this point.

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 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.



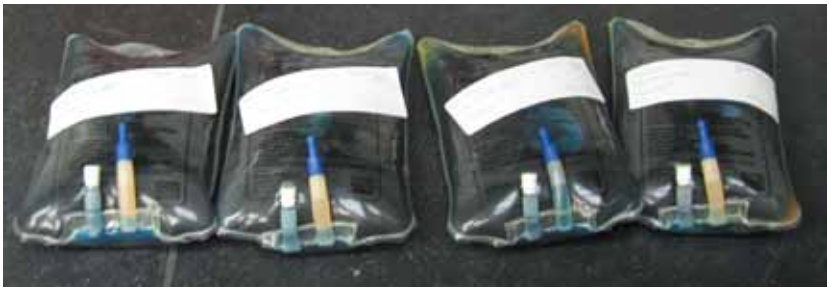
2.2 MAREK'S VACCINE PREPARATION

Use only a clean and sterile 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 dedicated 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.
    1. Add the vaccine dye to the diluent
    2. Add antibiotic, only when prescribed by a veterinarian, at the recommended dose
- Record all additives on the diluent bag
- Prepared a water thaw bath with:
    1. Clean water and chlorine disinfectant (final concentration of 200 ppm)
    2. Set water temperature to 27º C (80º F)
- Remove the vials to be reconstituted from the liquid nitrogen tank.
- Place the vials in the prepared water bath (27º C) (80º F) and allow to thaw (approximately 70 to 90 seconds depending on the dose)
- Once thawed, remove the vials from the thaw 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).
- Using a sterile 20 ml syringe with and 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
- 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.
- Gently massage and invert the diluent bag to ensure vaccine is mixed thoroughly (do not shake vigorously).
- Then, 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. Then gently withdraw all of the liquid back into the syringe. Repeat this for all of the vials used and then return the contents of the syringe to the diluent bag.

- Swirl and invert the diluent bag again to ensure vaccine is mixed thoroughly (do not shake vigorously).
- Never force vaccine through a syringe, as this will break down vaccine cells, and dramatically reduces the level of vaccine content.
- Record the contents and time that the vaccine was prepared on the diluent bag.
- Discard all used needles, syringes, and vials in an appropriate waste receptacle.

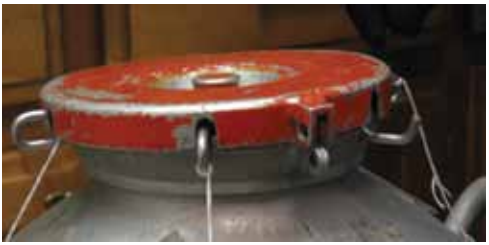


2.3 MAREK'S VACCINE STORAGE

Marek's Disease vaccines are 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 for any form of administration. The nitrogen tanks must also be properly maintained to insure the vaccines stay at a constant temperature.

2.4 NITROGEN STORAGE TANK MAINTENANCE

- Avoid moving the storage tank abruptly and avoid falls or bumps, which might lead to breaking its 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 well placed and well adjusted.
- Damage to the external or internal parts and to the neck tube may result in loss of tank vacuum and consequently in tank failure.
- After using all the vaccine ampules, the tank must not be out of nitrogen, because the thermal shock that might occur when refilling can cause 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 under the direct action of the liquid nitrogen.
- The level of liquid nitrogen must never be under 30 cm, as measured with a suitable measuring stick. The nitrogen level must be checked daily;
- Use Personal Protective Equipment (PPE) when measuring the level of nitrogen.



Incorrect tank lid placement
(not completely closed)

Always wear safety glasses and insulated gloves while handling vaccine from liquid nitrogen containers.

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 the neck injection. A skilled operator can vaccinate about 1600-2000 chicks/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 course of the day. Burred or bent needles must be replaced immediately.

Before Vaccination

- Calibrate all vaccinators pre-vaccination for accuracy
  - Verify the position of the needles
  - Provide plenty of new sterile needles
  - Check all vaccinators for dose accuracy
  - Check the pneumatic pressure
- 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 inadvertently, the vaccine will flow to the bottom (the cap of the vial), and be visualized.
- 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.
- Put on safety goggles and insulating gloves.

Vaccine Administration

- Begin the vaccination process with properly sanitized equipment.
- Hook up the diluted vaccine to the vaccination equipment and 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 30-45 minutes. Should the vaccination personnel need to stop or interrupt the procedure at any time, keep track of the interruption and do not allow the use of vaccine that has been sitting for more than 45 minutes.
- A chick sample may be taken per vaccinator to insure the quality of vaccination. Because dye has been added to the vaccine, one can look for evidence of dye in the SC tissue. Count the numbers of chicks with SC dye for every 100 chicks sampled and determine the percent chicks missed. Correct any problems immediately. The inspection must be done within 15 minutes post-vaccination or else the dye will no longer be visible under the skin.
- Determine the 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 is properly calibrated to deliver the prescribed volume of vaccine. Verify that the prescribed air pressure is correct (most machines operate with 75 psi).
- 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

- Insure proper cleaning, sanitation, sterilization and maintenance of the vaccination equipment at the end of the day.
- Discard all unused vaccine, including vaccine left over during personnel “breaks”.

2.6 SPRAY VACCINATION IN HATCHERY

In many areas, chicks are vaccinated with live vaccines using a spray cabinet that administers a defined amount of water-borne 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.

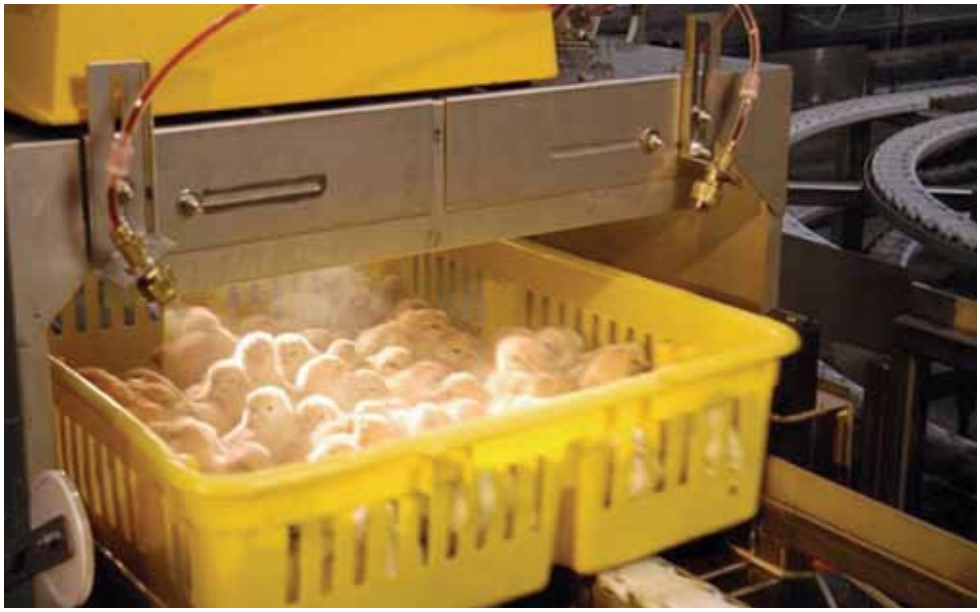


Photo courtesy of Dr. Linnea Newman

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 and spray equipment used.
- A particle size of 100-300 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.
- Items to monitor include the air pressure, nozzle spray pattern, volume delivered per nozzle in every actuation, orientation of the nozzles, and height of the chick box.

Important points for coccidiosis vaccination by spray cabinet:

- Coccidiosis vaccines must be stirred or agitated gently continuously to insure 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 a spray 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 and consumption of the vaccine.
- After vaccination, the chick boxes should be placed in an area with abundant light in order to continue stimulating vaccine consumption by preening.

3. FIELD VACCINATION

There are various ways to mass apply vaccinations to poultry in production housing situations. In developed poultry industries, the emphasis is on effective application with the lowest labor costs. In countries where labor is inexpensive and readily available, application strategies that maximize the immune response can be selected. Disease challenges in every production area will additionally dictate 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 types of housing (floor, slats, cages), water systems (open, closed, hand), and age of bird.

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 manufacturer's instructions for the particular equipment you are using. 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 if possible when a flock is vaccinated.

Equipment

- Two to three backpack sprayers.
- Vaccine storage - Insulated cooler with ice or cold packs.
- Distilled water for mixing.

Before Vaccination

- Spray 1 gallon of 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 other birds, between 30-60 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.

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 warmer than 80° F (27º C) and probably no cooler than 60° F (16º C) although the cool side is less critical, as long as it is not freezing.
- Pour enough water into the sprayer tanks to allow the vaccinators to walk the length of the house twice SLOWLY without running out of vaccine. (minimum 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(s) 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.
- Record the serial number and expiration date for the vaccines used.

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.
- After vaccination is completed be sure to restore proper ventilation.

Vaccine Administration

- Walking 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 three feet (1 m) above the birds heads.
- Keep a constant pressure of 4.5-5.0 Bars (65-75 PSI).

Post Vaccination

- Reset all curtains and fans to previous positions.
- Properly dispose of all empty vaccine vials, water jugs, etc.

Sprayer Maintenance

- Fully charge batteries prior to use.
- Change batteries after spraying 30 gallons of water.
- Thoroughly rinse the tank with one 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 the bleach solution.
- Periodically check all hose and connections for signs of wear. Replace as needed.

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 hour to insure 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, standard guidelines are available based on bird type, age and temperature (Table 2).

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 age and ambient temperature. It's important to note at higher temperatures, birds need double the water:

TABLE 2

		(gal/1000 birds/day)
Broiler Age in Weeks	**70º F (22º C)**	**90º F (32º C)**
1	10	20
2	16	31
3	25	49
4	33	65
5	40	72
6	46	90
7	51	100
8	55	108

Before Vaccination

- Always administer the oral vaccine on the day 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.
- Sufficient drinker space is required to allow free access to the vaccine solution.

Vaccine Preparation

- The addition of skim milk powder to the water 20-30 minutes prior to adding the vaccine is recommended as a stabilizer. Add the skim milk powder at the rate of 1lb/50 gal (500 g/200 L).
- Open the vaccine vial by removing the aluminum seal and the rubber stopper. Using the 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.
- Mix with the vaccination and the stock solution after adding vaccine stabilizer, following manufacturer's directions. When the vaccination 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.
- Walk the birds to check if they are all drinking water. If using hand drinkers, redistribute drinkers if necessary.
- Note that the birds must drink all the vaccine solution in no more than two hours, and never in less than 1 hour.

Post Vaccination

- Record all vaccine information as well as any problems which may occur 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 particular 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

- Mix into vaccination water a vaccine stabilizer following manufacturer's instructions.
- Add the vaccine vials and blue dye according to the calculation.

Vaccine Administration

- Open the water tank valve in order for 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

- The drinker lines are raised to prevent drinking by the birds in the 1-2 hours prior to vaccination.
- Flush the drinker lines with fresh water to minimize the amount of unwanted residues.

Vaccine Mixing

- **Calculate the amount of water needed so the vaccine is consumed between 60 and 90 minutes. This amount should be approximately 30% of the daily intake. If the water restriction period was excessive, the birds will be too thirsty and the vaccine will be consumed too quickly, and every bird will 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 container(s) 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 lines is open to improve flow.
- One vaccinator 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 of the vaccine doses are consumed.
- Walk through the birds at least 2-3 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 VACCINE

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 eye dropped.
- 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 2º and 8º C (36º to 45º F).
- Open the diluent bottle and, using a syringe, remove 3 ml of diluent, inject into the lyophilized vaccine vial. Some vaccines come with a special adapter to mix diluent and vaccine - just 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 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.

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.

3.4 WING WEB VACCINATION

This route is commonly used for Fowl Pox, Avian Encephalomielitis, Chicken Anemia and Live Fowl Cholera.

Vaccine Preparation

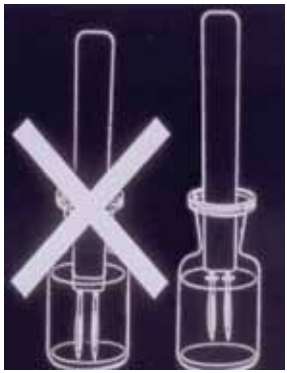
- The preparation of this vaccine is similar to that of the eye-drop vaccine. The vaccine is lyophilized and must be reconstituted in the same manner as other vaccines.
- Only use the specific diluent which comes packaged with the vaccine.
- Shake the vaccine vial carefully, turning the vial from one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

Wing web applicators

Vaccine Administration

- Administer the vaccine in the center of the wing web, using a two-pronged needle applicator or other wing web applicator (Grant innoculator or others).
- Remove the feathers located on the wing web before exposing it for vaccination.
- 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 the needle every 500 birds. The used needles can be disinfected and used again as long as they remain sharpened.
- If during the application the wing vein is punctured, immediately change the needle and repeat vaccination.

Take care to not dip wing web applicators too deep into vaccine – this wastes vaccine doses.



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.

3.5 INJECTABLE (INACTIVATED) VACCINES

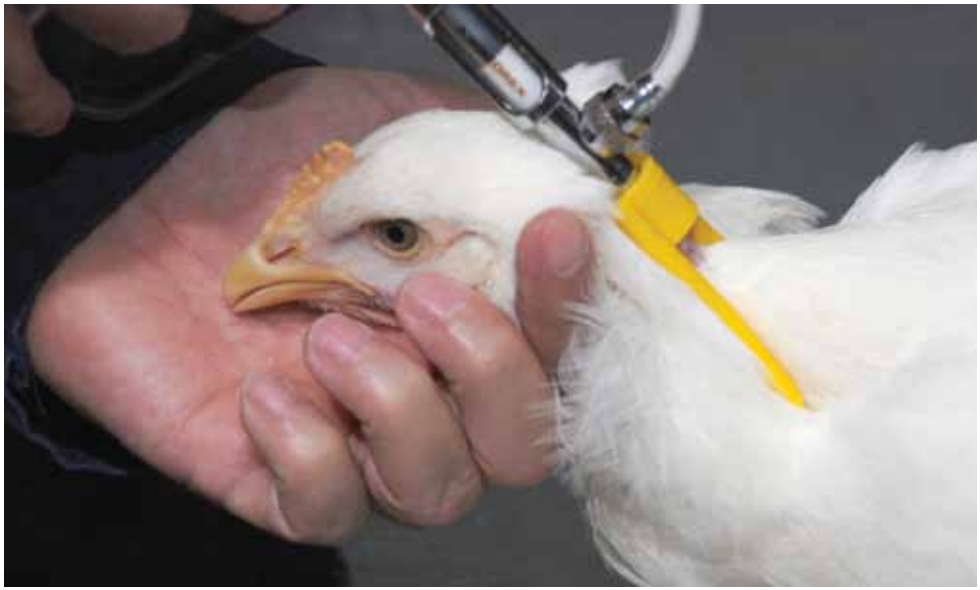
Injectable vaccines must be manually injected into each bird using an 18 gauge by 1/4" needle. There are two major injection methods in avian species to allow suitable vaccination:

Intramuscular (IM) – into the muscle
Subcutaneous (SC) – under the skin

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.

TABLE 3

Subcutaneous	Intramuscular
Neck	Breast
Inguinal Fold	Thigh
	Leg
	Wing
	Tail Head



Crew Safety
Accidental human injection with oil emulsion products is a serious potential danger when injecting this group of products. When this occurs, immediate medical attention should be administered to the injured personnel. When these products are injected into the hands or fingers, 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 reduce the potential for human injection to occur. 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

- Remove the vaccine from the refrigerator and leave at room temperature for at least 12 hours in order to reduce the viscosity of the chilled mineral oil, thus making the administration easier and preventing intense local reactions.
- Oil emulsion vaccines can also be warmed using a warm water bath prior to injection – do not exceed 100° F (37° C) for more than 5 hours.
- Gently agitate the vaccine container before and during the vaccination process to homogenize the contents.

Vaccine Administration

- Administer the vaccine by using only the labeled dose at the chosen site of injection.
- Needles should be regularly replaced, at least once every 500 birds.
- Make sure that there is no air in the tube when the vaccine is administered.

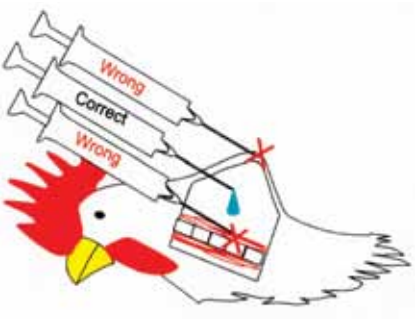
Post Vaccination

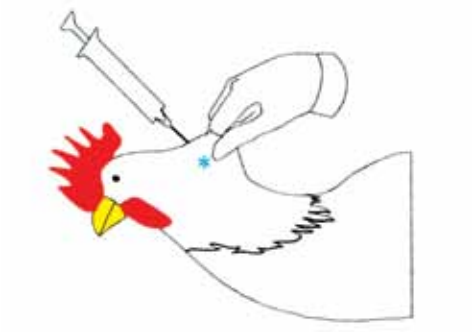
- Record vaccine information and any problems that may occur regarding the birds or the vaccination process.
- After vaccination, all needles, syringes and plastic tubes must be washed prior to sterilization.
- Sterilize all equipment which will be used in the vaccination, using autoclave, alcohol or boiling water.

3.6 INSTRUCTIONS FOR EACH SITE OF ADMINISTRATION

Neck

The skin on the back of the neck should be lifted up 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.





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.

Breast

Vaccine is injected into the superficial pectoral muscle about 1 to 1.5 inches (3 to 5 cm) lateral to the keel bone, depending on the age of the bird. The needle should be directed caudally at a 45º 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 is directed 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 is made into the large muscle group on the under side of the wing. The needle is pointed toward the body. Avoid major vessels 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 pointed cranially. Care should be taken to not withdraw the needle too quickly, which can lead to leakage of vaccine out of the injection site.

Tailhead–circled areas are ideal injection locations.

4. VACCINATION QUALITY CONTROL

The best vaccination program can only be achieved with proper administration and monitoring to insure 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. Train one crew member to be in charge of quality control. This person will evaluate birds during vaccination sessions for wet feathers, hemorrhage, 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 the vaccines – both live and killed – to assist in observation of vaccine at the time of administration:
    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 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 7-10 days following administration. Select and examine 50-100 birds chosen randomly throughout the house.

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

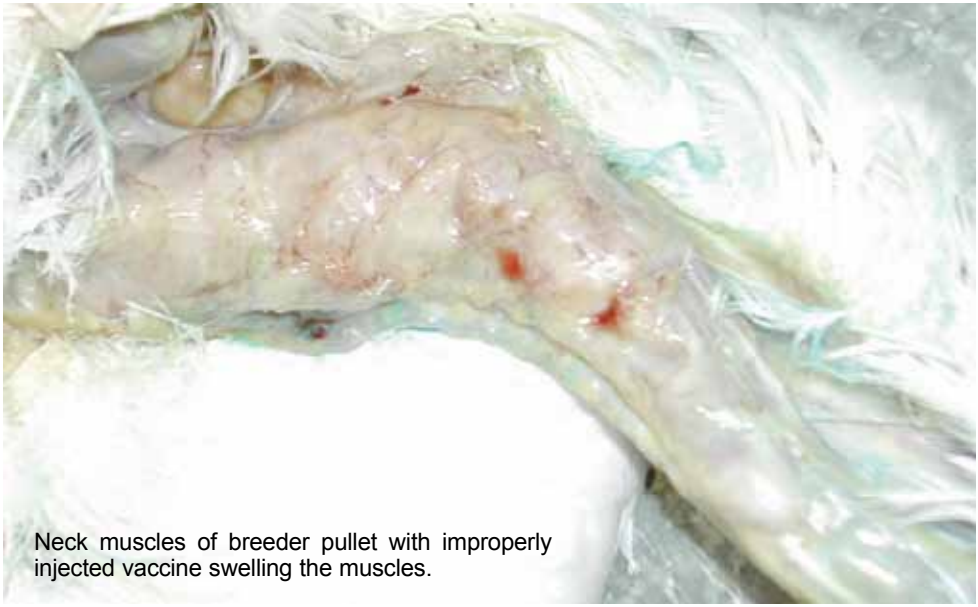
TABLE 4 Example of a Quality Assurance Sheet

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.



Neck muscles of breeder pullet with improperly injected vaccine swelling the muscles.

3. If the needle is inserted too deeply, the needle will pass through the muscle layer and vaccine can be injected into the spinal cord. Birds usually die within a few minutes after injection into the spine.
4. Birds injected too close to the head will develop swollen heads which can impair feed consumption and vision. Injecting too low results in swelling over the back. Flockmates may peck at these noticeable swellings causing more problems.

Bird with swollen head following SC injections that were too high on the neck.

Bird with crooked neck following improper SC injections that were administered too deeply into the neck muscle.

5. When injection occurs through the side, off mid line, large vessels and soft tissues 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.

Subcutaneous bleeding from puncture of the blood vessels.

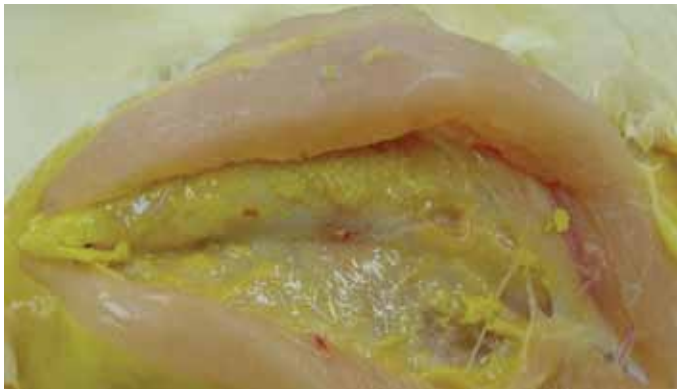
Large mass in area of thymus – birds were injected off mid-line

6. Also, inserting the needle through the side can cause the needle to pass through both layers of skin. Vaccine ends up wetting the feathers on the opposite side of the neck and the bird does not develop immunity.

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

4.2 INTRAMUSCULAR (IM) BREAST INJECTION

Intramuscular (IM) breast injection is an easier technique with increased accuracy, but can have its own problems. Adverse lesions, in the form of granulomas, may remain in the muscle and be found at spent fowl processing. Vaccinators can 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 going off-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

4.3 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 will commonly occur, so it is not a common choice. The leg is often selected as the site of injection for cage-reared layer pullets – the leg can be easily accessed with minimal stress on the pullets. Moving of pullets will exacerbate the leg swelling that can be observed in vaccinated birds, so stress post-vaccination should be minimized. The use of a concentrated presentation of product (0.25ml dose) will also minimize reaction if planning to inject into small muscle locations.

Excessive reaction to injection in the leg

4.4 MONITORING THE VACCINATION PROGRAM

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

The most common serological test used for this purpose is ELISA (Enzyme Linked Immuno Sorbent Assay). A variety of kits are available for numerous antigens through a number of 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. Poor vaccine administration can raise the %CV and lower GMT of flocks sampled. This may be explained by high numbers of missed birds, vaccine leakage or improper location of injection. The duration of titer levels can also be impacted by improper vaccination as titers wane over time 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 MG.

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 particular 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	-

5. NOTES

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