

Appendix 1: Post-mortem technique for domestic fowl

The use of a consistent routine of post-mortem examination will greatly enhance your ability to recognise abnormalities in organs and tissues. This sequence of dissection for the domestic fowl allows observation of all body systems, and outlines suitable methods for collection of specimens for laboratory examination.

When selecting birds for autopsy, take live birds that are showing typical signs, rather than those that are *in extremis* or dead. In these, the primary disease may be obscured by secondary diseases or by post-mortem decomposition.

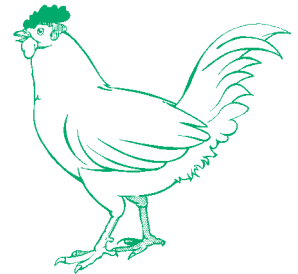
Examine the bird for clinical abnormalities before it is killed. This may indicate a particular system or organ that needs special attention during the post-mortem examination.

Humane killing of birds

It is important to kill birds in a humane, efficient manner which does not itself cause changes that might confuse the diagnosis.

Cervical dislocation

- Grasp the legs and primary wing feathers with one hand, so the bird cannot flutter.
- With the other hand, grasp the bird's head from above, holding the head between the first (index) and second fingers. Curve the fingers along the bottoms of the jaw. This avoids pressure on the larynx and tongue when the neck is broken.
- Hold the bird across your body, with its head downwards. No undue force is used at any time up to this point. Bend the bird's head backwards.
- Break the bird's neck using a fairly strong rapid stretching action, keeping the head bent backwards. The bird will lose consciousness immediately, but will make strong reflex movements for about 2 minutes after neck dislocation. While struggling continues, keep the bird immobilised by maintaining your grip on the wing bases. Elevate the head to lessen the likelihood of inhalation of crop content which may be regurgitated.



Intravenous injection of air

- Grasp the bird by the wing bases with the left hand and immobilise it over the edge of a table.
- Pluck a few feathers over the brachial vein.
- Compress the base of the wing with the left index finger to distend the brachial vein.
- Place the needle into the vein and rapidly inject 6–7 mL of air. Reflex struggling is brief and there is no trauma to neck structures. The bird does not regurgitate, as it may after cervical dislocation. Again, the wing bases should be held firmly to prevent the carcass escaping.

Materials:

A sharp knife

A pair of scissors

A pair of shears (or garden secateurs)

A pair of forceps

Bottles containing 50% glycerine in saline

Bottles containing 10% formalin (Remember that the volume of 10% formalin used should be at least 10 times that of the tissue to be fixed.)

A coolbox with ice or ice-packs (if possible)

Post-mortem technique for domestic fowl

- Examine the exterior of the carcass:
 - the vent for discharges;
 - the skin by reflecting the feathers, which may hide skin lesions or external parasites (see Permin and Hansen 1998 for more detail on the diagnosis of poultry parasites); and
 - the orifices of the head for discharges and the colour of the mucous membranes.
- Position the carcass on its back with legs towards you.
- Push a sharp knife through the fold produced by lifting the skin on the posterior part of the sternum. Draw the knife back through the fold, producing a v-shaped incision. Enlarge this until the flap of skin that has been freed can be grasped firmly and drawn forward with the left hand, while holding the legs in the right hand. Continue the skin incision along the neck to the head using the knife.

- Reflect the skin from the upper part of the legs, and dislocate the hip joints. The carcass can now lie flat. The general colour and condition of the musculature is noted at this point.
- Rotate the carcass so that the head lies towards you. Reflect the skin from the structures of the neck.
- Open the mouth and cut through the left temporo-mandibular joint with shears. Cut open the mouth with scissors, continuing the incision through the wall of the pharynx and oesophagus as far as the crop. Open and examine for changes.
- Cut horizontally through the beak just above the nostrils with shears to open the nasal cavities and infra-orbital sinuses. Open and examine for changes.
- Remove the small cartilagenous nasal turbinates using small scissors or a scalpel blade.
- Using small or sharp-pointed scissors, enter the larynx and open the trachea using only the tips of the scissors. Take care not to damage the mucosa. Spread open the trachea and examine the mucosa for exudate and inflammation.
- Rotate the carcass back to its original position with the tail towards you.
- Open the abdomen with scissors, starting just above the pubis. Continue the abdominal incision through the costochondral junctions of the last few ribs on the right-hand side of the bird. Use the scissors to cut the pectoral muscles of the left side of the thorax.
- Cut the remaining ribs, the coracoid bone and the clavicle on the left side using shears. Cut the remaining ligaments and muscles and reflect the sternum to your left until the coracoid bone on the other side breaks. This allows the contents of the thorax and abdomen to be displayed.
- Cut the omentum and reflect. The first structures to be displayed are the abdominal airsacs; these are easily destroyed and should be examined at this early stage of the abdominal dissection. They should be very thin and transparent; a pale mucoid thickened appearance suggests chronic respiratory disease.
- Draw the gizzard out to your left. Hold the small intestine by its mesentery and draw out the intestine to the left, freeing mesenteric attachments and airsacs.

Most of the abdominal viscera and organs are now exposed (Figure 16), and the dissection has reached what can be called the Display Stage. This is a most important stage of dissection in any species and is the time for recording the general state of nutrition, presence or absence of anaemia, exudates in serous cavities, malposition of viscera, etc.

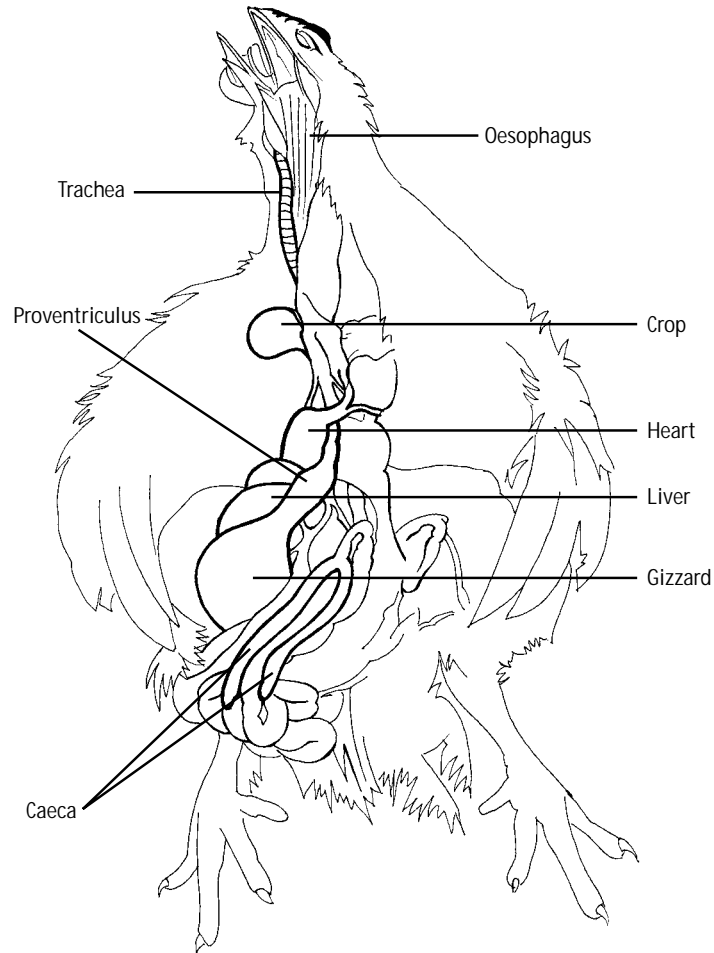


Figure 16: *The display stage of the necropsy showing the major organs of the chicken. The trachea, proventriculus and caeca should be examined for lesions.*

- Free the intestine by cutting the colon just before it enters the cloaca. Cut the colonic, caecal and small intestinal mesenteries close to the gut and draw the bowel out to the left. Free the paired caeca.

It is best not to separate the two arms of the u-shaped duodenum, for the pancreas lies between and is easily damaged. The two arms of the duodenum can be opened and examined and may then be fixed as a whole for histological examination.

- Open the remaining small intestine with the scissors, taking care not to damage the underlying mucosa. Any part of the mucosa that shows abnormality can be examined microscopically by taking a scraping of the full thickness of the mucosa, using a blade of the scissors or a scalpel blade. Transfer the mucosal sample to a microscope slide. Apply a coverslip and carefully press onto the sample using a pointed instrument, not your dirty glove. Microscopic examination of this wet preparation will reveal the presence of various stages of coccidiosis.
- Open the intestine along its entire length. Examine the junctions of the small intestine and the colon at the base of the caeca. Note the caecal tonsils (small nodules of lymphoid tissue) at this junction. These may contain small red spots which suggest local haemorrhage, but are in fact normal.
- Open the paired caeca. Scrapings of the mucosa may be examined for caecal coccidiosis. Always examine the very tips of the caeca, where the small nematode *Heterakis gallinae* may be found.
- Cut the heavy muscular wall of the gizzard with a knife and extend the incision up through the proventriculus and lower oesophagus to the crop. The lining of the gizzard should be tough and tightly adherent to the mucosa. If it strips easily to reveal oedema or underlying haemorrhage in a freshly dead bird, toxic damage may be suspected.
- Examine the crop for presence and type of feed and any degeneration of the mucosa.
- Inspect the spleen by reflecting the gizzard to the left. Reflect the opened gizzard and proventriculus to the left.
- Examine the heart. Note the size and contents, if any, of the pericardial sac. An increase in size of the heart in relation to the carcass gives the best indication

of the presence of heart failure. Remove the heart by cutting through the vessels and atria at its base. There will be froth in the right atrium if the bird was killed by an intravenous injection of air.

- Cut the myocardium of the right and left ventricle from base to apex, and examine the interior of the heart. For histological examination, the whole open heart should be fixed in formalin. In cases of suspected septicaemia, blood can be aseptically aspirated before removing the heart.
- Inspect the liver *in situ* and note its colour, shape and size relative to the carcass. Normal liver is very fragile and should be handled gently. The texture of the organ is noted during handling, and the consistency of the liver is noted while cutting a block for histological examination. Cut the liver from its attachments. The surface may be cleaned by wiping with the knife blade. This is preferable to washing with water which causes osmotic damage to the tissue.
- Note the size of the gall bladder. Remember that the gall bladder has not been stimulated to empty in birds that have not been eating, and may appear overfull.
- Dissect the lungs away from the ribs and roof of the thorax. Normal aerated lung can be fixed by floating the organ unsliced in formalin, as the fixative diffuses readily through air-filled tissue.
- Examine the ovary which often contains wrinkled, discoloured, involuting ova in birds or normal hens that have recently gone off lay. Reflect the ovary to reveal the adrenal glands.
- Remove the ovary and remains of the abdominal airsacs to reveal the kidneys. Note their size, shape and colour. Check the ureters for the presence of excessive amounts of white urates, which might indicate nephrosis. This should be done before the kidneys are removed. Normal kidney is very friable.
- Examine the sciatic nerve plexus which can be observed on each side of the spinal column. Check the spinal column for deformity at this stage.
- Display the continuation of the sciatic nerve in each leg by reflecting the adductor muscle of the thigh. The normal unstretched nerve should be glistening white, with fine cross striations.
- Examine the fine intercostal nerves, and the brachial plexus on each side, dorsal to the thoracic airsacs. Particular attention to nerves is necessary in the

diagnosis of Marek's disease, a viral infection which causes enlargement and greyish discolouration of affected nerves.

- Examine the spinal and vagus nerves in the neck.
- In young birds examine the thymus, the thyroid and parathyroid glands. The thyroids and parathyroids lie together on each side of the neck close to the origin of the common carotid arteries.
- Examine the leg joints. Open the tibio-tarsal joint by cutting the medial collateral, and dorsal tibio-tarsal ligaments and dislocating the joint. Pass the blade between the posterior end of the tibia and the sesamoid bone in the Achilles tendon. The stifle joint is opened in a similar manner.
- Examine the growth plates of long limb bones especially in young lame meat birds that have grown rapidly. Expose the proximal end of the tibia, and shave off part of the bone to display the growth plate.
- Examine the bone marrow by splitting the femur. The marrow in the normal bird is red.
- Remove the head by cutting through the atlanto-occipital joint.
- Remove the skin from the head and open the cranium using pointed shears. Begin just above the occipital condyles lateral to the foramen magnum. Then extend the incisions on each side to meet above the eyes. Care must be taken to avoid damage to the underlying brain. Remove the cranial cap carefully. Cut away the remnants of the dura matter and observe the brain.
- Remove the brain by cutting the cranial nerves beneath it. Gently displace the brain back until it can be extracted and all surfaces examined.
- In young birds, open the bursa of Fabricius through its opening to the cloaca.

Based on the technique used by Professor Roger Kelly. Adapted by Dr Mary Young.
The Division of Veterinary Pathology and Anatomy, The University of Queensland, Australia



Appendix 2: Collection of blood from the wing vein of chickens

This technique uses a needle and syringe. A 25 G (0.50 x 16 mm) needle is used for chicks under 4 weeks of age, and a 23 G (0.65 x 32 mm) needle for older chickens. Plastic syringes of 1.0 or 2.5 mL capacity are convenient. Both needles and syringes can be washed for reuse.

If you have an assistant

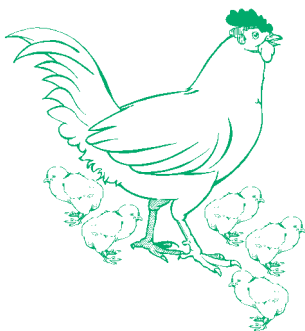
- Ask the assistant to hold the chicken horizontally against her or him with its head to her right.
- Pull the right wing out towards you, if necessary pluck away the small feathers from the underside overlying the humerus, and swab with 70% alcohol. The wing vein, named in various text-books as the brachial, ulnar or cutaneous ulnar, is clearly visible running between the biceps and triceps muscles.
- Insert the needle under the tendon of the pronator muscle, in the triangle formed where the wing vein bifurcates (see Figure 15), pointing the needle proximally i.e. in the direction of the blood flow. Do not go too deep or the needle will scrape the humerus and the chicken will struggle. Likewise keep clear of the ulnar nerve. With a little gentle probing you should enter the vein easily. This approach from under the tendon makes it easier to enter the vein than does aiming directly for it, and also tends to steady the needle if the bird moves.

If working alone

- Sit with the chicken horizontal between your thighs, head away from you, lying half on its back and half turned on its right side.
- Clamp down its legs with your left elbow (if you are right-handed) and its neck with your left forearm, and with your left hand spread out its left wing.
- With your right hand, proceed as above.

This works with all chickens except the dedicated strugglers. Some people prefer to hold the birds with the head towards them; if you can perfect both techniques you will have two veins to choose from.

Withdraw blood by gentle suction since the veins on chickens collapse readily. After the needle is removed, apply pressure to the vein for a few seconds to discourage further bleeding. Immediately label the syringe with the number of the chicken.



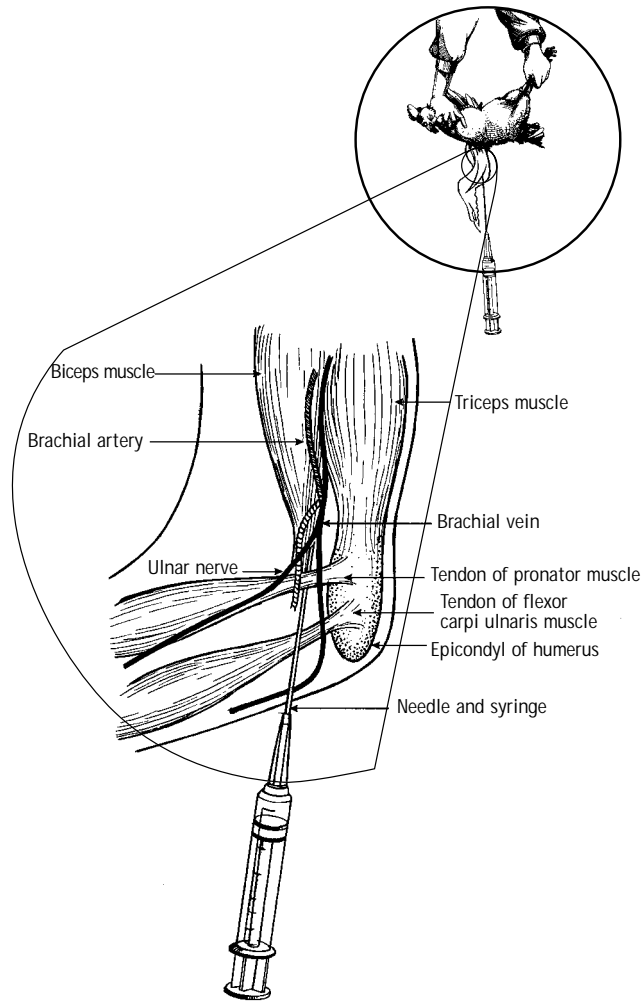


Figure 17: *Illustration of a convenient anatomical site for bleeding chickens (adapted from J. Samuel).*

If the blood is for serum collection, leave it in the syringe and store the syringe in a slanting position, with the needle end pointing upwards. Leave an air space between the blood and the end of the syringe. After collection, if possible, leave the syringes in a warm room at 37°C for one hour to assist coagulation. If the

blood is for the preparation of red blood cells, the collection will have been made into an anticoagulant. Mix the blood gently while it is in the syringe, remove the needle and transfer the blood to a vessel with a screw cap. If the blood is discharged through the needle, there is a chance that some blood cells will haemolyse.

It is possible to pierce a wing vein with a needle and then to collect the freely flowing blood into a small vial. This delivers a less satisfactory sample that will invariably be contaminated with bacteria. Also the chicken is likely to be discoloured with blood, and some owners will object to this.

Based on the technique used by Dr Janeen Samuel (Australia) and Dr Rini Dharsana (Indonesia).



Appendix 3: Calibration and care of Eye-droppers

Eye-droppers

Eye-droppers are made of flexible plastic, preferably low density polyethylene. The ideal eye-dropper has a removable tip, protected by a screw top cap. A suitable eye-dropper should:

- hold a suitable volume of vaccine;
- not inactivate (destroy) the vaccine virus; and
- deliver drops of an appropriate size.

Volume of droppers

Eye-droppers of up to 30 mL capacity can be used. It is not necessary to place a full 30 mL of vaccine in these droppers. The volume of vaccine that is used will depend on the number of chickens to be vaccinated, and the size of the drop delivered by the dropper.

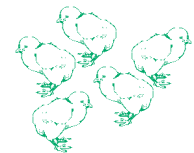
Testing for antiviral activity

Vaccine virus will be killed on exposure to some plastics. A sample of a new batch of eye-droppers should be tested before use in the field. This test must be done in a laboratory that is able to measure the infectivity of the vaccine virus.

Vaccine virus should be diluted for use and then divided into two parts. The diluent should contain antibiotics as the laboratory will require vaccine free of contamination when the virus content is measured in eggs. Place half the vaccine in the eye-dropper and half in a stoppered, sterile glass test tube (or leave it in the vaccine vial). Store both overnight in a cool, dark location. The two preparations are then tested to confirm that there is little or no difference in virus content between the vaccine stored in the eye-dropper and that stored in the test tube.

Size of drops

The volume of diluent used to reconstitute freeze-dried vaccine, or to dilute liquid vaccine, will depend on the size of the drop that is formed by the eye-dropper. It



is the outside diameter of the tip, not the inside diameter, that determines drop size. It is best to use an eye-dropper that produces more than 40 drops per mL. If the eye-dropper produces 66 drops per mL (an ideal number) it means that each drop is approximately 15 μ L. This volume is ideal for the small eye of a chicken.

Human eye-droppers are not as convenient for use in chickens. These often produce drops of 25 μ L to 35 μ L. Such drops are large compared to the size of a chicken's eye and splashing of the drop and wastage of the vaccine can occur.

Each new batch of eye-droppers should be calibrated to ensure that chickens receive the correct dose of vaccine.

Calibration Method Number 1 — for use with freeze-dried vaccine

1. Remove the tip of the eye-dropper (Figure 18, step 1), add 1 mL of water to the dropper (steps 2 to 5) and then replace the tip securely (step 6).
2. Hold the eye-dropper upside down, squeeze the dropper very gently and count the number of drops that fall from the tip (step 7). Remember that the eye-dropper should be held in the vertical position (see Figure 6 in Section 5.1.1). It is generally advisable to repeat this process three times and to use the average number of drops in the calculation below.
3. Use the following formula to calculate the volume of diluent required to dilute the number of doses of the vaccine per vial and the eye-dropper in use:

$$\text{Volume of diluent (mL)} = \frac{\text{No. of doses of vaccine per vial}}{\text{No. of drops formed per mL}}$$

Example 1: How much diluent should be added to a vial containing 250 doses of ND vaccine given that 1 mL of water in the eye-dropper yielded 50 drops?

$$\begin{aligned} \text{Volume of diluent (mL)} &= \frac{250 \text{ doses per vial}}{50 \text{ drops per mL}} \\ &= 5 \text{ mL per vial} \end{aligned}$$

Example 2: How much diluent should be added to a vial containing 100 doses of ND vaccine given that 1 mL of water in the eye-dropper yielded 37 drops?

$$\begin{aligned} \text{Volume of diluent (mL)} &= \frac{100 \text{ doses per vial}}{37 \text{ drops per mL}} \\ &= 2.7 \text{ mL per vial} \end{aligned}$$

METHOD 1

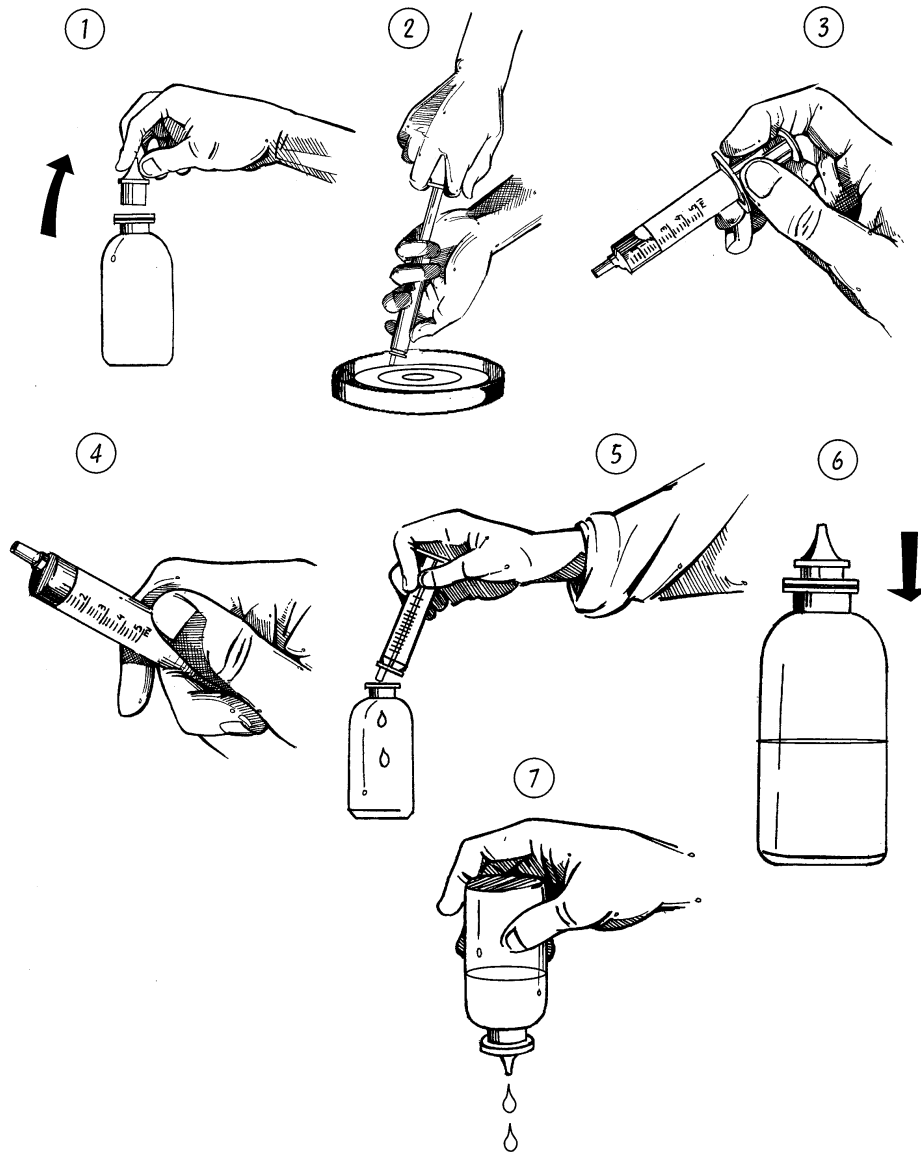


Figure 18: Calibration method number 1.

Calibration Method Number 2 — for use with freeze-dried vaccine

This method is easier for people less familiar with syringes and mathematical calculations. It is better if two people to work together.

1. Check the vaccine label to determine the number of doses per vial.
2. Remove the tip of the eye-dropper (Figure 19, step 1), fill the eye-dropper with water (step 2) and replace the tip (step 3).
3. Remove the plunger from a 10 mL or 20 mL syringe (step 4) and hold the syringe vertically with the tip down. The tip should be closed with a finger or a thumb (step 5).
4. Hold the eye-dropper vertically, squeeze the eye-dropper very gently and commence counting drops into the syringe (step 6). Continue counting until the number of drops equals the number of doses contained in the vaccine vial. Many people find it easier to count the drops in groups of ten and record the number of groups. For instance, for a 250 dose vial, count 25 groups of 10 drops to give a total of 250 drops. Working in pairs, people count to 10 and then make a mark on the ground.
5. Hold the syringe vertically and check the level of the water against the marks on the syringe. This is the volume required to dilute the vaccine. Repeat three times.

If it is necessary to use glass eye-droppers with a rubber bulb, this method of calibration can be used.

METHOD 2

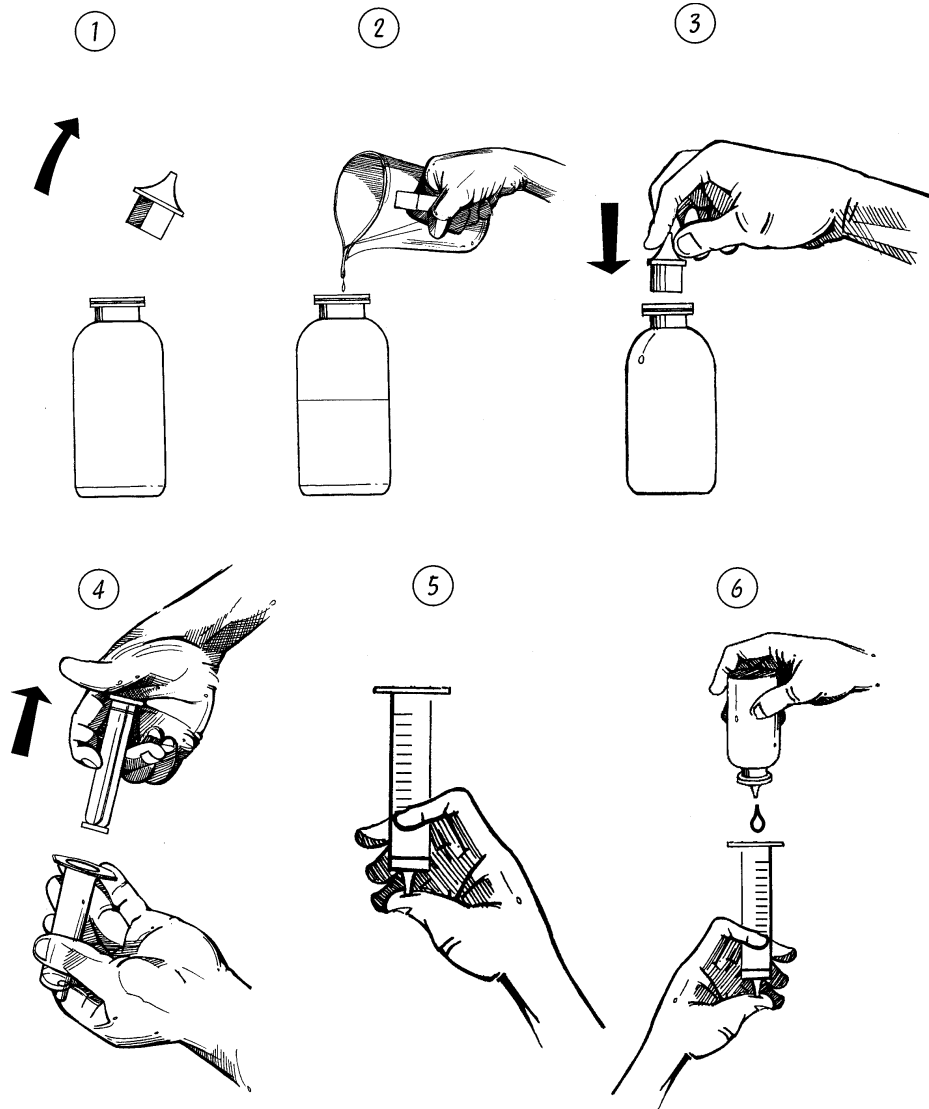


Figure 19: Calibration method number 2.

Calibration Method Number 3 — for use with “wet” vaccine

The method described below should be considered a guide rather than a formal “calibration” method and may be used when working with locally-produced “wet” I-2 ND vaccine. It will be useful in circumstances where those involved with fieldwork would prefer not to perform complex calculations. A more accurate method for use with “wet” vaccines may be found in the companion ACIAR ND laboratory manual.

1. Remove the tip of the eye-dropper (Figure 19, step 1), fill the eye-dropper with water (step 2) and replace the tip (step 3).
2. Remove the plunger from a 5 mL or 10 mL syringe (step 4) and hold the syringe vertically with the tip down. The tip should be closed with a finger or a thumb (step 5).
3. Hold the eye-dropper vertically, squeeze the eye-dropper very gently and commence counting drops into the syringe (step 6).
4. Count the drops until the water in the syringe has reached the 1 mL mark.
5. Record the number of drops. Repeat this process twice to confirm that the number of drops counted is correct.

If 50 or less drops were required to fill the syringe to the 1 mL mark, then the eye-dropper is suitable for administration of the “wet” I-2 ND vaccine by single eye-drop application. Eye-droppers that require considerably less than 50 drops to yield 1 mL, will deliver a higher dose of the vaccine. This will result in a greater immune response by the bird and will not cause any harmful reactions as the I-2 vaccine remains innocuous even in large doses.

This rule may be used providing that the following conditions are met:

- The eye-dropper is made of a suitable plastic and has been approved by the laboratory producing the I-2 vaccine.
- The I-2 vaccine is prepared by mixing equal volumes of allantoic fluid and a stabiliser (such as 2% gelatin).
- The average titre of allantoic fluid is 10^9 EID₅₀/mL of I-2 virus. The laboratory distributes the vaccine such that it contains 10^7 EID₅₀ of I-2 virus per dose to ensure that after transport each bird will receive a dose of 10^6 EID₅₀ that is required to provoke an adequate immune response.

This method is based on the fact that 10 μL of allantoic fluid with a titre of 10^9 EID₅₀/mL will contain 10^7 EID₅₀ of I-2 virus. When this allantoic fluid is mixed with an equal volume of a stabiliser, 10^7 EID₅₀ of I-2 virus will be contained in 20 μL . Fifty drops containing 20 μL each are required to make 1 mL.

How to care for plastic eye-droppers

To ensure a long life for eye-droppers, they must be cleaned and stored correctly after use.

1. Wash in cool, clean water only. Do not use HOT water.
2. Do not use treated tap water. If you only have access to treated tap water, it is advisable to let it stand overnight to allow the chlorine to evaporate.
3. Do not use disinfectants as they will inactivate the vaccine virus.
4. Do not clean the tip of the eye-dropper with anything abrasive.
5. Do not force anything into the tip of the eye-dropper that will enlarge the opening.
6. Allow the eye-dropper to dry thoroughly and then wrap in a dry clean cloth.
7. Store away from direct sunlight, sources of heat, rats and mice!



Appendix 4: The role of community livestock workers in the control of Newcastle disease

It is now well recognised that any non-compulsory program that does not have community participation is unlikely to be sustainable in the long term. In addition, government veterinary and extension services in most countries are unable to provide cost-effective services such as routine vaccination to all local communities, especially those in remote areas. Therefore, community participation is crucial to the development of a sustainable program to control ND in village chickens.

In many developing countries, the work of government veterinary and extension staff has been complemented at the village level by persons from the local community, the Community Livestock Workers (CLWs). A CLW is a man or a woman selected by the local community or appointed with their agreement to deal with animal health and production in the community (FAO 1994). S/he must be able to communicate effectively in the language used by farmers, read labels and instructions, and keep appropriate records.

In situations where most farmers raise only poultry, it may be best to commence with the training of community vaccinators. These vaccinators may receive further training to become CLWs should the community demand such a service.

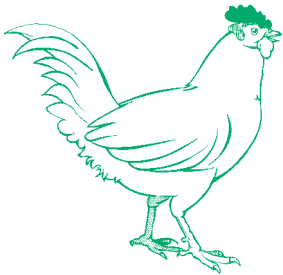
Many factors affect the success of CLW programs. These include:

Social factors

- The program must be based on community needs and use existing structures or organisations.
- The community must be involved in all parts and stages of the program.
- The CLW must be chosen and respected by the community.
- There should be an ongoing exchange of information between all stakeholders.

Technical factors

- CLWs and their trainers must identify and respect local knowledge.
- They must be able to fulfill their technical responsibilities.



- There must be collaboration with beneficiaries and sources of technical support to ensure that CLWs offer appropriate technical advice.

Institutional factors

- The CLW program must cooperate with all existing institutional structures (government, traditional, local project, government veterinary and livestock extension services, NGOs) in the planning and implementation of the program.

Economic factors

- To ensure economic sustainability, the local community must be involved in the development of cost-recovery mechanisms for the program.
- Charging for services (financial contribution by the beneficiaries to cover the cost of the vaccine and its administration by a CLW) is essential to the success of the program. To start with, charges may represent only partial cost-recovery.
- CLWs will obtain most benefit from ND control activities if they also raise village chickens. Protecting their own birds from ND will provide a greater economic return than the money earned vaccinating the birds of others.

Environmental factors

- The program should not contribute to environmental degradation.
- Care with the use of chemicals and antibiotics should be emphasised.

Compared to government veterinary and extension staff, CLWs

- provide more cost-efficient services, and transport and labour costs are reduced;
- are more flexible in their working hours, e.g. they are available for weekend and after-hours work; and
- are more accessible to village poultry owners.

To ensure sustainability of the program, CLWs must:

- be assured of a reliable supply of vaccine (and other necessary inputs);
- receive appropriate training;
- be answerable to their community;

- be able to monitor their own work;
- be provided with incentive in cash or kind; and
- receive good technical follow-up and support.

The training of CLWs is an important component of the ND control program. Factors to be considered include:

- who does the training?
- where should the training be conducted?
- who is responsible for post-training supervision?
- who is responsible for monitoring?
- who is responsible for evaluation?

The training program for CLWs involved in control of ND should include:

- Features of a chicken — simple anatomy
— recognition of healthy and sick chickens
- Handling of a chicken
- Husbandry — housing: ventilation, cleaning, predators
— nutrition: young chicks, use of supplements
- Diseases of chickens — clinical signs, field diagnosis, treatment and control of:
 - Newcastle disease
 - External parasites
 - Internal parasites (coccidiosis, helminths)
 - Fowl cholera, Fowl pox
- Vaccination techniques — eye drop and drinking water for ND vaccines
- Record keeping — number of cases
— diagnosis and treatment of cases
— outcome of treatment
— inventory of stock (pharmaceuticals, etc.)
— vaccinations performed
— payment received

In order to perform eye drop vaccination, CLWs must be able to:

- read numbers on a syringe;
- understand the meaning of the lines and intervals between the numbered lines on a syringe;
- read and check the number of doses of ND vaccine per vial and the expiry date of the vaccine;
- use the syringe to put the appropriate volume of water into a vial and draw vaccine out (if using vaccine that requires dilution);
- check that the vaccine is properly diluted;
- shake vial completely to dissolve all vaccine;
- assemble an eye-dropper;
- hold the eye-dropper vertically to form a drop of the correct size;
- check that the correct number of drops leave the eye-dropper;
- hold a chicken gently and calmly; and
- clean an eye-dropper and syringe correctly.

The ND vaccination kit for CLWs should contain:

- syringe (10 mL or smaller if appropriate), needle optional;
- calibrated eye-dropper;
- ND vaccine
- coolbox and ice pack or damp cloth and basket;
- record book and pencil; and
- chicken marker-leg band, wing tag, coloured thread or cord etc.

Indicators of success to be used by CLWs to evaluate their work:

- an increase in the number of chickens per family/household;
- farmers continue to participate in subsequent vaccination campaigns;
- new farmers present their chickens for vaccination at each campaign; and
- payment received from farmers for the vaccination of their chickens is sufficient to buy vaccine for the following campaign and to cover any transport or labour costs involved.

Appendix 5: Comparison of some strains of Newcastle disease virus

Strain designation	Conventional classification ^a	Use	Mean death time ^b	ICPI ^c	IVPI ^d	k ^e	Virulence sequence ^f
I-2	Avirulent	Village vaccine	>150	0	0	NR ^g	No
V4	Avirulent	Village and commercial vaccine	>150	0.16	0	0.23	No
Hitchner B1	Lentogenic	Commercial vaccine	120	0.20	0	NR	No
La Sota	Lentogenic	Commercial vaccine	103	0.40	0	2.08	No
Komarov	Mesogenic	Commercial vaccine	69	1.41	0	NR	Yes
Mukteswar	Mesogenic	Commercial vaccine	46	1.40	0	NR	Yes
Herts 33/56	Velogenic	Challenge strain	48	1.88	2.7	0.86	Yes

^a These useful terms are no longer used by OIE

^b Time in hours for a minimal lethal dose to kill chicken embryos

^c Intracerebral pathogenicity index, ranging from 0 (least pathogenic) to 2 (most pathogenic)

^d Intravenous pathogenicity index, ranging from 0 (least pathogenic) to 3 (most pathogenic)

^e Rate constant of thermostability of infectivity at 56°C. Lower figures indicate greater heat stability

^f The amino acid sequence ¹¹²RRQR(orK)RF¹¹⁷ at the cleavage site of the F protein

^g Not recorded

Data derived from various sources:

- Alexander, D.J. 1997. Newcastle disease and other avian paramyxoviridae infections. In: Diseases of Poultry, 10th Ed., B.W. Calnek (ed.) London, Mosby-Wolfe, 541–569.
- Alexander, D.J. and Allan, W.H. 1973. Newcastle disease. The nature of the virus strains. Bulletin of the Office International des Epizooties, 79: 15–26.
- Collins, M.S., Bashiruddin, J.B. and Alexander, D.J. 1993. Deduced amino acid sequences at the fusion protein cleavage site of Newcastle disease viruses showing variations in antigenicity and pathogenicity. Archives of Virology, 128: 363–370.
- Ideris, I. 1989. Vaccination of Village Chickens Against Newcastle Disease. Ph.D. Thesis. Universiti Pertanian Malaysia. Serdang, Malaysia. 281 p.
- Lomniczi, B. 1975. Thermostability of Newcastle disease virus strains of different virulence. Archives of Virology, 47: 249–255.
- Ru, M. (pers. comm. 1999)
- Wambura, P. (pers. comm. 2000)

Appendix 6: Questions and answers

Q: Why village chickens?

A: Village chickens are important because of their high numbers and their wide ownership by rural folk. They provide an important source of protein for families and they can be sold to generate funds.

Q: Why Newcastle disease?

A: It is the major problem identified by village chicken farmers and it can kill almost all susceptible chickens during an outbreak.

Q: Why the need to vaccinate?

A: The only way to control the disease is to vaccinate because there is no known treatment.

Q: Is vaccination free?

A: No, there will be a small charge per chicken.

Q: Can chickens be eaten or sold after vaccination?

A: Yes.

Q: How many times must chickens be vaccinated?

A: It depends on the route of administration:

- eye drop: every 3 to 4 months;
- drinking water or suitable food: two initial vaccinations, then every 3 months.



Q: Will the vaccination harm chickens?

A: No, the NDV4-HR and I-2 vaccines will not harm the chickens but the birds need to be handled gently to avoid stress.

Q: Will the vaccine protect all chickens against all diseases?

A: No, the vaccine is only to prevent ND.

Q: Will all the chickens survive ND after vaccination?

A: It is not possible to guarantee 100% protection because small numbers of chickens may not respond to the vaccine especially if they are malnourished.

Q: Will the NDV4-HR and I-2 vaccines harm humans?

A: No, there are no reported cases of the vaccine harming humans.

Q: Can farmers use the vaccine by themselves?

A: Yes, after receiving basic training.



Appendix 7: Suggestions for a village poultry questionnaire

Date:

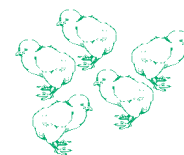
Name of interviewer:

Number of questionnaire:

Introduction: Village poultry are an important part of rural life in [name of country or region]. In order to assist village poultry farmers increase their production, we would like to know more about the major problems associated with raising village poultry. The responses given by farmers to this questionnaire will assist the Livestock Services Division to prepare an assistance package.

1. Livestock kept:

Species	Number	Where did you get them? What breed?	Who takes care of them?
Chickens			
Ducks			
Pigs			
Goats			
Sheep			
Rabbits			
Guinea pigs			
Cattle			
Other — what?			



2. How many chickens do you have?

Category	Number
Adult females	
Adult males	
Growers	
Chicks	

3. When was the last time that your animals received treatment?

What was the treatment?

Who gave the treatment?

4. What are the tasks associated with keeping village chickens and who is responsible for doing them?

Person	Tasks (give water/food, build the chicken house, etc)
Husband	
Wife	
Son(s)	
Daughter(s)	
Other — who?	

5. Who owns the chickens? (Mark the correct response with an "X")

Husband	
Wife	
Son(s)	
Daughter(s)	
Other — who?	

6. Daily routine for village chickens:

Activity(ies)	Time/Frequency	Who is responsible?
Shut the chickens in at night		
Let chickens out in morning		
Cleaning the chicken house		
Give water		
Give food		
Other — what?		

7. Where do your chickens sleep?

Location	Who made it? With what materials
Tree	
Chicken house on the ground	
Elevated chicken house	
Other — where?	

Parameter	Number
8. How many eggs on average does a hen lay per clutch?	
9. How many eggs on average hatch per clutch?	
10. How many chicks on average survive the first two months?	
11. At what age do chickens first lay eggs?	

12. Are you satisfied with the production of your village chickens?

Yes/No (circle the correct response)

Why? Do you

	Chicken	Eggs
Sell		
Eat regularly		
Use for ceremonies		
Other, what?		

13. What do you do with the chickens and eggs?

	Chicken	Egg
Never sell		
Sell, for how much?		
Exchange for other products — what?		
Where do you sell them?		

14. How many chickens and eggs has your family eaten over the last month?
Who ate them?

	No. eaten	Eaten by whom?
Chickens		
Eggs		

15. When and why do you sell your chickens and eggs?

16. How many chickens and eggs have you sold in the last six months?

17. In your opinion, what are the main causes of chicken mortality?

Birds of prey	
Cats and dogs	
Wild mammals	
Theft	
Accidents	
Lack of feed	
Diseases	

18. How many of your birds have died in the last six months?

	From disease	Slaughter	Other causes
	Chicks Adults	Chicks Adults	Chicks Adults
Chickens			
Ducks			
Other — what?			

19. What do you do with your chickens when they are sick?

Eat them	
Sell them	
Treat them	
Other — what?	

20. What treatment do you give your birds? How do you prepare the treatment?

	Conventional	Traditional
Treatment		
How to prepare and administer		

21. Where do you get this treatment from?

Veterinary Services		Traditional healer	
Pharmacy		NGO/Project	
Shop/market		Other — where?	

22. What type of food do you give your chickens?

Type of food	Frequency	Time of year
Nothing		
Maize		
Rice		
Food scraps — what?		
Wheat		
Maize bran		
Other — what?		

23. Do you give water to your birds? Yes/No. If yes, where does the water come from? What type of container do you put the water in?

Water source		Container	
Borehole		Plastic bowl	
Well		Metal bowl	
River/stream		Ceramic bowl	
Used		Tin	
Rainwater		Bamboo trough	
Other		Other	

24. What is the local name for Newcastle disease?

25. Can you describe the symptoms of Newcastle disease?

26. In which month(s) of the year is Newcastle disease more likely to occur? Place an X in the box next to the appropriate month(s).

January		July	
February		August	
March		September	
April		October	
May		November	
June		December	

27. Did you know that there are vaccines that can prevent Newcastle disease?
Yes/No

28. Have you already participated in a Newcastle disease vaccination campaign?
Yes/No

29. If yes, what were the results?

30. Other comments?

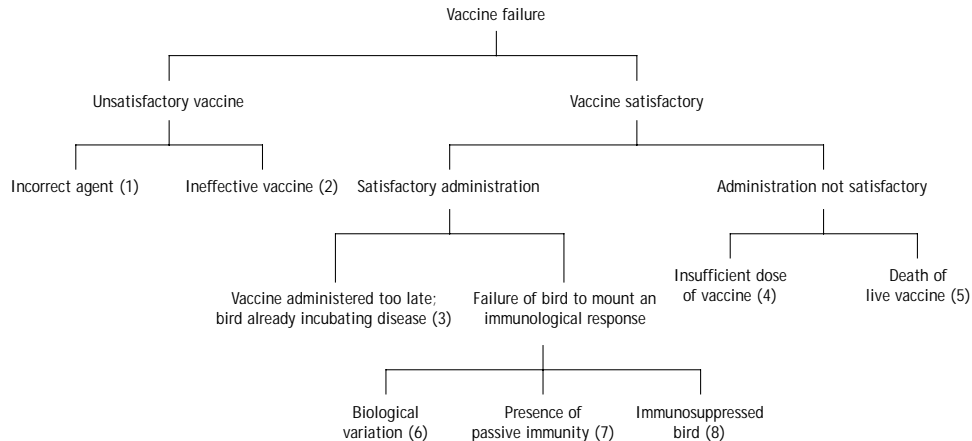
31. Personal details:

Name:	Village:
District:	Province:
Male/Female:	Age:
Ethnic group:	Local languages:
Who is the head of your family:	

Thank you for answering this questionnaire. Your comments will be of great assistance to the Veterinary Services Department in its preparation of a program to improve village chicken production.



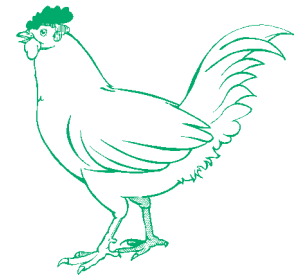
Appendix 8: Reasons for vaccine failure



Adapted from Tizard, I. 1987. *Veterinary Immunology: An Introduction*. London, W.B. Saunders.

For ND vaccines:

1. This is unlikely with ND vaccines due to the lack of antigenic variation between strains. Mislabelling of the vaccine is also unlikely.
2. Counterfeit vaccine.
3. Vaccinating in the face of an outbreak.
4. Eye-dropper not correctly calibrated; bird did not drink sufficient water, reduction of vaccine titre because of inadequate storage.
5. Improper vaccine conservation — exposed to sunlight; exposed to extremely high temperatures during transport or storage; held outside the cold chain beyond the recommended period. Vaccine mixed with an inappropriate food carrier, e.g. maize.
6. A very small percentage of birds will not mount an adequate immune response post vaccination.
7. Chicks up to the age of three weeks may have passive immunity to ND that will interfere with vaccination.
8. Malnourished bird; infection with immunosuppressive diseases such as Infectious Bursal Disease; certain parasitic infestations.



Appendix 9: The design and implementation of vaccine trials

Although the efficacy of thermostable ND vaccines is now proven, some occasions may still arise when vaccine trials need to be done. The objectives of the trials should be determined and the necessary preparations undertaken to ensure that the funds invested in the trials result in a successful outcome. The trial must be designed in accordance with the objectives. More information concerning laboratory techniques may be found in the companion ACIAR manual on the small-scale production of thermostable ND vaccine or via the website listed on the last page of this manual.

Pre-check

Materials:

Laboratory equipment;

Laboratory reagents, e.g. phosphate buffered saline, anticoagulant, etc.;

Reliable electricity supply (working back-up generator);

Funds for: — chickens, brooder, chicken house, feeder;
 — waterer and rations
 — personnel (including overtime for weekend work)
 — additional equipment
 — office supplies
 — vaccine

Trained personnel — veterinary and support staff.

Designing the experiment

Routes of administration of the vaccine should correspond with what is feasible in the field:

- chicken housing-ease of access to vaccinate chickens individually;
- possible food carriers-not recommended but some may want to try;
- water sources-options for administering vaccine via drinking water, availability of surface water, and for dilution of the vaccine;
- economic issues-how many times a year are farmers willing to pay for the vaccine.



Vaccine Laboratory Trials

Possible objectives

- To compare the levels of protection afforded by different routes of administration and different administration regimes.
- To determine the potency of a new batch of vaccine.
- To train laboratory staff.
- To investigate possible foods suitable for use as carriers for the vaccine.
- To confirm that the vaccine strain of the ND virus provides protection against local strains (this is not a priority, as to date all vaccine ND virus strains protect against all field strains).

Materials:

- day old chicks (minimum of 10 per group, best to order in excess of final needs);
- balanced ration;
- chicken house, with brooder, feeders and waterers
 - capable of separating treatment and control groups (best kept in separate houses with a different animal attendant looking after the control group to decrease the risk of the vaccine virus spreading to the control birds)
 - chickens kept on litter not in cages;
- wing tags;
- vaccine;
- ND virus challenge strain (if challenge is to be done; not necessary if antibody titres only are being used to monitor response to vaccination);
- standard positive and negative sera;
- blood collection equipment: syringes, needles, Eppendorf tubes and cryotubes with screw tops;

- laboratory equipment: pipettors, pipette tips, reagents, reagent troughs, egg incubators, centrifuge, centrifuge tubes, microtitre plates (96 well, V bottom), antibiotics, gelatin, skim milk powder, fertile eggs, candling lamps, 70% alcohol, etc; and
- record books and pens.

Experimental protocol

One possible design for a vaccine laboratory trial where only one vaccine and one route of administration is involved is shown in Figure 20.

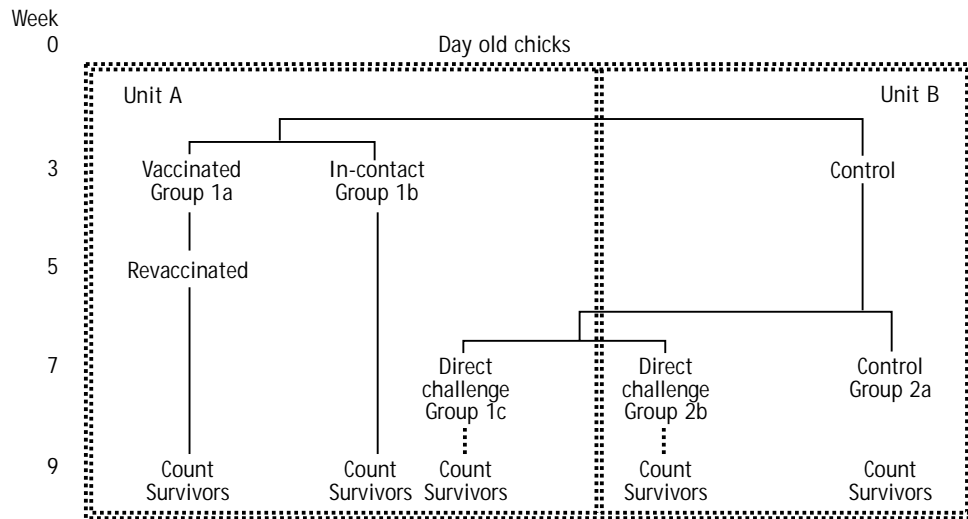


Figure 20: Design of laboratory trials with live ND vaccine. The double lines indicate the housing of the groups in two different units.

- Day 1** (i) place chicks in brooder
- Day 21** (i) allocate chicks randomly to experimental groups (equal numbers per group) with surplus chicks going to control group;
(3 wks) (ii) place groups into experimental chicken house with control group in a separate building if possible;
(iii) tag chickens and collect serum sample from each;

- (iv) vaccinate test groups, record vaccine batch number and expiry date
 - food carrier
 - drinking water
 - eye drop
- (v) the inclusion of 'in-contact' chickens with vaccinated chickens is optional (i.e. unvaccinated chickens housed together with vaccinated chickens to check for horizontal spread of vaccine virus).

Day 35 (i) collect serum samples from all chickens;
(5 wks) (ii) repeat vaccinations.

Day 49 (i) collect serum samples from all chickens (experiment may end here
(7 wks) if no challenge is to be performed or serum antibody levels may be monitored over time to determine rate of decline of antibodies)
(ii) introduce challenge strain by contact with inoculated control chickens (if challenge is to be performed)

Day 63 (i) collect serum samples from all surviving chickens
(9 wks)

Post trial (i) haemagglutination inhibition (HI) tests performed on all sera [using 4 HA units];
(ii) calculate geometric mean titres for each group; and
(iii) store sera.

At the end you should know:

- antibody titre after a single vaccination;
- antibody titre after a second vaccination;
- antibody titre that indicates resistance to challenge;
- antibody titres that will indicate chickens that have survived challenge in the field; and
- level of protection that can be expected from various vaccination regimes.

Vaccine field trial

Possible objectives:

- to confirm that the vaccine is effective under local field conditions;
- to compare different routes of administration under local field conditions;
- to determine the best intervals for re-vaccination in the trial site; and
- to train field and laboratory staff.

Personnel: Partners in trial

Village chicken farmers (female and male), village headman, local assistant, Veterinary Services staff, Extension Services staff, Laboratory staff.

Materials:

- hard cover record book for each trial site, pens, pencils;
- trial ND vaccine;
- wing tags;
- record sheets/books for records of farmers and tagged chickens;
- syringes, needles, racks, cotton wool, alcohol;
- jars with 10% formalin, jars with 50% glycerine;
- eye-droppers;
- serum tubes, Eppendorf tubes;
- permanent markers or adhesive tape and pen; and
- coolbox and icepack for transport of sera.

Methods

i) Begin extension activities in the area well before you intend to commence the trial.

- Meet with Village Headman and village representatives to discuss the objectives of the project and to seek their cooperation. The participation of female village poultry farmers is to be encouraged.

- Ensure that villagers understand that it is a trial that is being done and that results cannot be guaranteed.
- Discuss the need to tag birds and take monthly blood samples, and the reasons for this activity.
- Explain that the vaccine will not produce 100% protection, and that the vaccine will protect against ND only.
- Check that the village chicken population is not experiencing a disease outbreak.

ii) Should the community agree to participate, select a local assistant to help with record keeping. Provide training for the local assistant and for those who wish to perform the vaccination of the birds.

iii) Allocate treatment groups to different farmer groups using a lottery draw conducted at a community meeting where representatives of all groups are present. Aim to have a minimum of 200 birds per treatment group.

iv) Decide whether or not to inform farmers which group is the control group. If farmers are not to be informed, then a mock vaccination should be undertaken in order to perform the blind trial.

v) Ensure that you allocate sufficient time to field activities, especially when starting new trials. It may be best to allow one day per treatment group initially as this would enable you to meet the farmers and work on the birds at a time convenient to the farmers.

vi) Make detailed records of:

- community meetings-comments, decisions taken, who has agreed to do what, who was present, etc.;
- vaccine usage-vaccine batch, expiry date and number of doses in the vial; how the vaccine was diluted (type of diluent used, volume of diluent, quantity of grain if used); administration route; vaccinator (Veterinary Services Department staff, farmers, etc.), vaccination dates; and
- participating farmers, number of chickens owned per farmer and tagged birds using the record sheets; endeavour to tag at least 50 birds per treatment group or 10% of birds, whichever is the greater number.

vii) Take baseline serum samples from tagged birds immediately prior to the first vaccination.

viii) Meet regularly with local trial assistant to help with any problems that may have arisen; check record books to ensure that all is in order.

ix) Ensure feedback of results to participating farmers. Results of HI tests (serum antibody titres indicating the level of protection against ND) should be discussed with farmers (Table 6).

Table 6: The immune status of birds to ND based on HI titres expressed as Log₂.

Immune status	HI titre (Log ₂)
Bird not protected	0–2
Bird protected	3 or more

Use visual means to convey results if necessary, e.g. to compare the HI titre of birds or different treatment groups, you can use stones, leaves, maize cobs, etc to represent one unit (Log₂). For example, one month after vaccination, the geometric mean titre (GMT) for the different treatment groups may be demonstrated by drawing on the ground with a stick to create rows for each group and indicating the level of protection by the number of stones:

Control	●
Eye drop	● ● ● ● ●
Drinking water	● ● ●
Food	● ●

x) Conduct regular community meetings. Farmers should be involved in the monitoring and evaluation of a trial. A number of indicators may be used to assess the impact of the trial, e.g. changes in flock mortality, percentage of chicks reared, changes in the number of eggs laid and their hatchability, changes in flock size, occurrence of recorded disease outbreaks, sales of chickens and eggs, changes in household income levels, demand for ND vaccine, and changes in livestock ownership patterns.

xi) Benefit:cost aspects of each of the treatment groups should be included in the community discussions as farmers will be expected to pay for further ND vaccine after the end of the trial.

xii) Always ensure that compensation of participating farmers has been discussed prior to the commencement of a trial.

xiii) Collection of post-mortem samples. It is advisable to collect samples from a representative number of birds dying in the trial area. Sample collection is detailed in Section 4 of this manual. Collect samples in formalin for histopathology and in glycerine for virus isolation if you are expecting delays in the delivery of samples to the central laboratory. It is often necessary to negotiate with farmers to obtain the samples you need.

xiv) The length of the trial will depend on the objectives. If the trial is to confirm that the vaccine is effective under field conditions, then the trial must run until there is a natural outbreak of ND. Alternatively, researchers could buy a minimum of ten birds from each treatment group, two to three months after vaccination, and take them back to the laboratory for challenge with virulent virus. If the trial seeks to determine the best intervals for re-vaccination in the area, then serology must be performed on identified birds at monthly intervals for at least one year.



Appendix 10: Sources of further information

Professor Peter Spradbrow
 Division of Veterinary Pathology and Anatomy
 The University of Queensland
 PO Box 125
 Kenmore Q 4069
 Australia
 Tel: +61-7-3365 5738
 Fax: +61-7-3365 5600
 E-mail: vppsprad@mailbox.uq.edu.au

Dr John Copland
 The Australian Centre for International Agricultural Research
 GPO Box 1571
 Canberra ACT 2601
 Australia
 Tel: +61-2-6217 0500
 Fax: +61-2-6217 0501
 E-mail: aciar@aciar.gov.au or copland@aciar.gov.au

Dr Robyn Alders
 National Veterinary Research Institute
 C.P. 1922
 Maputo
 Mozambique
 Tel: +258-1-475171
 Fax: +258-1-475172
 E-mail: robyn@tropical.co.mz or robyn_alders@yahoo.co.uk

The International Network for Family Poultry Development
 c/o Professor Funso Sonaiya
 Department of Animal Science
 Obafemi Awolowa University
 Ile-Ife, Nigeria
 E-mail: fsonaiya@oauife.edu.ng
<http://www.fao.org/ag/aga/agap/lpa/fampo1/fampo.htm>
 Improvements in Rural Poultry in Developing Countries Website
<http://www.vsap.uq.edu.au/RuralPoultry>

