Department of Primary Industries

Procedure

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AVIAN INFLUENZA GENERIC SURVEILLANCE PROCEDURE

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AUTHORISED BY (DPI) A/Group Director Animal Biosecurity and Welfare	AUTHORISED DATE 15 March 2016
AUTHORISED BY (LLS) Executive Manager LLS	AUTHORISED DATE 15 March 2016
ISSUED BY Primary Industries, Biosecurity NSW	

REVISION HISTORY

VERSION	DATE	AMENDMENTS	
		SECTION	DETAILS
1	15/03/16	New procedure	

NEXT REVIEW DATE: 15/03/18

Purpose

The purpose of this procedure is to define the surveillance actions that are to be undertaken in the event of an outbreak of highly pathogenic avian influenza (HPAI) or low pathogenic avian influenza (LPAI) H5/H7 in New South Wales (NSW).

Scope

This procedure applies to staff of NSW Department of Primary Industries (DPI) and to staff of Local Land Services (LLS) in their role as Stock Inspectors enforcing the *Stock Diseases Act 1923* and *Animal Diseases and Animal Pests (Emergency Outbreaks) Act 1991*.

Warnings

The Work Health and Safety Act 2011 (WHS Act) places an obligation on the agency (Department of Industry/LLS) as a Person Conducting a Business or Undertaking (PCBU) and workers to provide a safe and healthy workplace. Safe Work Method Statements (SWMS) that support activities included in this policy must be used in identifying, assessing and controlling risks.

Table of Contents

1. PUBLIC HEALTH IMPLICATIONS

All avian influenza (AI) viruses have the potential to infect people. Appropriate workplace health and safety (WHS) measures, in line with known or theoretical risk, must be implemented with advice from Health Protection NSW.

Workers must be protected from infection with AI viruses wherever they have contact with infected poultry, products and premises. Protection includes vaccination with the currently available seasonal influenza vaccine and wearing appropriate personal protective equipment (PPE), in accordance with the national WHS guidelines for AI.

Workers involved in eradication activities will require training and supervision to ensure that all activities are managed appropriately with regard to WHS. Workers showing symptoms consistent with influenza must not come into contact with birds.

The agency and workers have a responsibility to provide a safe and healthy workplace for non-government workers (e.g. poultry owners, poultry workers, private veterinarians, contractors) delegated to perform tasks during an emergency animal disease (EAD) response. Prompt access to influenza vaccination must be offered to unvaccinated non-government workers.

2. OVERVIEW

The purpose of surveillance in the initial phase of an outbreak of AI is to identify the source of infection, determine the extent of spread and assess the impact of control activities. Later in the outbreak, the purpose is to give confidence that the true extent of infection has been determined leading to proof of freedom (POF) of disease and the lifting of quarantine restrictions.

Highly pathogenic avian influenza

In line with the AUSVETPLAN Disease Strategy Avian Influenza, active surveillance should be initiated as soon as HPAI is suspected. Initially, the location of all commercial and backyard poultry, zoo birds and large aggregations of caged birds, and pigs in the Restricted Area (RA), and all commercial and large aggregations of caged birds in the Control Area (CA) should be identified and mapped. A sample of birds of any domestic species that die in the RA should be investigated for AI, with specimens submitted to the State Veterinary Diagnostic Laboratory (SVDL) for virus isolation.

Intensive surveillance aims to identify potential new cases of AI. Due to the risk of spread of the AI virus by workers, equipment and vehicles measures should be adopted to enable continuing surveillance while minimising multiple visits by Inspectors and other authorised workers to premises in the RA and CA.

Measures should include:

- monitoring of dead bird pick-ups (laboratory submissions for suspicious cases);
- health reporting by telephone, fax, email or text messages;
- telephone surveys; and
- serological and/or virological sampling.

Field investigation visits can then be arranged to any potential new cases identified.

Surveillance of free-range poultry flocks of chickens, turkeys, ostriches and ducks in the CA, will serve as effective sentinels for the passage of AI viruses from waterfowl to poultry.

All reports of a decline in the health of birds (e.g. decrease in feed or water consumption, decrease or cessation in egg production, increase in mortality/morbidity, depression, respiratory disease) should be investigated.

Note: health status changes should not be expected in most instances in domestic ducks, geese, emus or ostriches. The trigger for investigations in these flocks should not be the reporting of clinical signs or mortality.

Note: waterfowl generally have a shorter shedding period for HPAI viruses compared to LPAI viruses.

Low pathogenic avian influenza (H5/H7)

In line with the AUSVETPLAN Disease Strategy Avian Influenza, if LPAI H5/H7 is detected in NSW poultry flocks, or in caged or zoo birds, the following risk factors will need to be assessed prior to a control/eradication and surveillance program being implemented:

- the species involved;
- the nature and severity of clinical disease;
- whether the AI virus produces clinical disease or infection in humans;
- the rapidity with which the AI virus is spreading within or between flocks;
- the proximity to commercial poultry or other significant avian establishments;
- the density of bird populations, especially poultry, in the area of the outbreak;
- the possibility of spread to other areas;
- the possibility of a mixed population of AI viruses being present, with apparent LPAI (H5/H7) viruses masking sub-populations of HPAI viruses;
- the impact that the disease will have on the marketing of poultry products;
- the possibility of creating a vaccination zone and process slaughtering of infected poultry flocks when major disease control activities can be undertaken, such as in the favourable, low-spread, summer months; and
- the costs and impacts of alternative response options.

Note: the appearance of clinical signs as a trigger for investigations is unlikely to be effective even in species that are clinically susceptible because infection may not produce clinical signs or only very mild clinical signs in poultry.

Note: most chickens excrete LPAI viruses for a week; a minority of chickens excrete LPAI viruses for up to 2 weeks.

2.1. Surveillance priorities

The priority risk premises include:

- commercial poultry premises and large aggregations of caged birds within the RA and CA;
- premises involved in the potential movement of fomites or poultry products from Infected Premises (IPs); and
- backyard poultry and zoo birds premises in the RA.

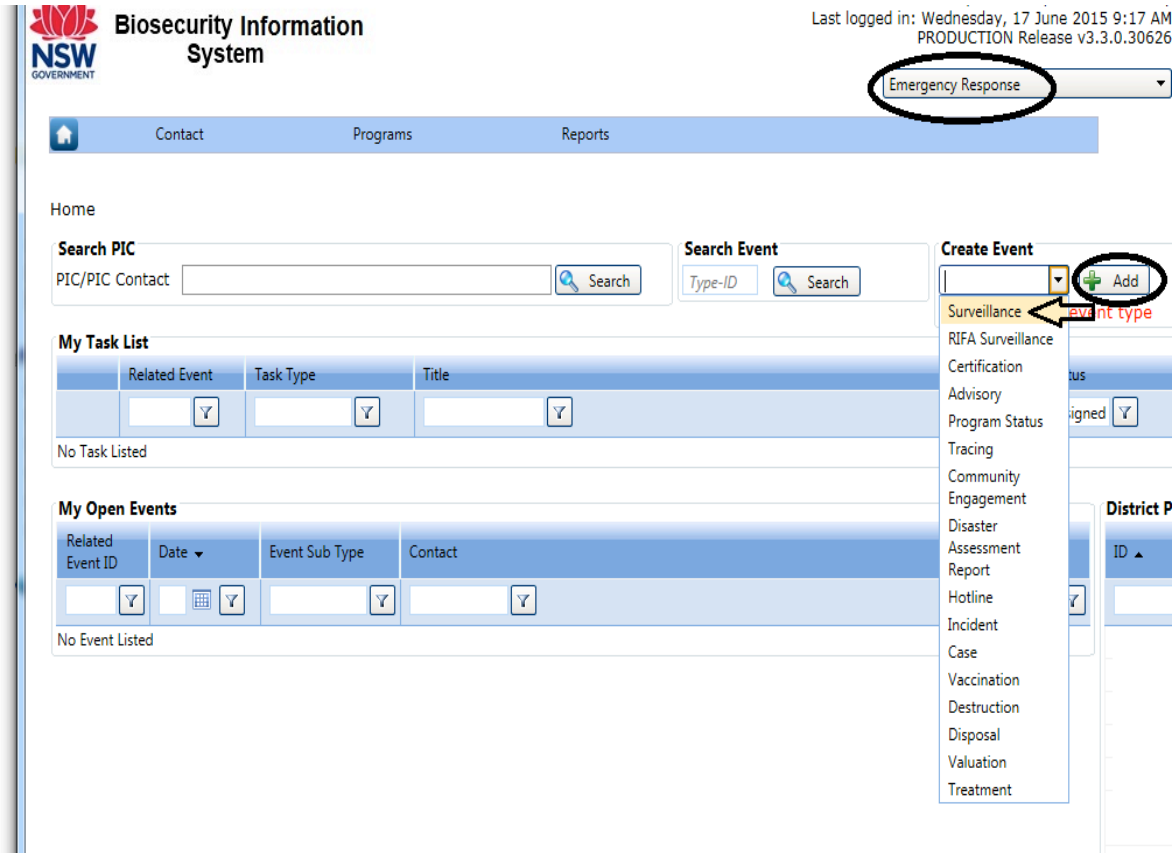
Priority premises for surveillance are, in order, premises in the same ownership as IPs, Suspect Premises (SPs), Dangerous Contact Premises (DCPs), Trace Premises (TPs), At-Risk Premises (ARPs) within the RA, Premises of Relevance (POR) within the CA, and selected wild duck or other wild bird populations in contact with poultry populations in the RA and CA.

2.2. Information management

Laboratory reporting should occur through Sample Manager. Veterinary Investigations (VI) should request that they are added to the laboratory report distribution list if they do not have access to Sample Manager.

The Livestock Health Management System (LHMS) should be used to record information collected on each property and to produce response statistics, surveillance and tracing schedules. Each risk premises in the RA, CA or OA, where necessary, will have a status consistent with the AUSVETPLAN guidance document for declared areas and allocation of premises classifications in an EAD response.

Surveillance data for the first visit should be added as a **Surveillance Event**. Record as much information as possible for each of the tabs under 'adding a surveillance event' to the LHMS emergency response component, as shown in the two images below.



A SO should contact the owner or person-in-charge of each stocked at-risk TP in the OA to arrange:

- daily health monitoring for 21 days after the last trace contact; and
- tracheal and cloacal swabs for PCR and blood sampling as soon as practicable after the trace is identified and 21 days after the last trace contact.

Consideration should be given to the size and type of bird before a decision is made as to whether or not tracheal and/or cloacal swabs for PCR testing are the appropriate method of sample collection. It may be necessary to collect an appropriate level of environmental samples i.e. faecal samples. The timing of sample collection should be based on species and AI virus pathogenicity.

All pigs should be monitored for disease consistent with influenza (e.g. high morbidity, low mortality, coughing, watery nasal discharge, laboured breathing, inappetence, fever, depression, stiffness). Testing will be necessary on any pigs showing clinical signs consistent with influenza infection.

Once negative results are available, the premises should be qualified as TPAN1 in LHMS. When monitoring and surveillance is complete the TP status is resolved.

Premises that have tested negative should be classified as Negatively Assessed (NA) with an appropriate qualifier.

Any report of disease or increased mortality should be investigated immediately.

3.3.2. Other premises

There are no special surveillance requirements for non-traced premises.

3.4. Health monitoring

3.4.1. Poultry

Health monitoring refers to the routine monitoring and reporting of the health of poultry flocks by the owner or person-in-charge of the premises.

A reporting procedure that includes the following observations should be established:

1. *Review of records and interviews of owners/persons-in-charge for the following:*
    - a sudden increase in deaths
    - a sudden decline in feed and/or water consumption
    - unusually quiet birds
    - unusually depressed birds
    - any decline in egg production from normal to cessation or the sudden appearance of eggs without shells or pale shell eggs
    - any birds with swollen heads/combs/wattles
    - any birds with nervous signs e.g. head shaking, head and neck tremors
    - abnormal position of head and neck in a reasonable % of birds
    - respiratory disease e.g. breathing difficulties, coughing, sneezing
    - watery diarrhoea
    - purplish patches on the legs and unfeathered skin

Mortality and production data should be provided to the epidemiology unit if suspicion of infection becomes apparent.

2. *Field autopsy findings, which include any of the following:*
    - severe swelling of combs and wattles
    - cyanosis of the comb
    - haemorrhage and necrosis of the comb
    - peri-orbital oedema
    - swelling of the shanks and feet
    - petechial haemorrhages on the viscera
    - catarrhal tracheitis
    - tracheal oedema
    - petechial tracheal haemorrhages
    - caseous tracheal exudate

The owner or person-in-charge should email, fax or text this information daily or provide a verbal report according to the schedule defined by the SO monitoring the premises.

3.4.2. Pigs

Health monitoring refers to the routine monitoring and reporting of the health of pigs by the owner or person-in-charge of the premises.

A reporting procedure that includes the following observations should be established:

1. *Review of records and interviews of owners/persons-in-charge for the following:*
    - any decline in feed and/or water consumption
    - morbidity rates and the relationship of the daily figures to normal
    - respiratory disease e.g. sneezing, coughing, nasal discharge, laboured breathing
    - unusually depressed pigs
    - stiff gait in a reasonable % of pigs
    - fever

The owner or person-in-charge should email, fax or text this information daily or provide a verbal report according to the schedule defined by the SO monitoring the premises.

3.5. Status qualifiers

A qualifier may be applied to premises previously defined as DCP, SP, TP or ARP that have been cleared of suspicion at the time of designation. It is a description to document progress in the response and in the POF phase.

4. SAMPLING PROCEDURES

4.1. Personal health and safety

The agency is responsible for providing instructions in the use of appropriate PPE to non-government personnel tasked with sampling birds as per these procedures.

Non-government personnel will be responsible for supplying their own PPE.

DPI and LLS staff who sample suspect birds and provide instruction to others must be trained and assessed as competent in the use of PPE and comply with the protocols in the policy Health and safety precautions for investigating zoonotic diseases and procedure Use of PPE.

4.2. Sample size and selection

Sample sizes for barn/litter and caged bird premises are outlined in the table below:

	Sample size (per shed*)	
	Assumptions for barn/litter birds : Sensitivity 98% Specificity 98% Design prevalence 15%	Assumptions for caged birds : Sensitivity 98% Specificity 98% Design prevalence 10%
Population per shed	***Sample size per shed***	***Sample size per shed***
≤ 40	All	All
41-60	31	41
61-100	33	55
101-200	35	60
> 200	40	64

*For surveillance purposes, sheds are to be treated as separate epidemiological units. Thus, a barn/litter bird premises with 10 sheds each with 10 000 birds would require 400 birds to be sampled.

On the day of sampling, collect birds that are recently dead or sick, but if there are insufficient numbers of these birds sample other live birds to make the required sample size. These live birds need to be collected from different parts of the shed.

The lower design prevalence (10%) is used for caged birds due to the lower 'within-shed' contact rates.

See Appendix 1 for a simplified version of sampling numbers and techniques for people in the field.

4.3. Sample type, collection and dispatch

4.3.1. Tracheal and cloacal swabs

For diagnostic investigations, sample 10 to 15 birds per shed targeting, where possible, sick or recently dead birds. For surveillance purposes, the sample size should be as per 4.2.

Collect both tracheal and cloacal swabs in PBGS transport medium, which can be obtained from the virology laboratory or the specimen receival and despatch unit at EMAI. These swabs should be kept separate and labelled in a manner that will link them to the individual bird e.g. 1T, 1C. Shed identification should also be added (e.g. S1T1 or S1C1 for a tracheal or cloacal swab from the first bird in shed 1) unless the samples are packed into separate secondary containers with labelling for the shed.

Complete the specimen advice form and ensure samples are labelled and packaged such that the shed and premises for each sample is clear. Ensure there is suitable coolant included. Samples should be kept cool, but not frozen.

Submit samples to the virology laboratory at EMAI.

Sample collection routines should be managed with the aim that samples are collected, dispatched and tested within a working day.

Sample types may be varied according to advice from the epidemiology unit or the virology laboratory depending on local circumstances.

4.3.2. Blood samples

Blood samples should be collected aseptically. Contamination of the container and stopper should be avoided. Blood and faecal matter should be removed prior to despatch, to reduce the risk of contamination of laboratory staff handling the specimens.

Use a separate sterile needle to avoid mechanically transmitting infectious agents from one bird to another.

Haemolysis occurs as a result of poor collection techniques, contaminated equipment or poor handling of the sample once it is collected.

Common causes of haemolysis include:

- use of non-sterile containers for collection or storage
- contamination by faecal and other material due to faulty aseptic techniques
- contamination of the sample by water
- a slow flow from the needle due to obstruction of the needle or failure to insert into mid-vein
- forcibly expelling blood through a needle
- heating of samples, usually in car boots or through back windows of cars, or after prolonged exposure to direct sunlight during collection
- freezing

Samples should be labelled serially (e.g. from 1 to 30) with a waterproof pen, preferably on an adhesive label. Do not label the stopper, which is removed during testing.

Samples should be allowed to clot before transporting them over any distance. Once the clot has retracted, blood samples should be held chilled to reduce contamination, haemolysis and autolysis.

All specimens should be clearly labelled and sent in a leak-proof container. Check that screw caps are tight.

All samples should be packed in insulated containers with sufficient ice bricks to ensure that they are still cold when received at the laboratory. However, care should be taken to prevent direct contact between coolant bricks and specimens, which may otherwise become frozen.

4.3.3. Clinical examination

If health monitoring raises concerns regarding the disease status of birds, a LLS veterinarian +/- an industry-nominated poultry veterinarian must visit the premises +/- conduct autopsies.

When examining health monitoring records, or inspecting birds, note the following:

- a sudden increase in deaths
- a sudden decline in feed and/or water consumption
- unusually quiet birds
- unusually depressed birds
- any decline in egg production from normal to cessation or the sudden appearance of eggs without shells or pale shell eggs
- any birds with swollen heads/combs/wattles

- any birds with flaccid pale combs or very dark combs and wattles
- any birds with nervous signs e.g. head shaking, head and neck tremors
- abnormal position of head and neck in a reasonable % of birds
- respiratory disease e.g. breathing difficulties, coughing, sneezing
- watery diarrhoea
- purplish patches on the legs and unfeathered skin

Mortality and production data should be provided to the epidemiology unit if suspicion of infection becomes apparent.

4.3.4. Post-mortem examination

If autopsies are conducted, note the following:

- petechial haemorrhages on viscera, skin and musculature
- catarrhal tracheitis
- tracheal oedema
- petechial tracheal haemorrhages
- caseous tracheal exudate

Veterinarians should collect a range of samples for virology and histopathology.

5. PREMISES CLASSIFICATION

Classification	Definition	How resolved
Approved Processing Facility (APF)	An abattoir, knackery, milk processing plant or other such facility that maintains increased biosecurity standards	N/A
At-risk Premises (ARP)	A premises in a RA that contains a live susceptible animal(s), but is not considered at the time of classification to be an IP, DCP, DCPF, SP or TP	When a RA is abolished
Dangerous Contact Premises (DCP)	A premises, apart from an abattoir, knackery or milk processing plant (or other such facility) that, after investigation and based on a risk assessment, is considered to contain a susceptible animal(s) not showing clinical signs, but considered highly likely to contain an infected animal(s) and/or contaminated animal products, wastes or things that present an unacceptable risk to the response if the risk is not addressed, and that therefore requires action to address the risk	If the presence of an infected animal(s) or contaminated animal products, wastes or things is confirmed, the premises would be designated as an IP If the presence of an infected animal(s) is not confirmed, but the likelihood is considered to remain high, the premises would continue to be designated as a DCP

Classification	Definition	How resolved
Dangerous Contact Processing Facility (DCPF)	An abattoir, knackery, milk processing plant or other such facility that, based on a risk assessment, appears highly likely to have received infected animals, or contaminated animal products, wastes or things, and that requires action to address the risk	If the presence of infected animals or contaminated animal products, wastes or things is confirmed, the premises would be designated as an IP If the presence of infected animals is not confirmed, but the likelihood is considered to remain high, the premises would continue to be designated as a DCPF
Infected Premises (IP)	A defined area (which may be all or part of a property) on which animals meeting the case definition are or were present, or the causative agent of the EAD is present, or there is reasonable suspicion that either is present, and that the relevant Chief Veterinary Officer or their delegate has declared to be an infected premises	The premises becomes a RP 21 days (or shorter period if agreed by the Consultative Committee for Emergency Animal Diseases) after completion of decontamination following full depopulation
Non-assessed Premises (NAP)	A temporary status for a premises located in the OA where the current presence of a susceptible animal(s) and/or risk products, wastes or things is unknown	Following investigation(s), a NAP that contains a susceptible animal(s) not known to have been exposed to the AI virus, but showing clinical signs similar to the case definition becomes a SP Following investigation(s), a NAP that contains no live susceptible animals or risk products, wastes or things becomes a ZP
Premises of Relevance (POR)	A premises in a CA that contains a live susceptible animal(s), but is not considered at the time of classification to be an IP, DCP, DCPF, SP or TP	When a CA is abolished
Resolved Premises (RP)	An IP, DCP or DCPF that has completed the required control measures and is subject to the procedures and restrictions appropriate to the area in which it is located	Remains a RP until POF testing completed with negative results
Suspect Premises (SP)	A temporary classification of a premises that contains a susceptible animal(s) not known to have been exposed to the disease agent, but showing clinical signs similar to the case definition, and that therefore requires investigation(s)	Following investigation(s), a SP becomes an IP if it meets the case definition or, if not, an ARP if in a RA or a POR if in a CA
Trace Premises (TP)	A temporary classification of a premises that contains susceptible animal(s) that tracing indicates may have been exposed to the disease agent, or contains contaminated animal products, wastes or things, and that requires investigation(s)	Following investigation(s), a TP becomes an IP if it meets the case definition or, if not, an ARP if in a RA or a POR if in a CA At-risk TPs in the OA that have tested negative should

Classification	Definition	How resolved
		be classified as negatively assessed (NA) with an appropriate qualifier. TP status is resolved when monitoring and surveillance is complete
Unknown status Premises (UP)	A premises within a declared area where the current presence of susceptible animals and/or risk products, wastes or things is unknown	Following investigation(s), an UP that contains a live susceptible animal(s), but is not considered at the time of classification to be an IP, DCP, DCPF, SP or TP becomes an ARP if in a RA or a POR if in a CA Following investigation(s), an UP that contains no live susceptible animal(s) becomes a ZP
Zero susceptible species Premises (ZP)	A premises that does not contain any susceptible animals or risk products, wastes or things	If restocked and located in a RA or a CA it becomes an ARP or a POR respectively

6. DEFINITIONS AND ACRONYMS

Approved Processing Plant	A plant designed to render dead bird carcases that has been assessed as low risk, on the basis that it follows approved protocols
Assessed negative (AN)	A qualifier that may be applied to ARP, POR and premises previously defined as SPs, TPs, DCPs or DCPF that have undergone an epidemiological and/or laboratory assessment and have been cleared of suspicion at the time of classification, and can progress to another status
Caged birds	Birds that are confined within an enclosure and maintained for purposes other than food production. Zoo birds are cage birds that are maintained at a zoo premises.
Control Area (CA)	A part of New South Wales that has been declared, pursuant to section 21 of the *Animal Diseases and Animal Pests (Emergency Outbreaks) Act 1991*, to be a control area in relation to avian influenza
Outside Area (OA)	The area of Australia outside the declared (control and restricted) areas
PCR	Polymerase chain reaction
Poultry	For the purposes of these procedures, poultry means one or more chickens, turkeys, guinea fowl, ducks, geese, quails, pigeons, pheasants, partridges, emus, and ostriches
Restricted Area (RA)	A part of New South Wales that has been declared, pursuant to section 15 of the *Animal Diseases and Animal Pests (Emergency Outbreaks) Act 1991*, to be a restricted area in relation to avian influenza
Risk enterprises	Private avian laboratories, cull hen collectors, dead bird pick-ups (but not processing plants)

Sample Manager	Laboratory specimen and test result database

7. LEGISLATION

- *Animal Diseases and Animal Pests (Emergency Outbreaks) Act 1991*
- *Stock Diseases Act 1923*

8. RELATED POLICIES AND PROCEDURES

- AUSVETPLAN Disease Strategy for Avian Influenza
- Policy: Health and Safety precautions for investigating zoonotic diseases
- Procedure: Use of PPE

9. CONTACT

Poultry Health Coordinator on (02) 4640 6308

10. APPENDIX 1: AVIAN INFLUENZA – GUIDE TO SAMPLE COLLECTION

Samples required for virus isolation are from the back of the throat/trachea and the cloaca of the bird. Each bird sampled must have a corresponding set of samples e.g. bird 1 – sample vials labelled T1 and C1.

Birds should be chosen evenly from within the various sheds to be a representative sample across each shed. Samples should either be labelled in a manner that will identify the shed of origin (e.g. S1T1) or packed separately (e.g. separate sealed bag) to samples from other sheds.

Swab sick or dead birds as a priority and then make up the required numbers with live birds.

If collecting samples for diagnostic purposes i.e. suspect AI, and need to prove it, then sample 10 to 15 birds from each shed.

If collecting samples for surveillance purposes in the RA, CA or TP i.e. AI has been proven on IPs and need to monitor for infection, then the following sample numbers are required:

- For sheds with 40 birds, sample all birds/shed (barn/litter and caged)
- For sheds with 41-60 birds, sample 31 birds/shed (barn/litter) or 41 birds/shed (caged)
- For properties with 61-100 birds, sample 33 birds/shed (barn/litter) or 55 birds/shed (caged)
- For sheds with 101-200 birds, sample 35 birds/shed (barn/litter) or 60 birds/shed (caged)
- For sheds with > 200 birds, the maximum sample size required is 40 birds/shed (barn/litter) or 64 birds/shed (caged)

Tracheal sampling procedures

To collect tracheal/throat swab samples, hold the bird's head up in a nearly vertical position with wings and feet restrained.

The bird should face the person swabbing the trachea.

Remove swab from package and handle the swab aseptically at all times (i.e. do not touch the fabric tip or allow it to come into contact with anything else).

Insert tip of the swab into the back of the throat near or into the opening to the trachea (windpipe).

Rotate swab tip against tracheal lining two or three times, and place directly into the liquid transport medium (PBGS) provided.

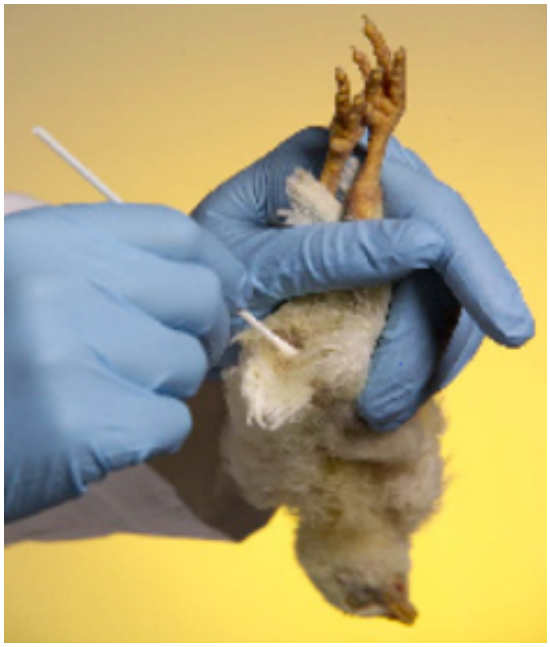
With the swab in the liquid medium, break the stem of the swab off by clamping it under the lid of the vial, leaving the swab tip in the tube. Ensure that the screw top on the vial is fully sealed and that the swab remains immersed in the liquid.

Repeat this procedure until all birds have been swabbed.

Cloacal sampling procedures

- Hold the bird's head down in a nearly vertical position with wings and feet restrained
- Face the bird's vent towards the person swabbing
- Locate and grasp tail feathers at the base and reflect away from you to locate cloaca
- Remove swab from package and handle the swab aseptically at all times (i.e. do not touch the fabric tip or allow it to come into contact with anything else)
- Insert tip into cloacal orifice (1 cm)
- Rotate swab tip against cloacal lining two or three times
- Remove swab, shake off excess faecal material, and place directly into the PBGS provided
- With the swab in the liquid medium, break the stem of the swab off by clamping it under the lid of the vial, leaving the swab in the tube
- Ensure that the screw top on the vial is fully sealed and that the swab remains immersed in the liquid
- Repeat this procedure until all birds have been swabbed

Tracheal swab collection



Cloacal swab collection

Blood collection procedures

The maximum amount of blood that can be safely collected from a clinically healthy bird is 1.5% of its body weight. Less blood should be collected from sick birds.

1. *The large vein under the wing (brachial vein)*
    - Place the bird on a table, setting it on its side
    - Lift up the wing with one hand and part the feathers along the wing. Water can be used to help keep the feathers separated
    - Place the needle at a slight angle, bevel up, against the vein on the underside of the wing. (The bevel is the side of the needle with the angle and the hole)

- Insert the needle into the vein and slowly withdraw blood
- Remove the needle and apply pressure to the vein for a few seconds (This will help to minimise the development of large haematomas, which can be common with poultry)
- Fill the appropriate vial 1/3 to 1/2 of its full volume (Allow the vacuum in the vial to empty the syringe, rather than pushing on the plunger, as this will prevent haemolysis)

2. *The vein on the side of the outstretched neck (jugular vein)*
   - Place the bird on a table, setting it on its side
   - Stretch out the neck with one hand and part the feathers along the neck (The right jugular vein is usually larger)
   - Place the needle at a slight angle, bevel up, against the vein
   - Puncture the vein and slowly withdraw blood
   - Remove the needle and apply pressure to the vein for a few seconds
   - Fill the appropriate vial 1/3 to 1/2 of its full volume

3. *The vein on the inner leg, above the hock (medial metatarsal vein)*
   - Place the bird on a table, setting it on its side
   - Stretch out the leg with one hand and part the feathers along the hock joint
   - Place the needle at a slight angle, bevel up, against the vein
   - Puncture the vein and slowly withdraw blood
   - Remove the syringe and apply pressure to the vein for a few seconds
   - Fill the appropriate vial 1/3 to 1/2 of its full volume

To obtain serum, place the blood vial on a slanted surface for 10 to 15 minutes to allow for clotting. The serum sample can now be spun for centrifugation. Vials containing the blood samples should be refrigerated and sent to the SVDL as soon as possible.