Project: Technical assistance to improve implementation of food safety standards and disease crisis preparedness

A sampling of poultry and wild birds in the case of HPAI

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Date: ……

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How should use the SOP

This SOP will be used by **member staff** of the CDCC, LDCC and EG and all others involved in the implementation of killing measures in affected establishments.

Where? Whom?

1. ‘central disease control centre’ - CDCC
2. ‘local disease control centre(s)’ - LDCC
3. expert group/s – EG, operational on request of the CDCC or LDCC

Definition of avian influenza

- "**avian influenza"** means an infection of poultry or other captive birds caused by any influenza A virus:
  a. of the subtypes H5 or H7; or
  b. with an intravenous pathogenicity index (IVPI) in six-week-old chickens greater than 1.2;
- "**highly pathogenic avian influenza (HPAI)**" means an infection of poultry or other captive birds caused by:
  a. avian influenza viruses of the subtypes H5 or H7 with genome sequences codifying for multiple basic amino acids at the cleavage site of the haemagglutinin molecule similar to that observed for other HPAI viruses, indicating that the haemagglutinin molecule can be cleaved by a host ubiquitous protease; or
  b. avian influenza viruses with an intravenous pathogenicity index in six-week old chickens greater than 1.2;

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The purposes of SOP

The purpose of this document is to provide a guideline for the LDCC and EG ‘officials’ to **ensure the proper identification of samples** to be collected, **correct handling and collecting** of samples, **safe packaging and transport** to the laboratory for diagnostic and **biosecurity measures** that need to be observed in the process.

Carcass

Swaps

Blood

General Guidelines for sample collecting

The establishment of laboratory diagnosis for HPAI is usually a matter of urgency and **samples shall be collected and transmitted without delay and in any case within 12 hours from collection**.

In this regard, the following main factors should be considered.

1. Procedures and guidelines for sample collection
2. Biosecurity during sampling
3. Principles for sample packaging
    a. Packaging
    b. Labelling
4. Storage and transport
5. Pathogenesis, circumstances and type of sample
6. Samples collecting

More detailed information on "**Samples collecting**" will be shared here.

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General overview

- For investigating of disease sampling is required:
  - when disease is suspected
  - to confirm or rule out the disease
  - derogation
  - repopulation
- Sampling can be for purpose of:
  - clinical or
  - laboratory examination

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General observations

- Trained personnel
- Sampling equipment and PPE is provided
- Sufficient (double) samples are collected
- Samples are packed in accordance with relevant standards
- Samples are identified in manner to provide traceability
- Immediate transport is organized
- Cold chain observed
- The laboratory is informed

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General biosafety

- Always sample health then sic animals
- Implement aseptic procedures applicable
- Do not contaminate the sample
- All samples should be considered infected
- Clean and wash the outer surface of the packaging and transporters
- Measures should be as well observed during transport and in laboratory

Packaging

For the packaging the following principles should be observed:

- always conduct the packaging of collected samples in a clean area, away from the sampling site
- the package shall be sturdy to avoid damage and leakage of specimens in case it is crushed
- minimum requirements for the transport of biological material follow the principle of triple packaging:
  - Primary receptacle;
  - Secondary packaging; and
  - Outer packaging.
- at least secondary or outer package shall be sturdy to avoid damage and leakage of specimens in case it is crushed.

Packaging

Primary receptacle

Secondary packaging

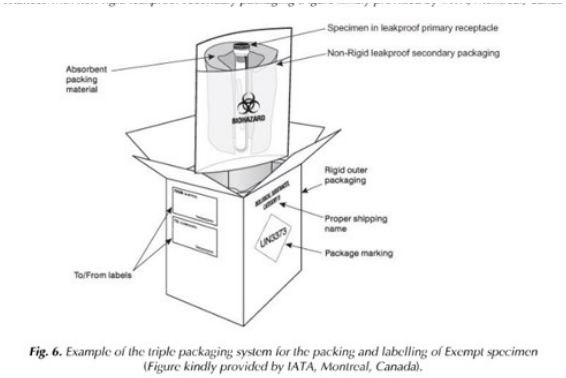
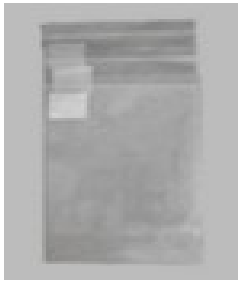
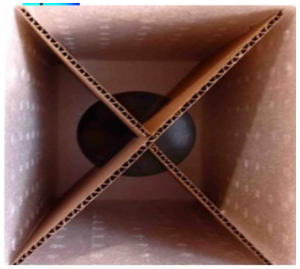


Fig. 6. Example of the triple packaging system for the packing and labelling of Exempt specimen (Figure kindly provided by IATA, Montreal, Canada).





Outer packaging

Labeling and transport

- All samples must be labeled to ensure traceability
- Specific form or minutes
- Plastic bag disinfected
- Dry ice
- Ice packs
- Refrigerated

Type of samples and circumstances

Reason for sampling	Sample	Sample volume and quantity	Minimum number of birds	Diagnostic technique
clinical suspicion (live or dead animal)	<u>Priority:</u> Sick or dead birds	Organs Whole sick bird or carcass	5 birds or carcasses	RT-qPCR RT-PCR Ab ELISA
	Tracheal /orotracheal swab cloacal swab	1 x tracheal/ orotracheal swab 1 x cloacal swab	20 birds	Virus isolation
	Whole blood from the wing vein	1-2 ml of blood	20 birds	
other reasons	Tracheal /orotracheal swab cloacal swab	1 x tracheal/ orotracheal swab 1 x cloacal swab	According to the Instructions on the prevention and elimination of avian influenza or recommendations from CDCC, LDCC	
	Whole blood from the wing vein	1-2 ml of blood		
	Serum	1-2 ml of blood		Competitio n ELISA

Samples of choice for HPAI and LPAI

Sample of choice for detection of:	Blood	Tracheal and cloacal swabs	Respiratory and digestive tract	Internal organs
Virus (HPAI)	2 dpi until death	2 -15 dpi (depending on strain), much longer in ducks and water birds	2-5 dpi (depending on strain) up to 14 dpi if bird recover	
Antibodies (HPAI)*	/	/		
Virus (LPAI)	2 dpi depending on the strain	2 dpi depending on the type		
Antibodies (LPAI)	7-14 dpi and can be detected for several months	/		

*Serology can be valuable for surveillance and demonstrating freedom from infection, but it is not very useful in diagnosing HPAI infections in highly susceptible birds, as they usually die before developing antibodies.

Collecting of blood samples from **domestic birds**

To obtain serum that will be tested for AI antibodies, an anticoagulant is not required and the blood is allowed to clot. Since highly pathogenic AI is an acute and lethal disease for most species, the detection of antibodies will only be helpful if low pathogenicity AI is suspected.

Those who bleed chickens must use a quick and effective technique. This will develop with practice and by applying the following advice.

- Handle the chickens gently
- Collect the blood samples quickly
- Take care not to damage the vein. Damaged veins will result in hematomas being formed
- Minimize the loss of blood

This minimizes trauma to the chickens and stress to their owners. The owners are then more likely to cooperate by supplying chickens for the collection of blood samples in the future.

Collecting of blood samples from **domestic birds**

Wing vein bleeding 1

Procedure:

- ask an assistant to hold the chicken horizontally on its back. The assistant uses one hand to hold the legs and places the other hand under the back to support the chicken
- pull a wing of the chicken out towards you
- note the wing vein, clearly visible running between the biceps and the triceps muscles. The wing vein forms a V (bifurcates). Note the tendon of the pronator muscle that runs across the V
- pluck away any small feathers that obscure the vein
- disinfect the area around the bleeding site by swabbing with 70 percent alcohol

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Collecting of blood samples from **domestic birds**

Wing vein bleeding 2

Procedure:

- insert the needle under the tendon. Direct the needle into the wing vein in the direction of the flow of blood. Do not insert the needle too deeply. Keep clear of the ulnar nerve(Images 1, 2)
- if blood does not flow make a very slight adjustment to reposition the end of the needle
- be patient and use gentle suction to withdraw the blood. Chicken veins collapse readily
- if a hematoma forms, try bleeding from the other wing
- after removing the needle, apply pressure to the vein for a few seconds to discourage further bleeding
- allow collected blood to clot at room temperature.

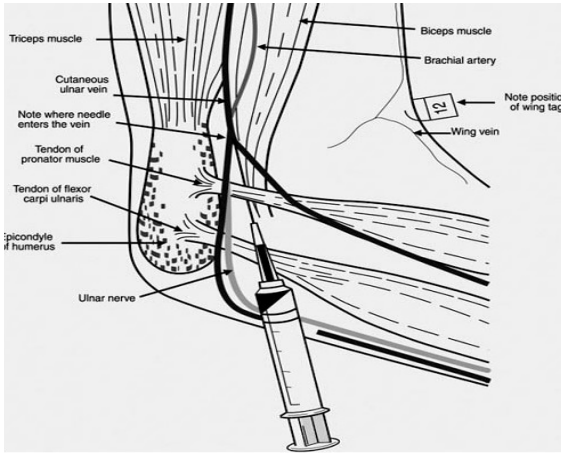

Image *1 – bleeding from the wing vein*

Image *2 Chicken bleeding from the wing vein*

Bleeding a chicken alone

Wing vein bleeding 3

The method is a modification of the procedure described above and is written for a right-handed person. Left-handed people soon make their modifications once they start bleeding chickens alone.

- sit on a chair. Turn the chicken on its back and place it on your thighs,
- arrange the chicken with its head away from you and place the right-wing securely between your thighs, (Image 3)
- use your left elbow to secure the legs by holding them down onto your left thigh,
- place your left forearm across the chicken and use your left hand to spread out the left-wing of the chicken, (Image 4)
- Use your right hand to bleed the chicken as described above (Image 5).

Image 3

Image 4

Image 5

Collecting of blood samples from **wild birds -1**

Blood can be collected from the jugular vein (right side of the bird's neck), brachial/ulnar vein (wing vein) (see image 6) or medial metatarsal vein (leg vein) using a 22 g (gauge), 23 g, 25 g, or 27 g hypodermic needs or butterfly needle and a 12 mL, 10 mL, 6 mL, 3 mL or 1 mL syringe, depending on the size of the bird and the amount of blood to be collected (see image 6).

In general, it is safe to collect 0.3-0.6 cc of blood per 100 g of body mass from live birds, however, it is always best to collect as little blood as is necessary to conduct the testing required.

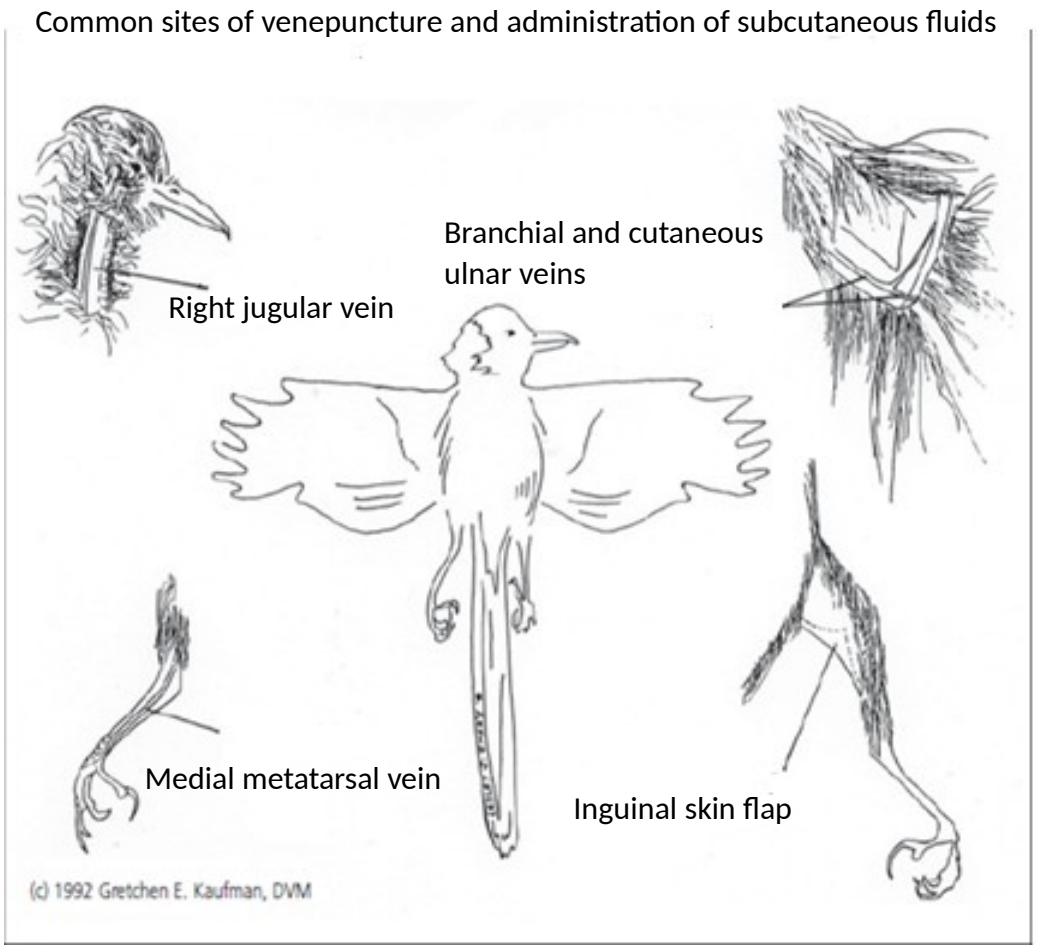

Image 6. Common sites of venipuncture and administration of subcutaneous fluids in birds

Collecting of blood samples from **wild birds - 2**

If you plan to do hematology tests in addition to disease surveillance, it is recommended that you use a 22 g (gauge) through 25 g needle as a 27 g needle or smaller damaged cells as they pass through this narrow diameter needle. After blood is collected, cover the venipuncture site with gauze and apply digital pressure until bleeding stops (30-60 seconds).

If it is not possible to collect a blood sample prior to euthanasia (greatly preferred) do so immediately after euthanasia via cardiac puncture. For duck-sized birds, insert a 4 cm (16 or 18 g) needle just below the keel but aim the tip of the needle cranially, towards the head at a 45-50^{o} angle, and withdraw blood.

Place blood into a serum separator tube (red top) and allow to clot at room temperature. Spin tubes in a portable centrifuge and transfer serum to a cryovial with a transfer pipette, or if unavailable, carefully pour off serum into the cryovial.

Source – FAO Manual for wild bird HPAI surveillance

Collecting swabs -1

1.wear appropriate PPE when handling birds and opening sample vials (see SOP for biosecurity when entering the establishment)

2.unwrap a Dacron swab from the stem-end of the packaging (choose appropriate size swab for bird) and be careful not to touch the swab tip

3.remove the swab and insert the entire tip of the swab into the cloaca. Use gentle pressure and in a circular motion, swab the inside of the cloaca two to four times

4.shake off any large (>0.5 cm) pieces of feces

5.open the cryovial and place the swab tip in the transport media approximately of the way towards the bottom of the cryovial

6.cut or snap the stem of the swab so that the swab remains in the vial and the cap can be screwed on tightly. The entire swab end and a portion of the stem should be left in the cryovial

Collecting swabs -2

7.wipe scissors with 70% alcohol if they were used to cut the swab stem

8.label the tube with appropriate information (sample ID and type of sample (cloacal vs tracheal)) making sure that the ID on the tube can be cross-referenced to the datasheet where additional information about the sample exists

9.record sample tube number on data sheet along with ID number, date, species, type of sample (cloacal vs tracheal), age, sex, location (GPS coordinates preferred), band number, comments, or other information

10.for tracheal swabs, repeat steps 1, 2, however instead of steps 3 and 4, gently insert the swab tip into the trachea by waiting until the bird breath and the cartilage protecting the trachea opens to allow the passage of air. Gently touch the swab tip to the back and sides of the trachea and remove it. Then follow steps 5-9

Collecting swabs - 3

Note that if birds are very small (passerines), it may not be possible to actually conduct a tracheal swab due to the narrow diameter of the opening into the trachea. In these cases, an oropharyngeal swab should be collected by gently rolling the swab tip around the inside of the bird's mouth contacting the upper and lower portions of the mouth, and behind the tongue.

If scissors or wire cutters are used to cut the swabs, they should be disinfected between bird samples. Other commercial swabs are pre-cut so they can easily be broken by hand.

Note that applicator sticks on many small-sized swabs may be metal. In this case, if wire cutters are not available, insert the swab in the viral transport medium, mix well, and discard the used swab in a disinfectant-filled container.

Source – FAO Manual for wild bird HPAI surveillance

Collecting swabs - 4

Label each sample so it can be cross-referenced with relevant information on the *Sick or Dead Bird Sample Collection Log* or a live bird data sheet as you collect each sample (Figure 4).

Samples must be sent to the laboratory with an accompanying letter correctly completed with all relevant data accompanied by a messenger and it is desirable to deliver samples by car .

Source – FAO Manual for wild bird HPAI surveillance

Figure 1
Oropharyngeal swab

Figure 2
Cloacal swab

Figure 3
Breaking the swab

Figure 4
Sample label

Reference materials

Local legal texts:

1. Legal text on Animal Health 41-2012 http://veteriner.gov.ct.tr/Mevzuat
2. -Instruction for the animal diseases control program http://veteriner.gov.ct.tr/Mevzuat

EU legislation:

3. Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law') (Text with EEA relevance) https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32016R0429&qid=1657187827264

4. COMMISSION DELEGATED REGULATION (EU) 2020/687 of 17 December 2019 supplementing Regulation (EU) 2016/429 of the European Parliament and the Council, as regards rules for the prevention and control of certain listed diseases https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32020R068

Reference materials

Other resources:

1. FAO (2007). Wild Birds and Avian Influenza: an introduction to applied field research and disease sampling techniques. Edited by D. Whitworth, S.H. Newman, T. Mundkur and P. Harris. FAO Animal Production and Health Manual, No. 5. Rome. http://www.fao.org/3/a1521e/a1521e.pdf,
2. OIE Avian Influeza, https://www.oie.int/en/disease/avian-influenza/,
3. OIE Terrestrial Manual - Chapter 1.1.3. – Transport of biological material, https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/1.01.03_TRANSPORT.pdf

Annex 1

a) Form of accompanying letter for samples from domestic birds

b) Form of accompanying letter for samples from wild birds

CONTACT

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THANK YOU FOR YOUR ATTENTION

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