Annex 12. Samples from animals and the environment

1) Animals

Specimens for isolation of H5N1 and other respiratory viruses and for the direct detection of viral antigen or nucleic acids from animals should generally be taken during the first three days after onset of clinical symptoms of influenza.

For serological surveillance studies at slaughterhouses or of free-flying wild birds that are bled and released, a single sample of serum is collected.

Sampling strategy for birds

For each affected species select up to three each of:

- dead birds (dead less than 24 hours)
- sick birds (suffering respiratory, neurological or gastro-intestinal disease, or moribund birds. Non-moribund, sick birds will be feverish (hot to the touch) while moribund birds may be hypothermic)
- apparently normal birds in direct contact with the currently sick birds.

If possible, also conduct a random survey of other birds that share the same habitat (cloacal swabs ± tracheal swabs only). Priority should be given to birds that share wetlands with affected birds since there is evidence that the main mode of transmission of AI between wild birds is probably faecal contamination of the environment (water or shore areas).

In birds, influenza can be an infection of both the respiratory tract and the large intestinal tract. “Classic” clinical signs of highly pathogenic influenza in chickens include conjunctivitis, respiratory distress, oedema or cyanosis of the comb and wattles, and subcutaneous ecchymosis or purpura. However some birds (such as ducks) may show minimal or no signs of illness.

Live animals

a) Birds

Sampling of birds for influenza infection should include:

- tracheal swab (the primary sample for domestic and intensively reared poultry)
- cloacal swab
- faecal specimen

The key to obtaining good samples from animals is proper restraint. At least two operators are needed for each live chicken sampled. Three and sometimes four operators are needed for larger birds (swans, large geese, storks etc).

To obtain the sample with minimal distress to the live bird , one assistant should hold the bird against their chest with the wings folded (helped by other assistants if required). (Cloacal samples can also be taken with the bird restrained in this way). An apron that cannot easily be

ripped by the bird's claws should be used. Do not use the wings or neck to restrain the bird nor hold the legs and do not hold the bird upside down while carrying it.

Tracheal swab

The individual taking the sample should pry open the beak with his/her free hand, insert a polyester swab into the trachea and gently swab the wall. Hold the swab in the same fashion as a pencil (see Figs 6 –8) . Withdraw the swab with gentle rotation and place it in VTM (see Annex 8).

Cloacal swab

Whenever possible, cloacal swabs should be collected from live or freshly dead/humanely killed birds. A cloacal swab is taken by inserting a swab into the vent and gently (live bird) or vigorously (dead bird) swabbing the mucosal wall. The swab should be deeply stained with faecal material. The swab is then placed in VTM (see Annex 8).

Blood samples

Blood sampling from live birds should only be undertaken by properly qualified individuals. (For taking blood samples from dead birds see below.)

Euthanasia

Birds suspected of suffering from HPAI should be killed by cervical dislocation (neck wringing) only. (Other methods present safety risks).

Immediately after the bird is dead perform a cardiac bleed to collect blood for serum separation. Select the size of needle used in proportion to the size of the bird. For duck-sized birds, aim a 1.5 inch needle just below the keel (breast bone), withdraw blood with minimal negative pressure and treat as described in section 3.e.

b) Mammals

In mammals influenza is primarily a respiratory tract infection (although there is some evidence that H5N1 infections may be systemic in cats). Suitable samples include:

- nasal swab
- throat swab
- rectal swab

The procedure for taking oro- and naso-pharyngeal and nasal swabs from humans has been described above (section 3.d). The technique for taking such samples from other mammals is similar, but restraint may be more difficult.

Dead animals

If dead birds/mammals are found during the investigation, and highly pathogenic avian influenza virus is suspected, representative internal organs should be sampled as well as the respiratory and intestinal tracts.

The specimens to be collected from dead animals should include:

- tracheal swab

- cloacal swab
- tissue (including trachea and lung. Should also include a piece of spleen and any obviously abnormal tissue)

Tracheal swabs from dead animals, including animals at slaughterhouses, can be taken after removal of the lungs and trachea from the carcass. The trachea is held in a gloved hand and the swab inserted to its maximal length with vigorous swabbing of the wall. The swab is then placed in VTM.

Tissue specimens should ideally be frozen immediately, without transport medium, and transported frozen (-70 °C or below). Alternatively such specimens can be preserved in 100% ethanol or in a commercially available non-toxic RNA preservative if no cold chain is available.

Sampling birds and mammals at post mortem examination

Wet the ventral surface of birds with clean water to avoid contaminating samples with down or feathers (dunk the carcass in a pail of water). Pluck as necessary to further minimize feather contamination. Open the abdomen taking care to avoid incising gut or vasculature. Cut along the ribs in order to lift the keel and breast toward the bird's head exposing the thoracic cavity.

Place swabs or tissue samples in viral transport medium (See Annex 8).

Sterilize instruments between each post mortem.

When VTM is not available, nasal turbinates or trachea may be a suitable substitute for a tracheal swab and faeces may be a suitable substitute for a cloacal swab. To take a tracheal sample, incise the skin of the neck and dissect until the trachea is identified. Transect a section that will fit into the cryovial. To take a nasal turbinate sample, cut off the upper beak or bill with rongeurs near the head and take a sample of the tissue from above the roof of the oral cavity.

2) Environmental samples

Environmental samples that should be collected when investigating deaths of poultry or of wild birds include:

- faecal (environmental)
- drinking-water of birds (e.g. from drinkers, drinking lines)
- soil

Faecal specimens

Faecal specimens from the cages of live poultry in bird markets, from poultry houses or from wild birds in the field should if possible be collected from freshly deposited wet faeces. The swab should be heavily coated with faeces and then placed in VTM (double the normal amounts of antibiotics and antifungals may be needed in media used for this purpose).

NB. Faecal specimens collected from cages or from the environment are often the only specimens available, but they cannot be assigned with certainty to the species of origin.

Drinking water

Collect water from ponds where water birds are found, from surface water where poultry are active and may drink, or from drinkers or drinking lines in poultry sheds. In each instance:

- Collect 5 ml of water
- Add to 5 ml of VTM
- Check pH with paper to ensure pH between 6 and 8
- If it is possible to test the water sample within 3 hours of collection, keep at ambient temperature. Otherwise freeze at -70 °C as soon as possible.
- Otherwise freeze at –70 °C as soon as possible.

When collecting the samples:

• From a tap or pump

- Remove any attachments
- Wipe, clean and disinfect (ideally flame) the water outlet
- Allow some water to flow before collection

• From a drinking line in a poultry shed

- Disinfect a drinking nipple and the nearby line with 1/100 bleach solution. Hold the nipple open with a pair of sterile forceps, let the water run for 5 seconds and then run the sample into a collecting bottle as above. Sample from at least five drinking nipples at different parts of the shed. Swab the forceps with 70% alcohol between taking each of the samples.

Soil

Collect soil from

- Areas where birds are active especially near domestic dwellings if birds have access and from areas around poultry sheds, lake shores, etc.
- Add 1–2 g to 10 ml of VTM
- Check pH with paper to ensure pH between 6 and 8
- Mix vigorously and re-check pH
- If the pH correct, freeze at -70 °C as soon as possible.
- If the pH is too low or too high, collect a large soil sample (e.g. 100 g) in a tube without VTM and freeze.