Specimen collection, storage and transport

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The results of any laboratory tests are only as good as the samples received in the laboratory.

*Talk to the lab staff to get information about:*

- what specimens are needed
  - Whole blood?
  - Serum?
  - Faeces?
  - Urine?
  - CSF?
  - Biopsy?
  - Other?
- when to collect them
- how to collect them
- collecting them safely
- how to store them
- how to ship them
- time schedule of these activities
(i) Preferred specimens

- **Upper respiratory tract**: posterior-pharyngeal (throat) swabs are the best specimen for detecting H5N1.
- **Lower respiratory tract**: If the patient is intubated, take a tracheal aspirate.
- **Blood**: serum (acute and convalescent if possible)
What specimens to collect from suspect cases of AI #2

(ii) Secondary specimens
(not essential but can be useful if materials are available)

• Plasma in EDTA
• Nasal swabs with nasal secretions (from the anterior turbinate area) (if other ‘flu is suspected)
• Faecal sample - if the patient has diarrhoea
• Spinal fluid if meningitis is suspected and a spinal tap is to be performed for diagnostic/therapeutic purposes
When to collect specimens?

You need to collect specimens at the right time if you want to get a result.
e.g. When to collect specimens from suspect cases of AI

- Take initial throat swab within 3 days of onset of symptoms.
- Collect acute phase serum sample during the first 5-7 days of infection and a convalescent sample after 3 - 4 weeks.
- Single serum samples. Best taken about 3 weeks after symptom onset.
- Take blood serum or plasma for viral RNA during the 1st week after symptoms develop.
- Ideally collect initial specimens before anti-viral therapy is begun. (Do not delay treatment in order to take samples).
- Collect specimens from deceased patients as soon as possible after death.

Fig 1: Virus excretion, viral RNA in blood and antibody response in H5N1 infection in humans
Label specimens correctly and fill in the forms completely!

- What type of specimen is it?
- Specimen number
- What information/tests do you want from the lab?
- Date
- Details of patient
  - Age
  - Sex
  - Details of illness
  - Name/number
  - Location - community/clinic/ward
- Who are you?
- How can the lab contact you?
- Label specimen on jar/tube NOT on the lid
Use the right sampling equipment etc.

- **Respiratory tract infections**
  - Samples for bacteriology - use cotton swabs or swabs with wooden sticks
  - Samples for virology - use only sterile dacron or rayon swabs with plastic shafts. Avoid calcium alginate or cotton swabs or swabs with wooden sticks, - may contain substances that inactivate viruses and inhibit PCR.

- **Blood**
  - Vacutainers (or similar) or syringes and needles.
  - Use a serum separator tube (SST) or a clotting tube (for serum) and an EDTA tube (for plasma)
  - Use appropriate transport media
Use the right sampling equipment etc.

- **Stools**
  - clean, dry, leak, proof-screw cap container and adhesive tape.
  - bacterial transport media (esp for rectal swabs from infants).
  - parasitology transport pack (10% formalin in water, polyvinyl isopropyl alcohol (PVA).

- **Urine**
  - Sterile plastic cup with lid (50 ml or more).
  - Clean, screw-top specimen transport containers (“universal” containers are often used).
  - Gauze pads.
  - Soap and clean water (or normal saline) if possible.
  - Urine collection bags may be necessary for infants.
Blood

• Venous blood for:
  – direct isolation of pathogen,
  – detection of genetic material (e.g. by PCR),
  – specific antibodies (by serology),
  – antigens or toxins (e.g. by immunofluorescence).

• Serum better than whole blood for viral pathogens.

• Paired sera for antibody assays (acute & convalescent).

• Collect by finger prick for slides for microscopy or for absorption onto special filter paper discs

• Take specimens for culture before giving antimicrobials
Stool specimens #1

• Collect:
  – as soon as possible after onset of diarrhoea (viruses < 48 hours, bacteria < 4 days)
  – before starting antimicrobials
• May need 2-3 specimens collected on separate days.
• Stool best for culture of bacteria (and viruses)
• Rectal swabs from infants may also be used for bacterial culture (not good for viruses and/or parasites)
Stool specimens #2

- Transport at 4–8°C. (e.g. *Shigella* spp. vv sensitive to raised temperature)
- Process within 1–2 days of collection.
- Cholera specimens do not need refrigeration.
  - With Cary-Blair transport medium, needs to reach the lab within 7 days.
  - Without transport medium, must reach lab within 2 hours
- Parasites - mix specimen with 10% formalin or PVA, 3:1 stool to preservative. Transport at ambient temperature in containers sealed in plastic bags
Do you need to sample anyone or anything other than the patient?

• Contacts?
  - early detection of infected individuals
  - searching for asymptomatic/subclinical cases
  - studies of the prevalence of infection.
  - assess possible susceptibility to infection

• Livestock?

• Wildlife?

• The environment?
Sampling Safety

- Use Personal Protective Equipment (PPE) – the level will depend on the circumstances
- Comply with all recommended infection control precautions including
  - personal hygiene measures
  - correct use of disinfectants.
- Ensure that
  - when the equipment is worn it protects you properly,
  - when you take it off you do not contaminate yourself or others
Sampling safety #2

- Sampling from the respiratory tract is hazardous:
  - operator is very close to patient
  - the procedure can generate aerosols and droplets.

- Always observe standard precautions when taking and handling blood specimens
Sampling safety #3

Monitoring personnel.

• If an incident that could lead to infection occurs during a sampling procedure monitor those involved for signs of illness for the known incubation period of the infection (including daily temperature measurement).

• Any evidence of illness within that period after exposure to a confirmed or suspected human case or to a potential animal source should be viewed as a suspected case of the disease and treated appropriately by a medical doctor.
Storing and shipping specimens

Specimens must always be stored and shipped under the appropriate conditions if a satisfactory result is to be obtained.

This frequently means some form of cold chain.
Storing specimens #1

- Do not freeze and thaw repeatedly (Take aliquots before specimens are frozen).
- Many viruses do not survive well at -20°C
- Samples in VTM (or blood sera/plasma) for viral isolation
  - if can be taken to the lab within 4 days, store at 4°C and freeze at -70°C on arrival
  - otherwise freeze at −70°C until transported to the lab
**Storing specimens #2**

- Store ethanol-preserved swabs at 4°C
- Blood serum for PCR or antibody determination is best frozen at −20 °C. but can be stored at 4°C for ca. 1 week.
- Do not store or ship samples for *virus isolation* in dry ice (solid CO₂) unless sealed in glass or sealed, taped and double plastic-bagged. (CO₂ can inactivate viruses).

*NB.* *Do not to seal any container with dry ice in it as it could explode.*
Packing and shipping specimens

- You cannot transport infectious substances or diagnostic specimens on passenger aircraft as carry-on baggage.
- Infectious substances cannot be shipped in diplomatic bags.
- Transport of specimens within national borders must comply with national regulations.
Infectious substances

• For the purposes of transport, infectious substances are defined as substances which are known or are reasonably expected to contain pathogens.

• *Pathogens* - microorganisms (including bacteria, viruses, rickettsiae, parasites, fungi) and other agents such as prions, which can cause disease in humans or animals.
Infectious substances: Categories

Category A
An infectious substance which is transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals.

Category B
An infectious substance which does not meet the criteria for inclusion in Category A.
Substances covered

1. *Lab cultures* - Category A or Category B, depending on the organism
2. *Specimens* - materials, collected directly from humans or animals, being transported for purposes such as research, diagnosis, investigational activities, disease treatment and prevention
3. *Biological products* - includes vaccines
4. *Genetically modified micro-organisms and organisms*
5. *Medical or clinical wastes*
Exempt Human/Animal Specimens

• Human or animal specimens (patient specimens) for which there is minimal likelihood that pathogens are present

• Must be transported in a leakproof package which is marked “Exempt human specimen” or “Exempt animal specimen”.

Exceptions

• Items that
  – do not contain infectious agents
  – contain neutralised/inactivated pathogens

• Environmental samples (including food and water) not posing a significant infection risk

• Blood or blood components for transfusion and/or transplantation

• Dried blood spots and faecal occult blood screening tests
Training

All personnel involved in shipping pathogens must undergo appropriate training.

• Category A
  – Special training - attend approved courses, pass exams

• Category B
  – Read the instructions!

If specimens are sent with other dangerous goods (e.g. flammable liquids, radioactive materials, liquefied gases etc.), personnel must be trained in the procedures for their transport.

Staffing implications
  – you may need a member of staff trained in Cat A shipment
  – local hospital, hospital or university lab may have such a person
To be used for all infectious substances. It consists of three layers:

1. **Primary leak-proof receptacle**
2. **Secondary leak-proof packaging**
3. **Outer cushioned packaging**
Marking

Each package must display the following information on the outer packaging or the overpack.

– the shipper’s and receivers names and addresses & tel nos
– the UN number
– temperature storage requirements
– the name, UN number & quantity of any refrigerant,
Documentation

• Declaration for Dangerous Goods

• An import and/or export permit and/or declaration if required.

• An air waybill or equivalent documents for road, rail and sea journeys.
Shipments should not be dispatched until:

• Arrangements have been made between the sender (shipper, consignor), carrier and receiver (consignee)
• The sender has confirmed that the material may be legally exported
• The receiver has confirmed that the material may be legally imported
• The carrier has confirmed that there will be no delay in the delivery of the package
The sender (shipper, consignor)

- Makes advance arrangements with;
  - the receiver including import/export permits
  - the carrier to ensure
    - that the shipment will be accepted
    - that the shipment is undertaken by the most direct route
- Prepares documentation
- Gets export & import authorizations
- Notifies the receiver of the arrangements well in advance of the expected arrival time
The carrier

• Provides advice to the sender about:
  – the necessary shipping documents and instructions for their completion
  – correct packaging
• May be able to provide a trained Cat A shipper
• Assists the sender in arranging the most direct routing
• Maintains and archives the shipment and transport documentation
• Carries the item!
The receiver (consignee)

- Obtains the necessary authorization(s) from national authorities for the importation of the material
- Provides the sender with the required documents
  - import permit(s),
  - letter(s) of authorization
  - other document(s) required by the national authorities
- Arranges for collection on arrival
- Acknowledge receipt to the sender
Sending specimens by airmail

• Cannot send Category A substances.
• Can send Category B substances by registered air mail
  – Use the basic triple packaging system
  – Labelling requirements
  – Local/international restrictions may be in force. Check with postal service.
• Exempt/exceptions – no problem
Refrigerants #1
wet ice/cold packs

- Easy to obtain
- Simple equipment
- Not very cold – unsuitable for many specimens
- Limited time
- Produces water – needs appropriate sealed containers (thermos)
Refrigerants #2
dry Ice (CO$_2$) and liquid nitrogen (N$_2$)

• Asphyxiants - must be handled in well ventilated areas.
• Very cold:
  - dry ice = -78.5$^\circ$C
  - liquid N$_2$ = -196$^\circ$C
• Wear appropriate protective equipment (e.g. gauntlets, footwear, apron, eye protection) especially when handling liquid nitrogen
• Need special packaging
• Containers must be able to vent evaporated gases to the air to prevent explosions
Dry Ice

- Can be sent into a country by the shipping company.
- Can sometimes get it from a local manufacturer, a brewery or from an importer of frozen products (e.g. ice cream).
- Make it as required by use of a dry ice maker - only possible if cylinders of liquid CO\(_2\) can be obtained.
- Specimens shipped in dry ice must be packed in specially insulated boxes capable of releasing CO\(_2\) gas. You can get these from shippers or from WHO.
- Mark the outer packaging with the hazard label for dry ice
Liquid nitrogen

- Special arrangements must be made in advance with the carrier.
- Primary receptacles must be capable of withstanding extremely low temperatures, and packaging and documentation requirements for liquid nitrogen must be observed.
- The outermost packaging must carry the hazard label for liquid nitrogen.
- For air transport, the handling label for cryogenic liquids must also be attached.
Liquid nitrogen

- Shipment in liquid nitrogen is done in a **dry shipper**.
- A charged dry shipper can be sent into a country by the shipping company or the receiving laboratory.
- Liquid N\textsubscript{2} can occasionally be obtained at international airports.
- Dry shippers should be marked with ownership details and arrangements made to return the shipper to the originating laboratory.