

Collecting, preserving and shipping specimens for the diagnosis of avian influenza A(H5N1) virus infection

Guide for field operations

October 2006



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This document is a work in progress and is based on the best information available at the time of production. The document will be updated regularly as more information becomes available.

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Abbreviations

AI	avian influenza A(H5N1)
ARO	Alert and Response Operations
BSA	bovine serum albumin
CDS	Communicable Disease Surveillance and Response
EDTA	ethylenediaminetetraacetic acid (anticoagulant used for blood samples)
EPR	Epidemic and Pandemic Alert and Response
HPAI	highly pathogenic avian influenza
H5N1	avian influenza subtype
IATA	International Air Transport Association
ICAO	International Civil Aviation Organization
NAMRU 3	Naval Medical Research Unit 3 (US Navy) based in Cairo, Egypt
PCR	polymerase chain reaction
PPE	personal protective equipment
RNA	ribonucleic acid
RT-PCR	reverse transcriptase PCR
UN	United Nations
VTM	viral transport medium
WHO	World Health Organization

1. Introduction

Highly pathogenic avian influenza (HPAI) caused by the A(H5N1) influenza subtype in animal populations, particularly wild waterfowl and domestic poultry such as chickens and ducks, poses a continuing global human public health risk. The virus has expanded its geographical range increasing the size of the population at risk. Each new human case gives the virus an opportunity to evolve towards a transmissible pandemic strain.

Collection of appropriate specimens from human and animal cases for rapid viral RNA detection by any qualified laboratory, together with rapid and precise characterization of virus isolates of the specimens at specialized reference laboratories, is essential for early detection of cases, proper management of patients and understanding the epidemiology of the disease. In addition, the development of resistance to antivirals can be determined, effective vaccines produced in a timely manner and quality control improved.

The rapid confirmation of the precise nature of isolates of the virus permits effective surveillance and in particular the proper documentation of the spread of the infection in human and animal populations and detection of changes in the virus that could indicate improved transmissibility to humans.

This protocol is designed to simplify the previously published guidelines on specimen collection, packing and shipment (see Annex 1) to allow for ease of use at field level and to provide the data needed to confirm the diagnosis of A(H5N1) infection as specified/described in the case definitions recently produced by WHO (see Annex 1 for link). It is a work in progress and may change as circumstances demand. It is intended to:

- describe the minimum number and types of specimens collected;
- enhance the chances of obtaining a positive result if the patient is infected with A(H5N1);
- allow the potential identification of respiratory pathogens other than A(H5N1) (including other strains of influenza virus);
- contribute to work designed to increase understanding of the pathogenesis of A(H5N1) disease including the potential duration of infectiousness.

As a WHO document, the protocol is designed primarily for sampling from humans by medical staff. Specimens from birds and mammals should ideally be taken by veterinarians, their assistants, or trained wildlife professionals and detailed guidelines are available on the FAO/OIE web site (see Annex 1 for links). However there may be instances where professionally trained persons are not available for sampling from animals and details of the specimens to take and how to take them are given in Annex 12. The same procedures for subsequent handling, storage and shipping of the specimens apply as for those taken from humans and the relevant parts of this protocol may be useful as an advisory document for the veterinary services.

This protocol covers the following aspects of sampling:

- the specimens required from humans;
- collection of the specimens;
- preservation before and for shipping;
- correct packing of the specimens;

- shipment to laboratories;
- the information that needs to accompany the shipment.

While this can act as a stand-alone document it is also intended to accompany a new WHO kit (Annex 4) which has been designed to facilitate all these aspects of sampling.

2. Safety

The majority of the human infections with influenza A(H5N1) virus to date have been caused by close (usually direct) contact with infected birds. Epidemiologic observations suggest human-to-human transmission of the virus by very close contact (e.g., face-to-face) with infected individuals may have occurred on some occasions. A(H5N1) infection is acquired by inhalation of infectious droplets or droplet nuclei or by direct (and possibly indirect) contact and self-inoculation of infectious virus into the nose, eye, or possibly mouth. Infection via the eyes could occur either by infection of the conjunctiva or (possibly) if the virus is washed through the lachrymal ducts and into the nasopharynx.

- There is some evidence that infection via the pharynx and the gastrointestinal tract may occur following ingestion of the virus.
- Infection across intact skin has not been described.
- The virus is transmitted on respiratory droplets larger than 5 microns and possibly by fomites. The relative importance of short-range fine particle (airborne) transmission in A(H5N1) infection is unknown.
- The virus cannot survive long in a dry environment but can survive for weeks in a moist environment protected from direct sunlight.
- Viral RNA and less often infectious A(H5N1) virus are detectable early in illness (see Fig 1). Infection from percutaneous inoculation of the virus during care of patients or specimen collection (needle sticks, cuts from contaminated surgical instruments) or during postmortem examination has not been recognized to date (although such injury obviously carries a real risk of transmission of known blood borne pathogens).

The use of personal protective equipment (PPE) is therefore mandatory if direct close contact with a patient is anticipated, when entering a room where aerosol-producing procedures in AI-infected patients are being performed, when sampling from wild birds or mammals and when taking specimens from poultry either in intensive rearing or laying facilities or from backyard flocks.

The level of PPE to be employed will be determined by the exposure risk. For example work in intensive poultry units, where virus contaminated material can accumulate and there is a great deal of potentially contaminated dust in the air, requires greater personal protective equipment levels than sampling from wild birds. Yuen & Wong (2005) recommend that: “In view of the high fatality of the disease, a combination of contact, droplet, and airborne precautions are recommended as long as resources allow despite the fact that the relative importance of these three modes in nosocomial transmission of avian influenza is still unknown.”

In general PPE should include:

- a suitable form of respiratory protection (Annex 6)
- non-sterile latex gloves (or equivalent if allergic)
- goggles or a face shield
- gown
- head covering.

It may also be necessary to include:

- impermeable apron
- suitable rubber boots.

High risk activities such as post mortem examination of a confirmed or strongly suspected human case, capture of birds in poultry sheds or on farms, euthanasia of infected or potentially infected birds or other animals, and procedures such as decontamination in an intensive agriculture system should only be conducted in a full body cover-all, with easily cleaned waterproof boots, heavy rubber gloves and eye protection. In poultry sheds or other situations where the virus load is likely to be high, the best form of respiratory protection is powered air pressure respirators (PAPR). If a PAPR is not available then a NIOSH-certified N95, EU FFP2, or equivalent respirator should be used.

PPE is essential to prevent infection during sampling but is not the whole answer. Those taking specimens should comply with all recommended infection control precautions including specific personal hygiene measures, and the correct use of disinfectants.

Further details of safety procedures are given in Annex 5 (Hand hygiene technique), Annex 6 (Respiratory protection) and Annex 7 (Disinfection).

Monitoring medical or veterinary personnel

1. If an incident that could lead to infection occurs during a sampling procedure (such as a breakdown of protective procedures) the staff member(s) involved should be monitored for signs of illness for a week (including daily temperature measurement). Post-exposure chemoprophylaxis with a neuraminidase inhibitor for 7 to 10 days is a consideration under such circumstances (see: *WHO rapid advice guidelines on pharmacological management of humans infected with avian influenza A(H5N1) virus*. Link provided in Annex 1).
2. All staff working with human or animal cases of AI should monitor their own health and any evidence of influenza-like illness within seven days of exposure to a confirmed or suspected human case or to a potential avian source should be viewed as suspected avian influenza and treated appropriately by a medical doctor.

3. Taking specimens

- A checklist for the various stages of taking, handling and shipping specimens is given in Annex 2. This should be filled in for each batch of specimens and kept for reference. Note that the checklist covers a wide range of activities from taking the specimen to activities such as taking aliquots from specimens (sub-sampling) which may occur before the specimen is sent to the reference laboratory.
- For each type of specimen two specimens should be taken in separate specimen tubes on each occasion that sampling is undertaken. One can be used for immediate analysis and the other retained for reference purposes, retesting, etc.
- Each patient or animal sampled or each environmental sample taken should be given a unique identifier number and accompanied by a field data sheet (Annex 9)
 - All specimens taken from that source should be marked with that unique identifier as well as any other numbers needed to identify the particular specimen.
 - This identifier should be used on all documentation concerning the specimen from that source.
 - Specimen tubes should also be marked with information about the type of specimen in the tube and the date when the specimen was taken.
 - Specimens of animal origin should be accompanied by a detailed epidemiological investigation form (see Annex 9).

a) What specimens to collect from suspect cases

1. Preferred samples

- **Upper respiratory tract** (take both types of specimen to allow detection of A(H5N1) and other influenza viruses):
 - Posterior-pharyngeal (throat) swabs are currently the highest yield upper respiratory tract specimen for detecting A(H5N1) (unlike human influenza). Naso-pharyngeal swabs may be collected if necessary (see below).
 - Nasal swabs with nasal secretions (from the anterior turbinate area) or nasopharyngeal aspirates or swabs are appropriate specimens for detecting human influenza A and B and therefore useful if the influenza is not due to A(H5N1).
- **Lower respiratory tract:**
 - If the patient is intubated, take a tracheal aspirate or collect a sample during bronchoalveolar lavage.
- **Blood:**
 - Serum (acute and convalescent if possible).

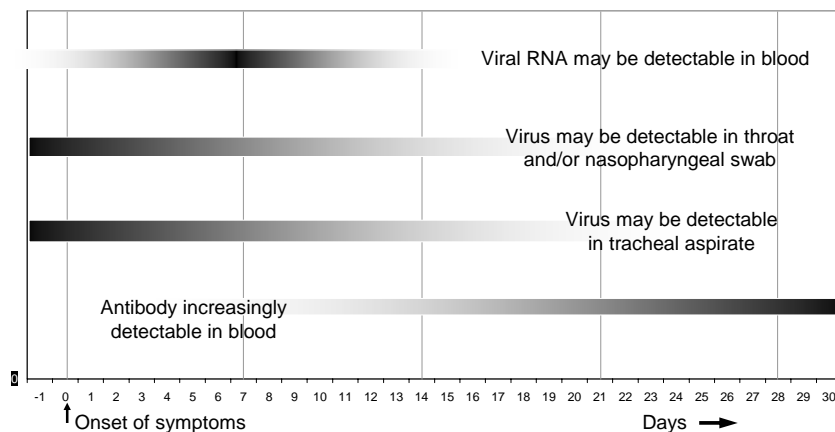
2. Secondary specimens (**these are not essential but can be useful if materials are available**)

- Plasma in EDTA (for detection of viral RNA)
- Rectal swab —especially if the patient has diarrhoea
- Spinal fluid if meningitis is suspected and a spinal tap is to be performed for diagnostic/therapeutic purposes.

b) When to collect the specimens from suspect cases

The figure below (Fig 1) is a summary of the data available at the time of the publication (October 2006) and will be changed as necessary as more data become available. It must be emphasized that the bars indicate **approximate** periods of time after onset of symptoms when taking specimens is **likely** to yield results and **not** periods when sampling will always be effective.

Fig 1: Virus excretion, viral RNA in blood and antibody response in H5N1 infection in humans



- A throat swab should be taken (if possible) within three days of onset of symptoms. Note that the virus is generally detectable in throat swabs from most patients from the point of onset of symptoms (or even just before) until towards the end of the second week, and infrequently beginning of the third week, after onset of symptoms. Cases whose initial specimens are negative for A/H5 but who continue to show symptoms suggestive of this type of infection and/or who have a history of exposure that would also support the diagnosis should therefore be sampled at least once again as soon as possible.
- Virus may be detectable in tracheal aspirates from onset of lower respiratory complaints (dyspnoea, difficulty breathing, marked cough) or pneumonia until the second or third week of illness.

- An acute phase serum sample should be taken seven days or less after symptom onset (this will usually be done when the patient presents and begins treatment) and a convalescent sample after 3 to 4 weeks. Note that the limited data available on antibody kinetics indicate development of positivity (initially ELISA and not necessarily neutralizing antibody) from day 10 onwards.
- Single serum samples. To be collected at day 14 or later after symptom onset since the likelihood of detecting neutralizing antibodies increases over time, certainly during the first 3 to 4 weeks after onset of symptoms.
- Blood serum or plasma for the detection of viral RNA should be taken during the first 7 to 9 days after the development of symptoms because the patient is most likely to be RNAemic (have detectable RNA in the bloodstream) at that time (Fig 1).
- Initial specimens (respiratory and blood) should ideally be collected from suspected patients before antiviral therapy is begun but treatment must not be delayed in order to take specimens. (Note that standard treatment may render throat swabs negative for virus after three or more days of treatment but probably has no effect on the development of neutralizing antibody).
- Specimens should be collected from deceased patients as soon as possible after death.

c) Sampling human contacts

Taking single respiratory tract or blood specimens from contacts of human cases who remain healthy in the days immediately after potential contact with HPAI is unlikely to yield useful results. Individuals who are contacts of suspect or known human patients or have had exposure to sick animals should be observed for seven days after the last contact (take the temperature daily). If they become ill with an influenza-like illness they should be sampled as outlined above.

Blood specimens for serological studies can usefully be taken from contacts for several reasons:

- as a tool for searching for asymptomatic/subclinical cases;
- for studies of the prevalence of A(H5N1) infection;
- to assess possible susceptibility to A(H5N1) infection.

d) Specimens from the respiratory tract

Sampling from the respiratory tract is hazardous as the operator is very close to the patient (Fig 2) and the procedure can generate aerosols and droplets. Full PPE is therefore essential.



Fig 2. Taking a throat swab - operator is close to the patient

Choose a sitting position for adults and a supine position for infants and younger children. Children often find sampling from the respiratory tract very distressing and need to be reassured. They may also need to be restrained during the sampling process.

If the child's parents or guardians are present they must be fully informed of what is to take place, and must be made aware that the child may become distressed. The parent(s) should not usually be in the room during the sampling procedure. The sampling procedure can generate aerosols which could present a risk to others in the immediate vicinity, and also the parent(s) may react to the child's distress by attempting to interfere with the sampling procedure and risk injury to the child. It is also not usually appropriate for a parent to help restrain the child, mainly because the assistant needs to be in PPE and the parent would not have been trained to don, wear and remove PPE safely. In addition, the parent could be upset by their child's distress and, if restraining the child, could release the child at a critical moment, which could risk injury to the child. (These are general considerations and should be adjusted accordingly to the local cultural sensitivity and social circumstances).

Very young children can be restrained by being held by an assistant against the assistant's chest with the child's arms restrained by the assistant's enfolding arms and the child's legs held between the assistant's legs (Fig 3).



Fig 3. Restraining a small child

Even older children will attempt to defend themselves with their hands or by leaning their head backwards. Lay the subject supine and with extended ('hold-up') positioning of the subject's arms above the head. The assistant holds the child's arms with elbows and arms pressed against the subject's forearms and the assistant also uses his/her palms to hold the subject's head in place (Fig 4 and Fig 5). An additional assistant may be needed to restrain the child's legs.



Fig 4 and Fig 5 restraining an older child

When taking throat (or nasal) swabs, the swabs must be held correctly. They should be held between the thumb and the first and second fingers with the shaft protruding beyond the web of the thumb (like a pencil) (Fig 6) and not between the thumb and forefinger with the base in the palm of the hand (Fig 7). The main reason is that if the patient makes a movement as a reaction to the swabbing the swab will slide out of harms way if held the first way (Fig 8 - with the patient represented by the open gloved hand of the operator) but not if held the second (Fig 9). In this case discomfort would be caused and the patient could be injured. In addition control over the swab is much greater if it is held correctly.



Fig 6. Swab held correctly



Fig 7. Swab held incorrectly



Fig 8. Correctly held swab can slide out of the way



Fig 9. Swab can injure patient

- Use only sterile dacron or rayon swabs with plastic shafts. Calcium alginate or cotton swabs, or swabs with wooden sticks may contain substances that inactivate some viruses and inhibit PCR testing and should only be used if dacron or rayon swabs are not available.
- Prepare two vials containing at least 2–3 ml of a suitable transport/preservative medium (Viral Transport Medium - VTM - Annex 8) for each specimen. These should be marked with:
 - the unique identifier
 - the specimen date
 - the type of specimen in the tube (e.g. blood serum, throat swab etc.).

Note: Always mark the tube itself with identifying details, never the cap as this can get switched during handling). Use an indelible and alcohol resistant marker pen. Be aware that stick-on labels can easily come off, especially when the specimen is chilled to very low temperatures. Relevant field data sheets (see Annex 9) should be filled in.

- Take two specimens and put one into each vial.
- If VTM is not available or alternatively specimens cannot be stored at appropriate temperatures (e.g. no freezers are available - see Table 1 below), swabs can be stored and shipped in absolute (100%) ethanol. (If pure ethanol cannot be used, 99% Industrial Methylated Spirit - without additives other than methanol - may be substituted). Put 1–2ml ethanol into a vial and put the swab tip into the tube (see below). Note that such specimens are suitable only for PCR.
- After a specimen is taken, the tip of the swab should be put into the vial and the shaft broken or cut off sufficiently short for the lid to be closed. Plastic swab handles usually have a weak point in them to allow them to be broken off for insertion into a specimen tube. Others have a handle made of a brittle plastic that will snap easily.

If the shaft cannot easily be broken off short enough to be put into a small tube such as a cryovial it will have to be cut. To do this:

- cut the shaft with scissors taking care not to touch the tip;
- allow the tip to slide into the VTM and then cap the tube. Do not let cut portions of the bag or wrap fall into the tube.

Sterilize the cutting edge of the scissors by the use of flame (e.g. by the use of a spirit burner, a Bunsen burner or another suitable heat source). Allow scissors to cool before reuse.

If this procedure cannot be followed, agitate the swab tip in the medium for 30 seconds and squeeze it against the side of the tube before removing it from the medium and disposing of it in a safe manner (not suitable for ethanol storage).

Posterior pharyngeal and nasopharyngeal swabs

Posterior pharyngeal swabs are the best upper respiratory tract to take because the evidence so far available suggests that they are more likely to be positive than anterior nasal swabs in sporadic A(H5N1) illness. However if difficulty is experienced in obtaining the former (e.g. from babies and young children) nasopharyngeal swabs should be obtained instead.

Posterior pharyngeal swab (throat swab)

- Hold the tongue out of the way with a tongue depressor;
- Use a sweeping motion to swab the posterior pharyngeal wall and tonsillar pillars. Have the subject say "aahh" to elevate the uvula. Avoid swabbing the soft palate and do not touch the tongue with the swab tip. (N.B. This procedure can induce the gag reflex);
- Put the swab into VTM.

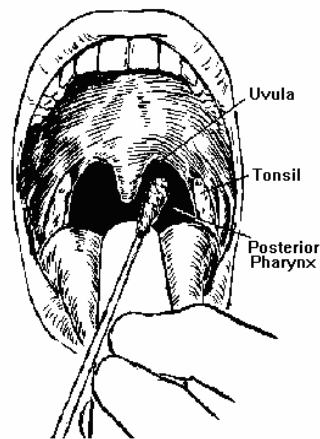


Fig 10. Taking a throat swab

Nasopharyngeal swab

- Insert a flexible, **fine-shafted** polyester swab into the nostril and back to the nasopharynx. The swab should be slid straight into the nostril with the patient's head held slightly back (Fig 11). The swab is inserted following the base of the nostril towards the auditory pit and will need to be inserted at least 5–6 cm in adults to ensure that it reaches the posterior pharynx. (Do NOT use rigid shafted swabs for this sampling method —a flexible shafted swab is essential).



Fig 11. Taking a nasopharyngeal swab

- Leave the swab in place for a few seconds
- Withdraw slowly with a rotating motion

- Put the swab into VTM
- A second swab should be used for the other nostril and put into a second tube. This can serve as the second sample from the patient.

Note: Nasopharyngeal sampling is an invasive process that can cause considerable distress to the patient.

Nasopharyngeal aspirate

- Easier and safer than swabbing in infants and young children.
- Use an aspiration trap. Insert silicon catheter in the nostril towards the auditory pit and aspirate secretion gently by suction (Fig 12).

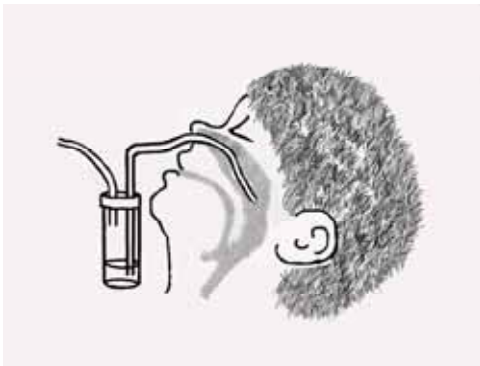


Fig 12. Nasopharyngeal aspiration

Anterior nasal swab

Use the same type of rigid swab as for sampling from the throat. Advance the swab tip past the vestibule (anterior nares) to the nasal mucosa (approximately 2–3 cm from the nostrils in adults) and gently rotate to collect nasal secretions from the anterior portions of the turbinate and septal mucosa (Fig 13).

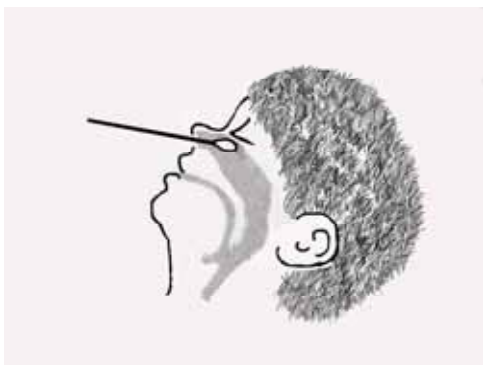


Fig 13. Anterior nasal swab

e) Blood specimens

- Standard precautions should always be observed when taking and handling blood specimens because the patient may be infected with a blood born pathogen (for example HIV or Hepatitis B).
- Use PPE — at least gloves (plus face-shields, masks and gowns if splashes are anticipated).

- Remove and discard PPE items **immediately** after completion of task.
- Perform hand hygiene every time gloves are removed.

The best "all round" specimen to collect is serum. Acute and convalescent sera are useful for detection of changes in antibody titre and serum can be used for detection of viral RNA. An acute-phase serum specimen should be taken soon after onset of clinical symptoms and not later than seven days after onset.

EDTA-anticoagulated plasma is also valuable for detection of viral RNA in blood and may be better than serum for this particular purpose since EDTA inactivates RNAses present in the specimen. Heparin is not suitable as an anticoagulant for this type of specimen because of potential inhibition of PCR reactions. Note that specimens for the detection of viral RNA in the blood should be collected during the first week after the development of symptoms (Fig 1).

At least 1ml of whole blood is needed to obtain a sufficient amount of serum or plasma for tests. This is the maximum that should be taken from infants. However larger specimens of 3–5 ml should be taken from older children and adults as this will allow a greater range of tests or repeat tests to be done.

A convalescent-phase serum specimen should ideally be collected 3–4 weeks after the onset of symptoms. When a patient is critically ill, a second ante-mortem specimen should be collected.

Blood should be collected either by use of a vacuum venepuncture system or syringes and needles. The specimens should be collected either in a serum separator tube (SST) or a clotting tube (for serum) and in an EDTA tube (for plasma).

Taking a blood specimen

1. Label the tubes, including the unique patient identification number, using an indelible marker pen. Always check to ensure that the correct tubes are used for each patient.
2. Place a tourniquet above the venepuncture site, palpate and locate the vein (Fig 14).



Fig 14. Tourniquet on, palpating the vein

3. Disinfect the venepuncture site meticulously with 70% isopropyl alcohol (an alcohol swab) or 10% polyvidone iodine by swabbing the skin concentrically from the centre of the venepuncture site outwards (Fig 15). Let the disinfectant evaporate. Do not re-palpate the vein.



Fig 15. Disinfecting the venepuncture site

4. Perform venepuncture. (Fig 16)



Fig 16. Venepuncture with a vacuum venepuncture system

5. If withdrawing blood with conventional disposable syringes, withdraw 3–5 ml of whole blood from adults and older children and 1ml from infants. Under asepsis, transfer the specimen to appropriate transport tubes. Secure caps tightly.
6. If withdrawing blood with a vacuum system (e.g. Vacutainer®), withdraw the desired amount of blood directly into each transport tube (Fig 17).



Fig 17. Drawing blood into a Vacutainer® serum-separator tube

7. Remove the tourniquet. Use a cotton swab to apply pressure to the venepuncture site until bleeding stops (Fig 18) and apply a band-aid.



Fig 18. Applying pressure to the venepuncture site

8. Never recap used sharps. Discard directly into a suitable container (a proper sharps disposal container if available or a container such as a coffee or other metal can which should be appropriately labelled before use).
9. Recheck that the tubes used for sampling have been correctly labelled.
10. After taking all the samples, complete the appropriate field data sheets or case investigation forms and the required laboratory request forms using the same identification numbers used on the tubes.

Separation of serum and plasma

Blood samples need to be centrifuged for at least five minutes at 1500g (3000 rpm). This requires an electric centrifuge (ideally with a swing out head rather than an angle head rotor). Hand centrifuges are not adequate for the separation of serum or plasma from red cells.

Serum separator tubes (SST)

The instructions for use of these tubes must be followed carefully if the tubes are to work properly. The tubes contain a gel (with an intermediate density between blood cells and blood plasma) and (usually) a coagulation (clot) activator (Fig 19).

- Put the blood sample into the tube and then follow the instructions for mixing the contents.
- Allow the clot to form (follow the instructions with the tube — do not cut the clotting process short).
- Centrifuge the tube according to the relevant instructions.

When a filled SST has been properly centrifuged, the sample will separate into a top layer of serum separated by a gel barrier from the cell/clot layer and the clot activator.

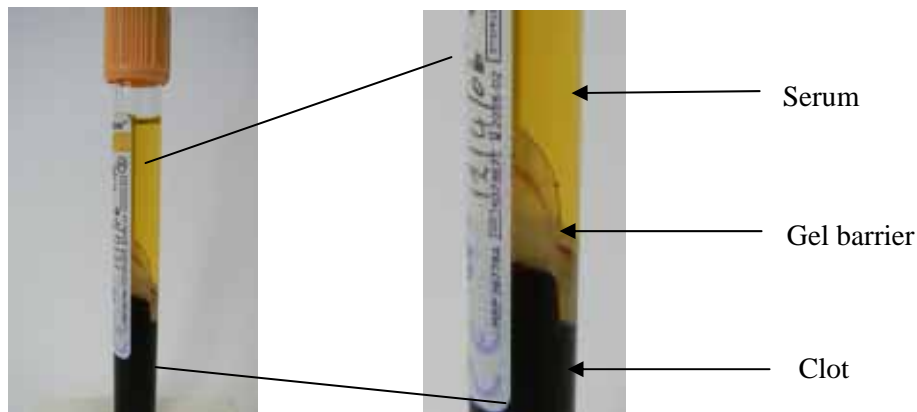


Fig 19. Serum separator tube

Clotting tubes

If a basic sampling tube without any additives is used the clot can be allowed to form overnight and the serum pipetted off the next day. Serum should not be left in contact with the clot for more than 12 hours, as lysis of the red cells can occur.

Whichever type of tube is used, once the serum has been separated it should be pipetted off without disturbing the gel barrier or the clot. Put the serum into a vial such as a cryovial (without VTM). Ideally vials for transport of serum should have external caps and internal O-ring seals. If there is no internal O-ring seal, ensure the cap is closed tightly and then sealed with an inert sealing film such as Parafilm®.

EDTA tubes

Centrifuge the tubes at high speed (ca 10 000 g) to compact the cellular fraction and then pipette off the plasma taking care not to draw blood cells off at the same time.

Filter paper

Blood or serum specimens can also be shipped in air dried form on filter paper discs or special filter paper strips (Nobuto strips). Volumes of 0.1 ml of whole blood or serum are put onto the strip which is then air dried. Strips of this sort can be stored for months at room temperature.

Transport

Blood specimen bottles and tubes should be transported upright and secured in a screw cap container or in a rack in a transport box. They should have enough absorbent paper around them to soak up all the liquid in case of spillage. See Table 1 below and the notes following the table for details of storage and shipment conditions.

Samples air dried on filter paper are exempt from shipping regulations and do not have to be sent to a laboratory via a specialist courier. They can be sent by airmail. (Note that to avoid damage, they should not be sent in hermetically sealed triple packages).

f) Specimens from patients who have died

- If the corpse has an endotracheal tube in place, collect a deep endotracheal aspirate. If the circumstance allows, perform tissue sampling by incision or by needle from the affected lung(s). The operator may use chest radiograph results to guide the sampling and aim for areas at the margins of interstitial infiltrates which is most likely the site of active virus replication for the best diagnostic yield. The lung tissue sample will provide excellent material for various laboratory tests including RT-PCR, virus isolation, histopathology, bacterial cultures, direct antigen detection or immunohistochemistry, and cytokine-chemokine analyses. The needle aspiration or the core needle sampling may give sufficient sample for microbiologic studies. Clean a small area on the lateral chest wall between two ribs and make a small incision between the ribs overlying the lungs with a sterile scalpel. Cut wedge sample(s) from the lung (1–2 cm³ minimum) or insert a large-bore needle (e.g. 18G) into the lung tissue and aspirate or cut available material into the needle/syringe. Put the specimen into VTM. The needle sampling should be performed as soon as possible after death.
- Throat swabs, nasopharyngeal aspirates or stool samples may be collected if time, sampling materials and safety considerations permit but this should not supersede or delay the collection and sending of the deep endotracheal or lung material.

4. Storing specimens

Table 1 below gives the different storage and shipment conditions that can be used and which methods are recommended (based on the likelihood of obtaining a positive A(H5N1) result on laboratory analysis).

Table 1. Suitability of various storage/shipment conditions for different specimen types

Storage/shipment conditions	Swabs or other specimens in VTM for isolation of virus	Swabs or other specimens in VTM for PCR	Swabs in ethanol for PCR**	Blood serum for virus isolation	Blood serum for PCR	Blood serum for antibodies
-70 °C or dry ice or Liquid N₂	SR	SR	N/A	SR	SR	SR
-20°C	NR	A	N/A	NR	A	SR
+4°C	A*	A	A	A***	A	A
Room temperature	NR	A	A	NR	A*	A*
Dried blood spot on filter paper	N/A	N/A	N/A	N/A	A	A

SR= Strongly recommended method A = Adequate method. NR = Not recommended N/A = Not applicable
 * For up to 7 days storage ** Where refrigeration is not available ***For up to 4 days storage

- Aliquots of specimens should be taken before the specimens are frozen.
- Repeated freezing and thawing of specimens must be avoided to prevent loss of infectivity. Note that certain types of freezer are designated "frost free" and these should not be used for specimen storage as the temperature cycling involved in keeping them free of ice accumulation can damage specimens.
- If specimens in VTM (or blood sera/plasma) for viral isolation can be taken to the laboratory within four days, they may be kept at +4 °C and frozen at -70 °C on arrival if they are to be stored. Otherwise they should be frozen at or below -70 °C until they can be transported to the laboratory. Freezing at -20 °C is not recommended because the virus does not survive well at this temperature, particularly in frost-free freezers.
- In the absence of freezers (or of VTM), ethanol-preserved swabs are a possible alternative. Storage of such specimens at +4 °C (in a standard refrigerator) is better than at room temperature.
- Blood serum samples should be frozen at -70 °C for PCR and at -20 °C or lower for antibody determination but they can be stored at +4 °C for approximately one week.

- Specimens for influenza virus isolation should not be stored or shipped in dry ice (solid carbon dioxide) unless they are sealed in glass or sealed, taped and double plastic-bagged. Carbon dioxide can rapidly inactivate influenza viruses if it gains access to the specimens. (Note: Take care not to place dry ice in an hermetically sealed containers as it could cause an explosion).

5. Taking aliquots of specimens

It is better to take more than one specimen when sampling from a patient than to subdivide specimens later. However this may not be possible and if specimens have to be sub-divided, the smallest volume of VTM or serum that should be stored is 0.5ml (thus a 3ml sample can be divided into six separate sub-samples). Fresh sterile or disposable pipettes should be used for each sample and these should be discarded safely (into 1/100 chlorine solution - see Annex 7).

Taking aliquots of samples should only be undertaken under appropriate levels of laboratory safety (e.g. preferably in a certified¹ a Class II biosafety cabinet).

Great care must be taken not to contaminate or cross contaminate specimens. This is especially so when they are intended for analysis by PCR because PCR procedures are especially vulnerable to cross contamination after amplification and uncapping of the tube.

Taking aliquots should be done before the specimen is frozen as repeated freezing and thawing of specimens can reduce the content of virus and should therefore be avoided.

6. Packing specimens and shipping them by air

The packing of specimens and their shipment to external laboratories by air is complex and is governed by international and national regulations and operator variations. These are summarized below. Links to other relevant documents are given in Annex 1. Readers are also referred to the WHO document *Guidance on regulations for the Transport of Infectious Substances* (Annex 10). The FAO/OIE reference laboratory requirements for shipment of animal specimens can be found on the FAO/OIE web site (see Annex 1 for the link).

International air transport of human specimens known or suspected to contain the avian influenza agent, or of specimens from avian influenza infected animals must follow the current edition of the International Air Transport Association (IATA) *Dangerous Goods Regulations (Infectious Substances Shipping Guidelines 2006)*. However be aware that specimens shipped by air need to be transported to and from the airport(s) (e.g. by road or by rail) and that different regulations may govern transport by such means — information should be obtained from the ministry of health or ministry of agriculture/chief veterinary office (for animals specimens) of the country(s) involved.

¹ Biological safety cabinets should be recertified annually by an independent qualified technician.

Shipment of specimens from humans and animals

There are two categories covering shipment of specimens by air:

- Category A covers infectious substances (included in an indicative list of specified pathogens) that are capable of causing permanent disability, life threatening or fatal disease in otherwise healthy humans or animals. Highly pathogenic avian influenza is part of the indicative list with the mention "cultures only" (more information can be found in Annexes 1 and 10). Thus only cultures of HPAI (i.e. virus isolates) must be transported as Category A.
- Category B covers all other infectious substances that are not included in Category A.

As far as shipment of human or animal samples suspected or confirmed to contain A(H5N1) viruses is concerned, **human blood and other human samples or animal blood and other animal samples** known or suspected to contain the A(H5N1) subtype, can be transported as "diagnostic specimens" (UN 3373) and are included in **Category B**. Note that individual airlines may adopt their own policies and these may be stricter than those issued by IATA.

Shipment of specimens in Category A requires shippers who have undergone special training. For the transport of Category B infectious substances there is a requirement for clear instructions on the use of the packaging to be supplied to the user; and this is regarded as sufficient "training" for the shipping of these substances. However, if such specimens are consigned with other dangerous goods (including **liquid nitrogen** or **dry ice**), then shippers trained in the proper procedures for the transport of those substances must be used.

Detailed instructions for packing specimens for shipment in the two categories can be found in the WHO document *Guidance on regulations for the Transport of Infectious Substances* (Annex 10).

Shipment of frozen specimens

Specimens that are to be shipped by air can be preserved either in dry ice or in liquid nitrogen. IATA regulations require that both types of shipments must be consigned by trained shippers. Details of the labelling required are given in the WHO document *Guidance on regulations for the Transport of Infectious Substances* (Annex 10).

Note

Dry ice and liquid nitrogen both give off gases that can cause asphyxiation and should only be handled in well ventilated areas. In addition any containers in which they are shipped must be able to vent evaporated gases to the air to avoid the risk of explosions.

Specimens stored and shipped at very low temperatures should be handled with appropriate protective gauntlets and eye protection should be worn, especially when handling liquid nitrogen.

Dry ice

Dry ice can be sent into a country in an appropriate container by the shipping company. Alternatively dry ice may sometimes be obtainable in-country from a dry ice manufacturer, a brewery or sometimes from an importer of frozen products such as ice cream. A third possibility is to make dry ice as required by use of a dry ice maker. This is only possible if cylinders of liquid CO₂ can be obtained in the country concerned (it can sometimes be obtained from hospitals or biological research institutes).

Specimens to be shipped in dry ice need to be shipped in specially insulated boxes capable of releasing gaseous CO₂. These can be obtained from shippers or provided by WHO or FAO's Animal Health Service. Note that dry ice is very cold and can injure unprotected skin.

Liquid nitrogen

Liquid nitrogen is a dangerous compound (it is very cold) and can cause injury to persons and severe damage to materials such as metals if spilt. For this reason shipment at liquid nitrogen temperatures is done in a device called a **dry shipper**. These are large Dewar flasks (vacuum flasks) that contain an absorbent material that will hold liquid nitrogen. The dry shipper is filled with liquid nitrogen and charged for the period specified by the manufacturer. Just before the specimens are put into the shipper, the excess nitrogen is poured out (see Annex 11 for the proper use of dry shippers).

Liquid nitrogen can sometimes be obtained at international airports — contact the engineering departments of international airlines operating from the relevant airports to see if this is possible. Liquid nitrogen can also be obtained from services that deal with artificial insemination (i.e. beef/dairy industry), and these are often strategically located in many rural areas for animal production and husbandry improvement programmes.

Dry shippers should be well marked with ownership details. Shipment procedures should always include arrangements to return the shipper to the originating laboratory.

Shipping specimens in alcohol

For specimens of Category A, follow Packing Instruction P602, (for details see *WHO document Guidance on regulations for the Transport of Infectious Substances*) respecting the volume limitations and adding the package orientation label if necessary (primary containers containing more than 50 ml).

For specimens in Category B, a quantity of 30 ml or less of dangerous goods included in Classes 3, 8 or 9 (i.e. alcohol) may be packed in each primary receptacle containing infectious substances. When these small quantities of dangerous goods are packed with infectious substances in accordance with Packing Instruction 650, no other requirements need to be met (for details see *WHO document Guidance on regulations for the Transport of Infectious Substances*).

Information about shipments of specimens

The recipients should be sent full details of the shipment in advance. Similar information should accompany the shipment. A form that can be used for this purpose is given in Annex 3². This form includes details of the materials included and details of the laboratory and of the person sending the specimens. The WHO Avian Influenza Response team in Geneva requests that a copy should be sent to them (AIRresponse@who.int) so that they can assist with problems should they occur and so that a complete listing of specimens shipped and results obtained can be maintained for detailed surveillance purposes.

Other relevant matters

- Passengers and crew members are specifically prohibited from transporting infectious substances or diagnostic specimens on passenger aircrafts either as or in carry-on baggage or checked baggage, or on their person.

² This form is not part of the international transport requirements but is designed to inform relevant individuals or organizations

- Infectious substances cannot be shipped in diplomatic bags.
- Transport of specimens within national borders should comply with the procedures detailed within each country's regulations.
- Authorization for shipment of specimens out of a country may be required. Contact the appropriate authorities (usually the ministry of health or ministry of agriculture/chief veterinary office) for further information. Wildlife samples may require clearance from the ministry of the environment or of natural resources.
- Appropriate import permission **must** be obtained from the recipient country before the specimens are shipped. Contact the recipient laboratory for help (usually reference laboratories have the appropriate import licences).

7. References

(See Annex 1 for links to key documents)

- IATA. *IATA Dangerous Goods Regulations*. 48th Edition, 2006. International Air Transport Association.
- ICAO. *Technical Instructions for the Safe Transport of Dangerous Goods by Air*. 2005–2006 Edition. International Civil Aviation Organization.
- Krafft AE, Russell KL, Hawksworth AW, McCall S, Irvine M, Daum LT et al. Evaluation of PCR testing of Ethanol-Fixed Nasal Swab Specimens as an Augmented Surveillance Strategy for Influenza Virus and Adenovirus Identification. *Journal of Clinical Microbiology*, 2005, 43:1768-1775.
- WHO. *Communicable Disease Control in Emergencies*. A field Manual. World Health Organization, Geneva, 2005.
- WHO. *Laboratory Biosafety Manual*. Third edition. World Health Organization, Geneva, 2004.
- Writing Committee of the World Health Organization (WHO) Consultation on Human Influenza A/H5. Avian Influenza A (H5N1) Infection in Humans. *New England Journal of Medicine*, 2005, 353:1374–1385.
- Wolff KL and Hudson BW. Paper strip sampling technique for the detection of antibody to the plague organism *Yersinia pestis*. *Applied Microbiology*, 1974, 28:323–325.
- World Alliance for Patient Safety 2005. WHO Guidelines on Hand Hygiene in Health Care (Advanced Draft): a Summary. WHO Geneva.
- Yuen KY and Wong SY. Human infection by avian influenza A H5N1. *Hong Kong Medical Journal*, 2005, 11:189–199.

Annex 1. Links to key documents

Avian Influenza, including influenza A (H5N1), in humans: WHO interim infection control guidelines for health care facilities

http://www.who.int/csr/disease/avian_influenza/guidelines/infectioncontrol1/en/index.html

Guidance on regulations for the Transport of Infectious Substances

(WHO/CDS/CSR/LYO/2005.22)

http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2005_22/en/index.html

Guidelines for the submission of diagnostic samples to reference laboratories: Avian

http://www.fao.org/docs/eims/upload/208595/gui_labsample_en.pdf

http://www.fao.org/ag/againfo/programmes/en/empres/documents/docs/gui_labsample/gui_labsample_en.htm

WHO case definitions for human infections with influenza A(H5N1) virus

http://www.who.int/csr/disease/avian_influenza/guidelines/case_definition2006_08_29/en/index.html

WHO guidelines for the collection of human specimens for laboratory diagnosis of avian influenza infection

http://www.who.int/csr/disease/avian_influenza/guidelines/humanspecimens/en/index.html

WHO guidelines for the storage and transport of human and animal specimens for laboratory diagnosis of suspected avian influenza A infection

http://www.who.int/csr/disease/avian_influenza/guidelines/transport/en/index.html

(Note: The advice on shipping in this document is outdated as the regulations have recently been changed)

WHO guidelines on hand hygiene in health care (advanced draft): a summary

http://www.who.int/patientsafety/events/05/HH_en.pdf

WHO laboratory biosafety guidelines for handling specimens suspected of containing avian influenza A virus

http://www.who.int/csr/disease/avian_influenza/guidelines/handlingspecimens/en/index.html

WHO laboratory guidelines for the collection of animal specimens for diagnosis of influenza infection

http://www.who.int/csr/disease/avian_influenza/guidelines/animalspecimens/en/index.html

WHO Rapid Advice Guidelines on pharmacological management of humans infected with avian influenza A(H5N1) virus

http://www.who.int/csr/disease/avian_influenza/guidelines/pharmamanagement/en/

WHO recommendations on the use of rapid testing for influenza diagnosis

http://www.who.int/csr/disease/avian_influenza/guidelines/rapid_testing/en/index.html

Annex 2. Checklist for specimen handling and shipment

Note: This form is not an official document but is intended to assist specimen tracking and quality control.

Checklist for specimen handling and shipment

Name(s) of person(s) taking and handling specimens			
Date specimens taken			
Place specimen(s) taken			
Type(s) of specimen(s) (blood, tissue, swab) and storage conditions			
Origin of specimen(s) (human, animal)			
Action	Yes	No	Comments
Safety			PPE used correctly? Any safety problems recorded?
Any shortages of equipment/reagents noted?			
Samples labelled according to protocol?			
Duplicate sample(s) taken?			
Aliquots of specimens taken at local laboratory?			If Yes, was this done before freezing?
Any other manipulations performed on sample at local level?			If Yes, what?
Specimens stored at local laboratory?			If Yes, under what conditions?
Specimens shipped to national laboratory?			If Yes: - Which laboratory? - To whom? - Under what conditions?
Specimens sub-sampled at national laboratory?			If Yes, by whom?

Specimens tested at local laboratory?			If Yes: - What test(s)? - What result? -Whom informed?
Specimens stored at national laboratory?			If Yes, under what conditions?
National government/MoH approval required for specimen shipment?			If Yes, has it been obtained?
Permission obtained for import of specimens by recipient nation?			
Shipper contacted?			If Yes, which shipper, and whom contacted?
AI Response contacted?			If Yes, whom contacted?
Specimens packed?			Notes:
Specimens shipped?			Notes:
Arrival of specimens at reference laboratory ?			Date (dd/mm/yy)
Results received from reference laboratory?			Date (dd/mm/yy)
Notes:			

Annex 3. Form to accompany specimens shipped by air

**For human specimens please also email a copy to AI Response in Geneva -
AIResponse@who.int**

Please note that this form is not an official requirement – it is intended to provide relevant information to the recipient and to AI Response or FAO.

Form for reporting shipment of specimens

Country sending specimens

Laboratory sending specimens	Address: Tel: Fax: Email:
------------------------------	------------------------------------

Contact details of person sending specimens	Name: Address: Tel: Fax: Email:
---	---

Destination laboratory

Contact at destination laboratory	Name: Address: Tel: Fax: Email
-----------------------------------	--

Date of shipment	dd/mm/yy
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Carrier

Details of local carrier representative	Name: Address: Tel: Fax: Email
---	--

Airline handling the shipment

Details of contact at airline	Name: Address: Tel: Fax: Email
-------------------------------	--

Category of specimens

Category A: Y N

Category B: Y N

Specimens frozen

Dry ice: Y N

Liquid nitrogen: Y N

Number of specimens

Type of specimens (swab, tissue, blood, etc.)
and ID numbers

Origin of specimens

Human: Y N

Animal: Y N

Notes:

Annex 4. Content of the WHO AI Investigation Kit

Item	Unit	Quantity
Boot covers, disposable, standard size	Pairs	30
Spray, antifog		5
Handwash, alcoholic, antiseptic and skin protective		5
Goggles, clear screen, reusable		15
Plastic bags, biohazard, 100pcs/roll	Roll	1
Face mask N95/FFP2, cup and folded model		60
Overall, complete with head cover, Cat III CE 0120 resistant to penetration by liquid. Particle tight, limited splash tight. Sizes M, L, XL		30 (10 of each size)
Gloves, examination, non sterile, sizes S, M, L	Box of 50 pairs	3 (1 box of each size)
WHO labelled adhesive tape	Roll	5
Bio-packaging (inner and outer unit) 1.5L with ice pack	Set of	1
Viral transport media, tubes		20
Pernasal swabs, sterile, plastic shaft		20
Dacron swabs, sterile, plastic shaft		20
Tamiflu® (oseltamivir) antiviral rug, 75mg, 10 capsules/box (Expiry date Nov 2010)	Boxes	30

Technical documentation supporting the kit:

1. WHO guidelines for investigation of human cases of avian influenza A(H5N1)
2. Tamiflu® - technical information and post exposure prophylaxis regimen
3. PPE guidance (Influenza H5N1: Infection Control Measures to Prevent Transmission within Health Care Settings)
4. Guidance on collection of specimens (this document).

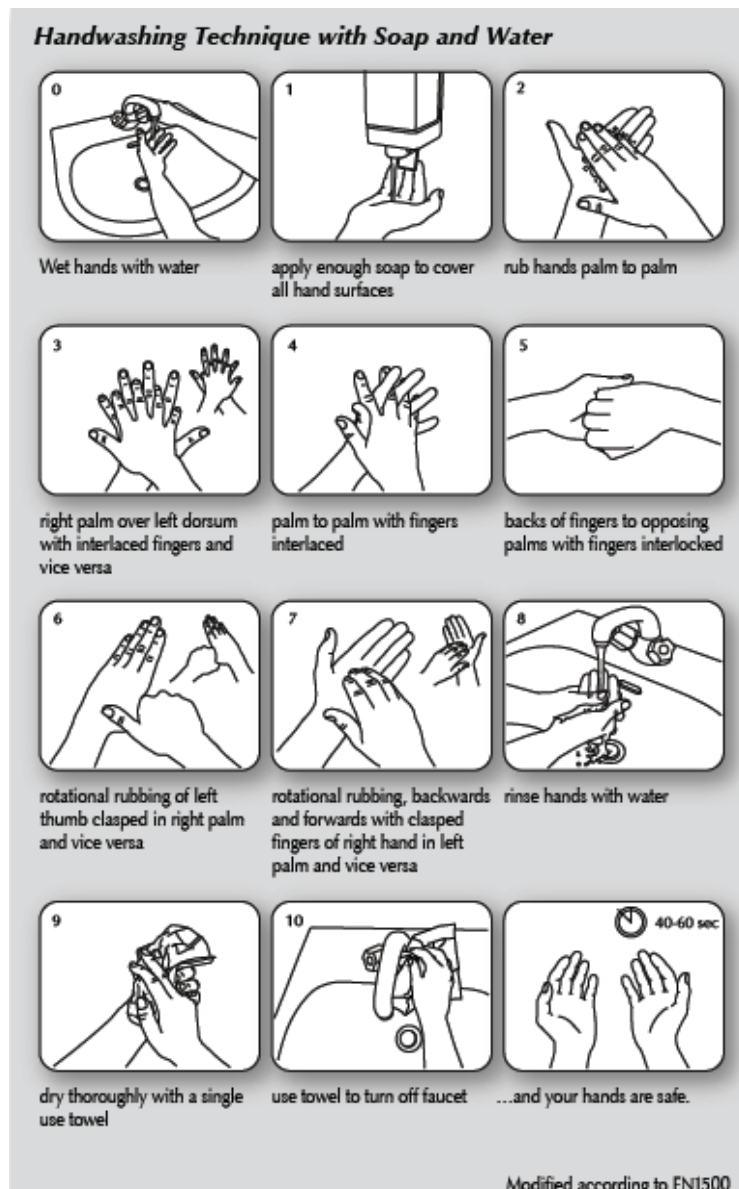
For information on how to obtain these kits please contact your local WHO office.

Annex 5. Hand hygiene technique

When they are visibly dirty or contaminated with proteinaceous material, or visibly soiled with blood or other body fluids, wash hands with soap and water. If hands are not visibly dirty, use an alcohol-based preparation. Ethyl alcohol has greater activity against viruses than isopropyl alcohol, therefore, ethyl alcohol-based hand disinfection products may be preferred over isopropyl alcohol products in settings where transmission of HPAI is likely.

a) Soap and water

Liquid, bar, leaflet or powdered forms of plain soap are acceptable when washing hands with a non-antimicrobial soap and water. Wet hands with water and apply the amount of product necessary to cover all surfaces. Vigorously perform rotational hand rubbing on both palms and interlace fingers to cover all surfaces.



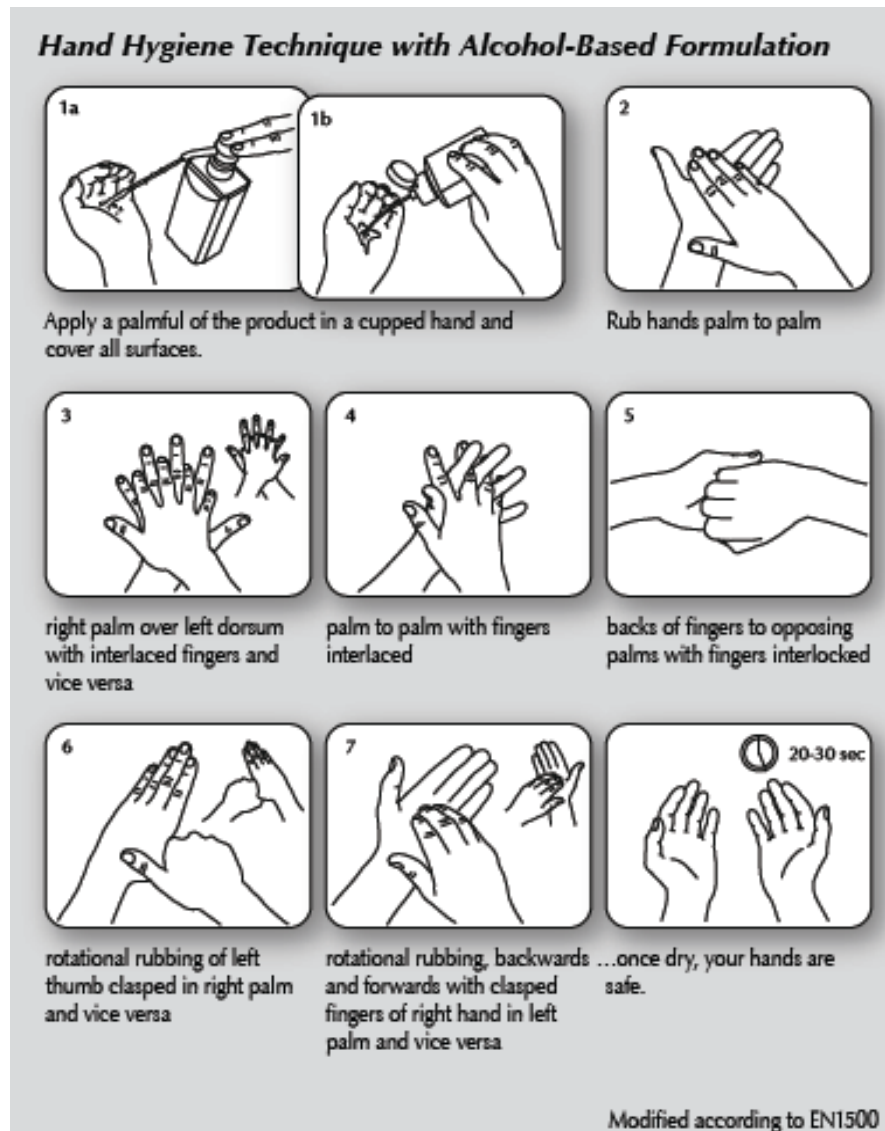
Rinse hands with water and dry thoroughly with a single use towel. Use the towel to turn off the tap/faucet. Make sure the hands are dry. Use a method that does not recontaminate hands. Make sure towels are not used multiple times or by multiple people.

Use running and clean water for hand hygiene whenever possible. Avoid using hot water, as repeated exposure to hot water may increase the risk of dermatitis.

When bar soap is used, small bars of soap in racks that facilitate drainage should be used.

b) Hand cleansers

When using an alcohol-based formulation (or another disinfectant based hand cleanser), apply a palmful of the product and cover all surfaces of the hands. Rub hands until they are dry.



Annex 6. Respiratory protection

The level of respiratory protection required when sampling depends on a number of factors including:

- the type of sample to be taken (e.g. sampling for blood is less risky than taking a throat swab which may cause the patient to cough);
- the situation (e.g. taking a swab from a dead bird in the open air requires less protection than sampling inside a poultry shed);
- the type of respiratory risk (droplets, aerosols and dusts require different types of protection).

There are many types of respirators and masks available and the different types offer very different levels of respiratory protection. However it must be accepted that in some situations high efficiency respirators will not be available and basic gauze masks may be all that can be used.

- Appropriate procedures should be used to select a particulate respirator that fits well and a user seal check (fit check) should be performed each time a disposable particulate respirator is worn.
- Disposable particulate respirators, although similar in appearance to surgical masks, differ significantly from surgical masks because they are specifically designed to protect the wearer from exposure to airborne infectious diseases by sealing tightly to the face and filtering infectious particles from the air.
- If a particulate respirator is not available, a tightly fitting surgical or procedure mask should be used.
- Surgical and procedure masks do not provide protection against small-particle aerosols (droplet nuclei), and aerosol-generating procedures should not be performed if a particulate respirator is not available.

Some common terms:

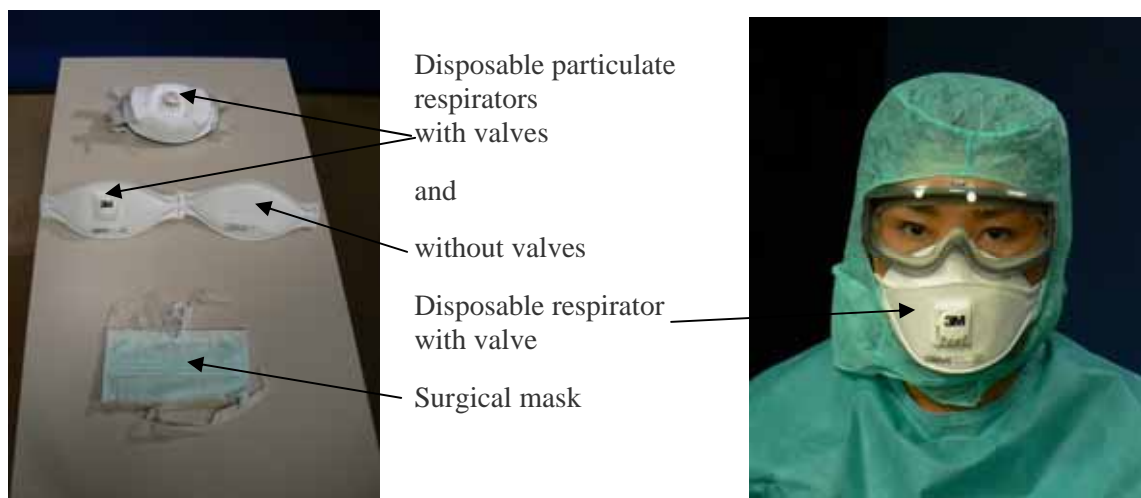
Fit test : evaluating the fit of a respirator on an individual.

High efficiency particulate air (HEPA) filter: means a filter that is at least 99.97% efficient in removing particles of 0.3 micrometers in diameter.

NIOSH: The National Institute for Occupational Safety and Health is the USA Federal agency responsible for conducting research and making recommendations for the prevention of work-related injury and illness.

Types of Respirators

a) Disposable particulate respirators



Disposable respirators of this sort are light weight and fairly comfortable to wear. The type with exhalation valves cannot be used when working in a sterile area such as an operating room since the exhalation valve allows droplets and particles exhaled by the user to escape. Since air has to be drawn actively into the mask, the mask will increase the work of breathing (when used properly).

Also it is almost impossible to prevent occasional leaks of contaminated air into the mask.



Particulate respirator, goggles, coverall, apron and gloves (suitable for work such as sampling birds in a chicken shed)

b) Half-Mask and Full-Mask replaceable particulate filter respirators

These are lightweight respirators with single or dual filters. These can be for specified chemicals or can be HEPA filters.



These respirators can be uncomfortable to wear for extended periods of time, communication may be difficult and they cannot be used in areas where a sterile field is required (operating theatre).

The full mask version can easily fog up in use and an antifogging adapter ensuring that exhaled air passes through the filters and not into the mask should be used.

c) Powered air-purifying respirator (PAPR)

PAPRs use a blower to force air through filters which remove contaminants before supplying the air to the wearer. They can be tight fitting (where the air is supplied to the face piece) or loose fitting (where the air is supplied into the helmet rather than the face piece). The latter are more comfortable.

PAPRs are more protective than a half-mask respirator and breathing resistance is lower. They are also comfortable to use (especially in hot areas) because air is forced into the mask by the blower, producing a cooling effect. They are of particular value for extended periods of work in hot and dusty environments such as culling in chicken sheds. They can be bulky and noisy and communication between individuals can be difficult.

PAPRs cannot be used where a sterile field is required because air can exit around the face seal or through an exhalation valve (tight fitting type).

The batteries must be recharged and maintained to ensure proper flow rates into the mask.

Annex 7. Disinfection

Disinfectants

Chlorine is the best disinfectant for use against A(H5N1) contamination. There are two main reasons for this:

1. In many countries it is the only cheap and easily available disinfectant effective against influenza viruses.
2. It is one of the few disinfectants that can safely be used in laboratories where PCR work is undertaken because it fragments nucleic acids. Other disinfectants such as quaternary ammonium compounds and alcohols precipitate nucleic acids and can give false results in PCR tests (see below).

The best compound for the preparation of chlorine solutions for disinfection is household bleach (also known by other names such as Chlorox®, Eau-de-Javel). Household bleach is a solution of sodium hypochlorite which generally contains 5% (50 g/litre or 50 000 ppm) available chlorine.

Note that:

- different products may contain different concentrations of available chlorine and the concentration should be checked before use;
- household bleach preparations can lose some of their chlorine over time. Use newly manufactured bleach if possible. If the bleach does not smell strongly of chlorine it may not be satisfactory for the purpose and should not be used;
- thick bleach solutions should **never** be used for disinfection purposes (other than in toilet bowls) as they contain potentially poisonous additives.

When preparing chlorine solutions for use note that:

- chlorine solutions gradually lose strength, and freshly diluted solutions must therefore be prepared daily;
- clear water should be used because organic matter destroys chlorine;
- 1:10 bleach solution is caustic. Avoid direct contact with skin and eyes;
- bleach solutions give off chlorine. Prepare them in a well ventilated area;
- use plastic containers for mixing and storing bleach solutions as metal containers are corroded rapidly and also affect the bleach.

Two different dilutions of bleach are used for disinfection.

- **1:10 bleach solution** (which contains 0.5% chlorine concentration), a strong disinfectant that is used to disinfect:
 - Excreta
 - Bodies
 - Spills of blood/body fluids
 - Vehicles and tires
 - It is also used to prepare 1:100 bleach solution
- **1:100 bleach solution** (which contains 0.05% chlorine concentration) which is used to disinfect:
 - Surfaces
 - Medical equipment
 - Bedding
 - Reusable protective clothing before it is laundered

Also recommended for:

- Rinsing gloves between contact with different patients (if new gloves are not available)
- Rinsing gloves, aprons, boots before leaving a patient's room
- Disinfecting contaminated waste before disposal

To prepare 1:10 bleach solution add one volume of household bleach (e.g. 1 litre) to nine volumes of clean water (e.g. 9 litres).

To prepare 1:100 bleach solution add one volume of 1:10 bleach solution (e.g. 1 litre) to nine volumes of clean water (e.g. 9 litres).

Note: 1:100 bleach solution can also be prepared directly from household bleach by adding 1 volume of household bleach to 99 volumes of clean water (e.g. 100 ml of bleach to 9.9 litres of clean water) but making it up from 1:10 bleach solution is much easier!).

There are some other products containing chlorine that can be used to make up disinfectant solutions. **These are not as suitable as household bleach for this purpose.** A table for the preparation of chlorine solutions from some of these compounds is given below.

Preparing chlorine solutions using products other than household bleach

Chlorine product	1:10 solution	1:100 solution
Calcium hypochlorite powder or granules (70%) (High Test Hypochlorite -HTH)	7 g (0.5 tablespoonful) per 1 litre of water	7 g (0.5 tablespoonful) per 10 litres of water
Bleaching powder (Chlorine of Lime) with 30% active chlorine	16 g (1 tablespoonful) per 1 litre of water	16 g (1 tablespoonful) per 10 litres of water

Disinfection

All objects that have come in contact with potentially infectious materials should be decontaminated.

Decontamination of surfaces

Wear an apron, heavy-duty gloves and other barrier protection if needed. Disinfect surfaces by wiping clean with 1:100 chlorine solution, then incinerate all absorbent material in heavy-duty garbage bags. The surfaces must be rinsed with clean water after disinfection.

Disinfecting surfaces in laboratories where PCR work is undertaken

Disinfect surfaces with 1:100 chlorine solution (more dilute solutions are not effective). The chlorine must subsequently be removed as it is caustic and may damage equipment. This may be done either by wiping the surfaces with clean water or with 70% alcohol (which also has a useful additional effect against most bacteria [not against bacterial spores] and vegetative fungi).

Decontamination of blood or body fluid spills

For spills, use 1:10 chlorine solution to inactivate pathogens before soaking up the fluid with absorbent materials. These absorbent materials must then be incinerated.

Disinfection of hands

The principal means for disinfecting hands is by washing with soap and water. If available, a commercial hand disinfectant containing alcohol, chlorhexidine or polyvidone iodine could be used. The use of strong chlorine solutions (such as 1:100 chlorine solution) should be avoided as it is dangerous).

Sterilization and reuse of instruments and materials

In field outbreak situations, sterilization and reuse of any instruments or materials is not generally advisable. However, it may be necessary to reuse instruments etc. and these should first be disinfected with chlorine, cleaned and then sterilized.

Items such as instruments used for autopsy should be disinfected with 1:10 chlorine solution or 70% ethanol.

Vehicles

Vehicles driven onto potentially infected poultry farms should be rigorously disinfected because influenza viruses may survive for weeks in cool, moist, dark conditions and can easily be spread via mud or faecal contamination on vehicle tires or sub frames. All gross contamination must be removed from vehicles with a power washer and then all surfaces that may have been splashed by mud or faeces on the farm must be sprayed down with 1:10 chlorine solution. Use of a tire bath with 1:10 chlorine for disinfection of tires is ideal (the chlorine solution should be replaced after every two or three vehicles as it will rapidly become exhausted). Operators of power washers must be very well protected due to the high risk of their being sprayed with contaminated material.

Annex 8. Viral transport media (VTM)

a) Specimens from humans

WHO HQ in Geneva has stocks of commercially prepared viral transport media (COPAN Universal Transport Medium).

Another suitable commercially available medium is Eagle Minimum Essential Medium (E-MEM)

Alternatively, VTM can be prepared locally. A suitable VTM for use in collecting throat and nasal swabs from human patients is prepared as follows:

- Add 10g veal infusion broth and 2g bovine albumin fraction V to sterile distilled water (to 400 ml).
- Add 0.8 ml gentamicin sulfate solution (50 mg/ml) and 3.2 ml amphotericin B (250 µg/ml)
- Sterilize by filtration.

b) Specimens from animals

WHO recommends two different transport media for use when taking samples from animals. Of these the transport medium based on tissue culture medium 199 (A) is widely used for collection and transport of clinical specimens from all species. A second medium, the glycerol-based medium given below (B), provides longer-term stability of specimens where cooling is not immediately possible; it is suitable for egg inoculation but not suited for tissue culture inoculation.

Antibiotics and antifungals reduce the risk of bacterial and fungal contamination. With increasing use of these agents in animal husbandry it has become necessary to use high concentrations of them in transport media.

Antibiotics lose their effect over time if they are kept at +4 °C and/or subjected to multiple freeze and thaw cycles. They should either be added to the transport medium when the samples are ready to be collected or added during preparation of the media which should then be frozen at -20 °C and thawed only when needed.

If attempts to isolate virus are to be made, transport media containing specimens should not be stored at -20°C but at -70°C or in a liquid nitrogen storage unit. Storage at -20 °C is adequate if the specimen is to be used for PCR tests only (See Section 4, Table 1).

A. Transport medium 199

1. Tissue culture medium 199 containing 0.5% bovine serum albumin (BSA)
2. To 1 litre of above add:
 - benzylpenicillin (2×10^6 IU/litre)
 - streptomycin (200 mg/litre)
 - polymyxin B (2×10^6 IU/litre)
 - gentamicin (250 mg/litre)
 - nystatin (0.5×10^6 IU/litre)

- ofloxacin hydrochloride (60 mg/litre), and
- sulfamethoxazole (0.2 g/litre)

3. Sterilize by filtration and distribute in 1.0–2.0 ml volumes in screw-capped tubes.

- OR -

B. PBS-Glycerol transport medium

1. Phosphate-buffered saline (PBS):

- NaCl 8g
- KCl 0.2g
- Na₂HPO₄ 1.44g
- KH₂PO₄ 0.24g
- Distilled water to make 1 litre

2. Autoclave PBS and mix 1:1 with sterile glycerol to make 1 litre

3. To 1 litre PBS/glycerol add:

- benzylpenicillin (2×10^6 IU/litre)
- streptomycin (200 mg/litre)
- polymyxin B (2×10^6 IU/litre)
- gentamicin (250 mg/litre)
- nystatin (0.5×10^6 IU/litre)
- ofloxacin hydrochloride (60 mg/litre), and
- sulfamethoxazole (0.2 g/litre)

Dispense 1.0–2.0 ml of transport medium into sterile plastic screw-cap vials (Cryovials). It is best to store these vials at –20 °C until used. However, they can be stored at +4 °C for 48–96 hours (optimally less than 48 hours) or at room temperature for short periods of 1–2 days.

Note:

Normal saline (NS) solution should **not** be used as a VTM. Adding BSA and antibiotics to NS changes the pH and this will destroy viruses.

Annex 9. Field data sheets

Annex 9. Field data sheets

Specimen collection form (human cases)

Name of person taken the specimen:		Contact details for specimen collector : (phone, email address)	
Date of birth of patient (dd/mm/yy):		Family name of patient:	
Sex of patient: M F	Nationality of patient:	Occupation of patient:	
Patient's hospital/clinic number:			
Patient's address (if available or a suitable contact address)			
House number or name:	Street name:	Town:	Country:
District:	Postal code:		
Place where specimens were taken: Hospital: Y N Clinic: Y N Home: Y N Other (specify): Y N			
Address (if different from above):			
Unique identifying number	Type of specimen	Date of collection	Clinical diagnosis
			Health of patient when specimen collected
			Remarks

Specimen collection form : Animal specimens

Please write additional details on the back of the form if there is not enough space below

Name , address and phone number of person collecting specimens:		Name , address and phone number of owner of animals/birds:		Date specimens collected (dd/mm/yy):					
		Location (give GIS coordinates if available)							
Description of husbandry practices		Details of any vaccination programmes that have been undertaken on the farm		Any treatments given to animals/birds involved. If Yes, give details					
Give any relevant history or epidemiological details		Any other animals/birds on the farm /in the house?		Numbers in herd/flock affected					
Unique ID number	Type of animal	Scientific name (Genus, species) (If not known take a digital photograph)	Local name	Age of animal (juvenile or adult is sufficient)	Sex of animal	Wild or domestic	If wild bird, is it migratory or resident	Health of animal H= Apparently healthy S=sick D=dead	Notes

Annex 10. Guidance on regulations for the Transport of Infectious Substances

WHO/CDS/CSR/LYO/2005.22

**Guidance on regulations for
the**

**Transport of Infectious
Substances**

September 2005



Communicable Disease Surveillance and Response

Guidance on regulations for the

Transport of Infectious Substances

2005



**World Health
Organization**

Communicable Disease Surveillance and Response

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Introduction

These guidelines provide practical guidance to facilitate compliance with current international regulations for the transport of infectious substances and patient specimens by all modes of transport, both nationally and internationally, and include the changes that apply from 1 January 2005. They replace the guidelines issued by the World Health Organization (WHO) in 1997 (document WHO/EMC/97.3). This publication, however, does not replace national and international transport regulations.

The latest regulations are based on a completely new system and are no longer related to the Risk Group concept used until the end of 2004. The rationale for the new system is set out in document WHO/CDS/CSR/LYO/2004.9 entitled *Background to the amendments adopted in the 13th revision of the United Nations Model Regulations guiding the transport of infectious substances* (http://www.who.int/csr/resources/publications/WHO_CDS_CSR_LYO_2004_9/en/).

The following guidelines provide information for classifying infectious substances for transportation and ensuring their safe packaging. They stress the importance of developing a working relationship between those involved – the sender, the carrier and the receiver – in order to provide for safe and expeditious transport of these materials.

Postal, airline and other transport industry personnel have concerns about the possibility of becoming infected as the result of exposure to infectious microorganisms that may escape from broken, leaking or improperly packaged material. The packaging of infectious substances for transport must therefore be designed to minimize the potential for damage during transport. In addition, the packaging must ensure the integrity of the materials and so, in turn, timely and accurate processing of specimens.

There are no recorded cases of illness attributable to the release of infectious substances or diagnostic specimens during transport, although there are reported incidents of damage to improperly and sometimes even properly packaged materials. The shipment of unmarked and unidentified infectious substances, improperly packaged, obviously increases the overall potential for exposure to all persons. Damage to packaging also means that samples dispatched for analysis, generally an urgent task, are unlikely to arrive at their destination on time.

International regulations

The international regulations for the transport of infectious substances by any mode of transport are based upon the Recommendations made by the Committee of Experts on the Transport of Dangerous Goods (UNCETDG), a committee of the United Nations Economic and Social Council. The Recommendations are presented in the form of Model Regulations. The United Nations Model Regulations are reflected in international law through international modal agreements (links to further information are provided in Annex 1):

Air The *Technical Instructions for the Safe Transport of Dangerous Goods by Air* published by the International Civil Aviation Organization (ICAO) are the legally binding international regulations. The International Air Transport Association (IATA) publishes Dangerous Goods Regulations (DGR) that incorporate the ICAO provisions and may add further restrictions (where necessary such restrictions are included in these guidelines). The ICAO rules apply on all international flights. For national flights, i.e. flights within one country, national civil aviation authorities apply national legislation. This is normally based on the ICAO provisions, but may incorporate variations. State and operator variations are published in the ICAO Technical Instructions and in the IATA Dangerous Goods Regulations.

- Rail** Regulations concerning the *International Carriage of Dangerous Goods by Rail* (RID) apply to countries in Europe, the Middle East and North Africa. RID also applies to domestic transport in the 25 countries of the European Union through Council Directive 96/49/EC.
- Road** The *European Agreement concerning the International Carriage of Dangerous Goods by Road* (ADR) applies to 40 countries. In addition, modified versions of the convention are being used by countries in South America and South-East Asia. ADR also applies to domestic transport in the 25 countries of the European Union through Council Directives 94/55/EC.
- Sea** The *International Maritime Dangerous Goods Code* published by the International Maritime Organization (IMO) is of mandatory application for all 155 contracting parties to the International Convention for the Safety of Life at Sea (SOLAS).
- Post** The *Letter post manual* published by the Universal Postal Union (UPU) reflects the United Nations Recommendations using the ICAO provisions as the basis for shipments.

The World Health Organization serves in an advisory capacity to UNCETDG and ICAO.

National regulations

Many countries adopt the United Nations Model Regulations in their entirety to stand as their national dangerous goods legislation. Some countries apply variations. National authorities should provide details of their own national requirements.

Note: These guidelines are based on the 13th revised edition of the United Nations Recommendations on the Transport of Dangerous Goods, the text of which is reflected in the 2005 editions of the international modal regulations, and in many sets of national legislation. In December 2004, UNCETDG agreed on further changes for the 14th edition. These changes do not come into force until 2007. However, some of them are covered in these guidelines as they are being permitted as options for air transport between 2005 and 2007, when they become mandatory. Shippers of infectious substances should check carefully whether such options are also permitted for land transport in the countries of origin and destination. If, in the future, further modifications are made to the section of the United Nations Recommendations that deals with infectious substances and patient specimens, the WHO guidelines will be updated accordingly.

Definitions and classification

In describing transport safety measures, the terms “infectious substances” and “infectious materials” are considered to be synonymous. The term “infectious substances” is used in this document. Text reproduced from the United Nations Model Regulations is italicized.

Infectious substances

For the purposes of transport, infectious substances are defined as substances which are known or are reasonably expected to contain pathogens. Pathogens are defined as microorganisms (including bacteria, viruses, rickettsiae, parasites, fungi) and other agents such as prions, which can cause disease in humans or animals. The definition is applied to all specimens except those explicitly excluded (see below). Infectious substances are divided into two categories.

Cultures (laboratory stocks)

Cultures are the result of a process by which pathogens are amplified and propagated in order to generate high concentration, thereby increasing the risk of infection when exposure to them occurs. This definition refers to cultures prepared for the intentional generation of pathogens and does not include cultures intended for diagnostic and clinical purposes.

The following revision of the definition has been adopted for the 14th edition of the United Nations Model Regulations. ICAO has approved the application of this new text for air transport from 2005, as described in the Addendum No.2 to Doc 9284-AN/905, published in May 2005:

Cultures are the result of a process by which pathogens are intentionally propagated. This definition does not include human or animal patient specimens as defined below. Cultures may be classified as Category A or Category B, depending on the microorganism concerned.

The following additional definition has been adopted for the 14th edition of the United Nations Model Regulations. ICAO has approved the application of this new text for air transport from 2005, as described in the Addendum No.2 to Doc 9284-AN/905, published in May 2005:

Patient specimens

These are human or animal materials, collected directly from humans or animals, including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluid swabs, and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment and prevention.

Biological products

Biological products are those products derived from living organisms which are manufactured and distributed in accordance with the requirements of appropriate national authorities, which may have special licensing requirements, and are used either for prevention, treatment, or diagnosis of disease in humans or animals, or for development, experimental or investigational purposes related thereto. They include, but are not limited to, finished or unfinished products such as vaccines.

Genetically modified microorganisms and organisms

Genetically modified microorganisms and organisms are microorganisms and organisms in which genetic material has been purposely altered through genetic engineering in a way that does not occur naturally. Those genetically modified microorganisms and organisms that do not meet the definition of an infectious substance shall be assigned to UN 3245 and shipped following Packing Instruction P904 (ICAO/IATA PI913) – this is not considered further in these guidelines.

Medical or clinical wastes

Medical or clinical wastes are wastes derived from the medical treatment of animals or humans or from bio-research. Medical or clinical wastes containing Category A infectious substances shall be assigned to UN 2814 or UN 2900 as appropriate. Medical or clinical wastes containing Category B infectious substances, or which are reasonably believed to have a low probability of containing infectious substances, shall be assigned to UN 3291 and shipped following Packing Instruction P621 (ICAO/IATA PI622) – this is not considered further in these guidelines.

Exemptions

Because of the low hazard they present, the following substances of biological origin are exempted from dangerous goods requirements and regulations:

- substances that do not contain infectious substances or will not cause disease in humans or animals
- substances containing microorganisms that are not pathogenic to humans or animals
- substances in a form in which any pathogens present have been neutralized or inactivated such that they no longer pose a health risk
- environmental samples (including food and water samples) that are not considered to pose a significant risk of infection
- blood and/or blood components collected and shipped for the purposes of transfusion and/or transplantation
- dried blood spots and faecal occult blood screening tests
- decontaminated medical or clinical wastes.

The following additional conditional exemptions have been adopted for the 14th edition of the United Nations Model Regulations. ICAO has approved the application of this new text for air transport from 2005, as described in the Addendum No.2 to Doc 9284-AN/905, published in May 2005. However, for the other modes of transport, this text will only be applicable in 2007. The following is an extract from the 14th edition of the United Nations Model Regulations.

Exempt Human/Animal Specimens

Human or animal specimens for which there is minimal likelihood that pathogens are present are not subject to these Regulations if the specimen is transported in a packaging which will prevent any leakage and which is marked with the words "Exempt human specimen" or "Exempt animal specimen", as appropriate. The packaging should meet the following conditions:

The packaging should consist of three components:

- a leak-proof primary receptacle(s);*
- a leak-proof secondary packaging; and*
- an outer packaging of adequate strength for its capacity, mass and intended use, and with at least one surface having minimum dimensions of 100 mm × 100 mm;*

For liquids, absorbent material in sufficient quantity to absorb the entire contents should be placed between the primary receptacle(s) and the secondary packaging so that, during transport, any release or leak of a liquid substance will not reach the outer packaging and will not compromise the integrity of the cushioning material;

When multiple fragile primary receptacles are placed in a single secondary packaging, they should be either individually wrapped or separated to prevent contact between them.

If such a packaging is used it should be marked "Exempt human specimen" or "Exempt animal specimen", as appropriate.

NOTE: *An element of professional judgment is required to determine if a substance is exempt under this paragraph. That judgment should be based on the known medical history, symptoms and individual circumstances of the source, human or animal, and endemic local conditions. Examples of specimens which may be transported under this paragraph include the blood or urine tests to monitor cholesterol levels, blood glucose levels, hormone levels, or prostate specific antibodies (PSA); those*

required to monitor organ function such as heart, liver or kidney function for humans or animals with non-infectious diseases, or therapeutic drug monitoring; those conducted for insurance or employment purposes and are intended to determine the presence of drugs or alcohol; pregnancy test; biopsies to detect cancer; and antibody detection in humans or animals.

Note: Air transport changes in 2005. ICAO has decided that it will make the above provisions mandatory in 2005 when they issue a second Addendum to the 2005/06 edition of the Technical Instructions in the summer of 2005. The addendum will make the above extract from the United Nations Recommendations mandatory, which means that if a medical judgement is made that the sample to be sent is not Category A or Category B then it may be sent in the packaging described above.

General preparation of shipments for transport

Because of the differences in the hazards posed by Category A infectious substances (UN 2814 and UN 2900) and Category B infectious substances (UN 3373), there are variations in the packaging, labelling and documentation requirements for the two categories. The packaging requirements are determined by UNCETDG and are set out as Packing Instructions P620 (PI602 for ICAO/IATA regulations) and P650, reproduced in Annexes 3 and 4, respectively. The requirements are subject to change and regular upgrade by the organizations mentioned. The current packaging requirements are described below.

Note 1: Hand carriage of Category A and Category B infectious substances and transport of these materials in diplomatic pouches are strictly prohibited by international air carriers.

Note 2: Inner packagings containing infectious substances shall not be consolidated with inner packagings containing unrelated types of goods.

Shippers of infectious substances shall ensure that packages are prepared in such a manner that they arrive at their destination in good condition and present no hazard to persons or animals during transport.

Basic triple packaging system

This system of packaging shall be used for all infectious substances. It consists of three layers as follows.

- **Primary receptacle.** A primary watertight, leak-proof receptacle containing the specimen. The receptacle is packaged with enough absorbent material to absorb all fluid in case of breakage.
- **Secondary packaging.** A second durable, watertight, leak-proof packaging to enclose and protect the primary receptacle(s). Several cushioned primary receptacles may be placed in one secondary packaging, but sufficient additional absorbent material shall be used to absorb all fluid in case of breakage.
- **Outer packaging.** Secondary packagings are placed in outer shipping packagings with suitable cushioning material. Outer packagings protect their contents from outside influences, such as physical damage, while in transit. The smallest overall external dimension shall be 10x10 cm.

Each completed package is normally required to be marked, labelled and accompanied with appropriate shipping documents (as applicable). The requirements for these aspects are described below.

Packaging, labelling and documentation requirements for infectious substances in Category A

Packaging

The basic triple packaging system is used with the following additional specifications.

Infectious substances in Category A may only be transported in packaging that meets the United Nations class 6.2 specifications and complies with Packing Instruction P620 (PI602) (see Annex 3; Figure 1). This ensures that strict performance criteria are met; tests for compliance with these criteria include a 9-metre drop test, a puncture test and a pressure test. The outer packaging shall bear the United Nations packaging specification marking (Figure 2), which indicates that the packaging has passed the performance tests to the satisfaction of the competent authority.

The primary receptacle or the secondary packaging shall be capable of withstanding a pressure differential of not less than 95 kPa. The United Nations packaging specification marking alone does not indicate that a test for this has been undertaken, and packaging users should ask their suppliers whether the completed package meets this requirement.

There is no comprehensive list of suppliers of packagings that comply with Packing Instruction P620 (PI602). However, an Internet search using a suitable international or national search engine usually provides appropriate information, as well as access to national regulations. Search phrases such as "UN packaging" and "UN infectious substance packaging" produce extensive results. Carriers and forwarding agents should also be able to supply details of local suppliers or local companies that can provide such information.

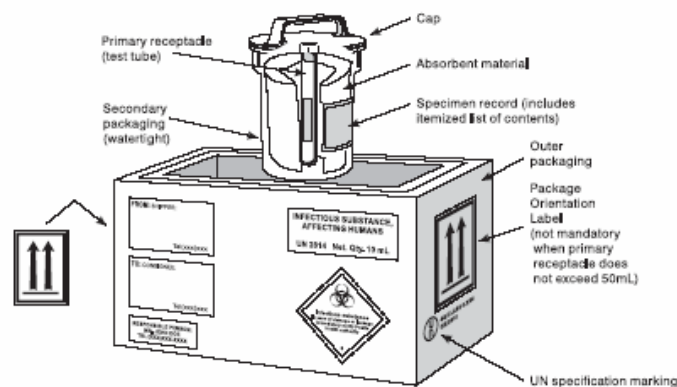


Figure 1. Example of triple packaging system for the packaging and labelling of Category A infectious substances (Figure kindly provided by IATA, Montreal, Canada)


	4G/Class 6.2/05/GB/2470
<p>This marking comprises:</p> <ul style="list-style-type: none">• the United Nations packaging symbol• an indication of the type of packaging (in this example a fibreboard box (4G))• an indication that the packaging has been specially tested to ensure that it meets the requirements for Category A infectious substances (Class 6.2)• the last two digits of the year of manufacture (in this example 2005)• the competent state authority that has authorized the allocation of the mark (in this example GB, signifying Great Britain)• the manufacturer's code specified by the competent authority (in this example 2470) <p>Users shall be provided with clear instructions as to how the package should be filled and prepared for transport.</p>	

Figure 2. Package specification marking for Category A infectious substances (UN 2814 and UN 2900)

The maximum net quantity of Category A infectious substances that can be contained in an outer shipping package is limited to 400 kg for solids and 450 l for liquids for surface transport (road, rail and sea). For air transport the limits per package are as follows:

- 50 ml or 50 g for passenger aircraft
- 4 l or 4 kg for cargo aircraft.

Any primary receptacle with a capacity of more than 50 ml shall be oriented in the outer packaging so that the closures are upwards. Orientation labels ("UP" arrows) shall be affixed to two opposite sides of the outer packaging.

Marking

Packages are marked to provide information about the contents of the package, the nature of the hazard, and the packaging standards applied. All markings on packages or overpacks shall be placed in such a way that they are clearly visible and not covered by any other label or marking. Each package shall display the following information on the outer packaging or the overpack.

- the shipper's (sender's, consignor's) name and address
- the telephone number of a responsible person, knowledgeable about the shipment
- the receiver's (consignee's) name and address
- the United Nations number followed by the proper shipping name (UN 2814 "INFECTIOUS SUBSTANCES AFFECTING HUMANS" or UN 2900 "INFECTIOUS SUBSTANCES AFFECTING ANIMALS", as appropriate). Technical names need not be shown on the package.
- temperature storage requirements (optional)
- when dry ice or liquid nitrogen is used: the technical name of the refrigerant, the appropriate United Nations number, and the net quantity

Labelling

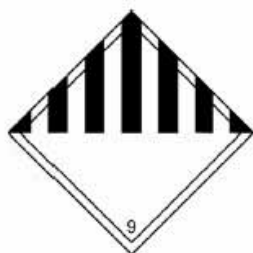
There are two types of labels: (a) hazard labels in the form of a square set at an angle of 45° (diamond-shaped) are required for most dangerous goods in all classes; (b) handling labels in various shapes are required, either alone or in addition to hazard labels, for some dangerous goods. Specific hazard label(s) shall be affixed to the outside of each package for all dangerous goods to be shipped (unless specifically exempted). The hazard labels shown in Figures 3–7 are of importance for infectious substances in Category A:



Label name:	Infectious substance
Minimum dimensions:	100 × 100 mm
(for small packages:	50 × 50 mm)
No. of labels per package:	1
Colour:	Black and white

The words “INFECTIOUS SUBSTANCE” shall be shown. The statement “In case of damage or leakage immediately notify a Public Health Authority” is required in some countries.

Figure 3. Hazard label for Category A infectious substances and for genetically modified microorganisms and organisms that meet the definition of an infectious substance, Category A



Label name:	Miscellaneous dangerous substances
Minimum dimensions:	100 × 100 mm
(for small packages:	50 × 50 mm)
No. of labels per package:	1
Colour:	Black and white

Figure 4. Hazard label for certain noninfectious genetically modified microorganisms and organisms (UN 3245) and for carbon dioxide, solid (dry ice) (UN 1845); substances packed in dry ice (see section on Refrigerants) shall bear this label in addition to the primary risk label (e.g. the label shown in Figure 3 for Category A infectious substances, the marking shown in Figure 10 for Category B infectious substances)



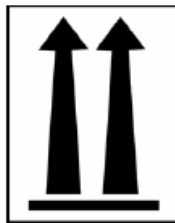
Label name:	Non flammable, non-toxic gas
Minimum dimensions:	100 × 100 mm
(for small packages:	50 × 50 mm)
No. of labels per package:	1
Colour:	Green and white or green and black

Figure 5. Hazard label for liquid nitrogen; substances packed using liquid nitrogen (see section on Refrigerants) shall bear this label in addition to the primary risk label (e.g. the label shown in Figure 3 for Category A infectious substances, the marking shown in Figure 10 for Category B infectious substances)



Label name:	Cryogenic liquid
Minimum dimensions:	Standard A7: 74 × 105 mm
No. of labels per package:	1
Colour:	Green and white

Figure 6. Handling label for cryogenic liquids; for transport by air, where cryogenic liquids (deeply refrigerated liquefied gases) are used (see section on Refrigerants), this label shall be affixed to insulated vessels or flasks used as outer packaging in addition to the labels or markings shown in Figures 3, 5 and 10, as appropriate



Label name:	Orientation label
Minimum dimensions:	Standard A7: 74 × 105 mm
No. per package:	2 on opposite sides
Colour:	Black and white or red and white

The words “THIS SIDE UP” or “THIS END UP” may also be displayed on the top cover of the package.

Figure 7. Orientation label to indicate position of closures on the primary receptacles; for the transport of quantities of liquid infectious substances in Category A that exceed 50 ml per package, this label shall be affixed to two opposite sides of the package with the arrows pointing in the right direction, in addition to the label shown in Figure 3

Instructions for the labelling of overpacks are given in the section on Overpacks.

Documentation

The following shipping documents are required.

To be prepared and signed by the shipper:

- for air: the shipper’s Declaration for Dangerous Goods (Figure 8 shows one example)
- a packing list/proforma invoice that includes the receiver’s address, the number of packages, detail of contents, weight, value (Note: for international transport, a minimal value shall be indicated, for customs purposes, if the items are supplied free of charge)
- an import and/or export permit and/or declaration if required.

To be prepared by the shipper or the shipper’s agent:

- an air waybill for air transport or equivalent documents for road, rail and sea journeys.

For UN 2814 and UN 2900, an itemized list of contents shall be enclosed between the secondary packaging and the outer packaging. When the infectious substance to be transported is unknown, but suspected of meeting the criteria for inclusion in category A and assignment to UN 2814 or UN 2900,

the words "suspected Category A infectious substance" shall be shown, in parentheses, following the proper shipping name on the document inside the outer packaging.


SHIPPER'S DECLARATION FOR DANGEROUS GOODS						
Shipper Hôpital des enfants 5, Rue des Mimosas 05234 Rivière Fleurie - Primance Dr Bedikian tel +0789 456 123			Air Waybill No. 543 7654 9876 Page 1 of 1 Pages Shipper's Reference Number (optional)			
Consignee Laboratorios Biovirobact 5, Calle Escherichia 98675 Eproveta - Polotos Dr Guarquir tel +0520 36 009 832						
Two completed and signed copies of this Declaration must be handed to the operator.			WARNING			
TRANSPORT DETAILS			Failure to comply in all respects with the applicable Dangerous Goods Regulations may be in breach of the applicable law, subject to legal penalties.			
This shipment is within the limitations prescribed for: (delete non-applicable)		Airport of Departure: VILLEBELLE				
PASSENGER AND CARGO AIRCRAFT		<input checked="" type="checkbox"/> PASSENGER AND CARGO AIRCRAFT ONLY				
Airport of Destination: VIALIS			Shipment type: (delete non-applicable) <input type="checkbox"/> NON-RADIOACTIVE <input checked="" type="checkbox"/> RADIOACTIVE			
NATURE AND QUANTITY OF DANGEROUS GOODS						
Dangerous Goods Identification						
UN or ID No.	Proper Shipping Name	Class or Division (Subsidiary risk)	Packing Group	Quantity and type of packing	Packing Inst.	Authorization
UN 2814	Infectious substance, affecting humans, (Ebola virus)	6.2		50 mL	602	
UN 1845	Dry ice	9	III	20 kg All packed in one fibreboard box	904	
Additional Handling Information Emergency contact: Dr Bedikian Tel +0789 456 123						
I hereby declare that the contents of this consignment are fully and accurately described above by the proper shipping name, and are classified, packaged, marked and labelled/placarded, and are in all respects in proper condition for transport according to applicable international and national governmental regulations. I declare that all of the applicable air transport requirements have been met.				Name/Title of Signatory: Dr Bedikian, Goods Receipt & Dispatch Place and Date: Rivière Fleurie, 18 May 2005 Signature (see warning above): 		

Figure 8. Example of a completed shipper's Declaration for Dangerous Goods

Packaging, labelling and documentation requirements for infectious substances in Category B

Packaging

The triple packaging system continues to apply, including for local surface transport. Testing documents are not required, however. It may be possible to source packagings locally rather than finding an authorized supplier, provided that the packaging manufacturer and the shipper can comply fully with the requirements of P650 (see Annex 4; Figure 9).

As for P620, there is no comprehensive list of suppliers of packagings that comply with Packing Instruction P650. However, an Internet search using a suitable international or national search engine usually provides appropriate information, as well as access to national regulations. Search phrases such as "UN packaging" and "UN infectious substance packaging" produce extensive results. Carriers and forwarding agents should also be able to supply details of local suppliers or local companies that can provide such information.

To ensure correct preparation for transport, packaging manufacturers and subsequent distributors shall provide clear instructions to the consignor or persons preparing packages (e.g. patients) on how the packaging should be filled and closed.

For surface transport there is no maximum quantity per package. For air transport:

- no primary receptacle shall exceed 1 l (for liquids) or 1 kg (for solids)
- the volume shipped per package shall not exceed 4 l or 4 kg.

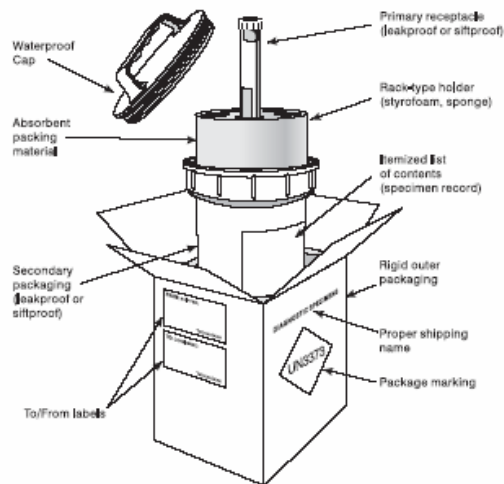


Figure 9. Example of the triple packaging system for the packing and labelling of Category B infectious substances (Figure kindly provided by IATA, Montreal, Canada)

Provided all the requirements of P650 are met, there are no other transport requirements. P650 incorporates all that is needed to make a shipment for Category B infectious substances.

Marking

Each package shall display the following information:

- for air: the shipper's (sender's, consignor's) name, address and telephone number
- for air: the telephone number of a responsible person, knowledgeable about the shipment
- the receiver's (consignee's) name, address and telephone number
- for air: the proper shipping name ("DIAGNOSTIC SPECIMENS" or "CLINICAL SPECIMENS" or "BIOLOGICAL SUBSTANCE, CATEGORY B")
- temperature storage requirements (optional).

The marking shown in Figure 10 is used for shipments of Category B infectious substances.



- Minimum dimension: the width of the line forming the square shall be at least 2 mm, and the letters and numbers shall be at least 6 mm high. For air transport, each side of the square shall have a length of at least 50 mm
- Colour: none specified, provided the mark is displayed on the external surface of the outer packaging on a background of contrasting colour and that it is clearly visible and legible
- For surface transport (by road, rail and sea): no other mark is required
- For air transport: the mark shall be shown but with the following additional information:
The words "DIAGNOSTIC SPECIMENS" or "CLINICAL SPECIMENS" in letters at least 6 mm high shall be displayed adjacent to the mark
From 2007 the name will be "BIOLOGICAL SUBSTANCE, CATEGORY B" for all modes of transport, but this shipping name can be used immediately without contravening the regulations

Figure 10. Marking for infectious substances of Category B and for genetically modified microorganisms or organisms that meet the definition of an infectious substance, Category B

Note: For air transport:

- When dry ice (solid carbon dioxide) is used (see section on Refrigerants), the label shown in Figure 4 shall be applied.
- For cryogenic liquids (see section on Refrigerants) the labels shown in Figures 5 and 6 shall also be affixed.

Documentation

Dangerous goods documentation (including a shipper's declaration) is not required for Category B infectious substances. The following shipping documents are required.

To be prepared and signed by the shipper (sender, consignor):

- for international shipments: a packing list/proforma invoice that includes the shipper's and the receiver's address, the number of packages, detail of contents, weight, value (Note: the statement "no commercial value" shall appear if the items are supplied free of charge)
- an import and/or export permit and/or declaration if required.

To be prepared by the shipper or the shipper's agent:

- an air waybill for air transport or equivalent documents for road, rail and sea journeys.

A flowchart to help with the classification of infectious substances and patient specimens is shown in Annex 5.

Overpacks

"Overpack" is the term used when several packages are combined to form one unit and sent to the same destination by a single shipper. When refrigerants are used to protect contents, the overpacks may comprise insulated vessels or flasks. Whenever an overpack is used, the required marks and labels shown on the outer packaging must be repeated on the outermost layer of the overpack. This requirement applies to infectious substances in Categories A and B. Overpacks are also required to be marked with the word "overpack".

Refrigerants

Refrigerants may be used to stabilize infectious substances in Categories A and B during transit.

Ice or dry ice shall be placed outside the secondary receptacle. Wet ice shall be placed in a leak-proof container; the outer packaging or overpack shall also be leak-proof. Dry ice must not be placed inside the primary or secondary receptacle because of the risk of explosions. A specially designed insulated packaging may be used to contain dry ice. The packaging must permit the release of carbon dioxide gas if dry ice is used. ICAO/IATA Packing Instruction 904 shall be observed.

The secondary receptacle shall be secured within the outer package to maintain the original orientation of the inner packages after the refrigerant has melted or dissipated.

If dry ice is used to ship infectious substances in Category A, the details shall appear on the shipper's Declaration for Dangerous Goods. In addition, the outermost packaging shall carry the hazard label for dry ice (see Figure 4) and the appropriate marking. If dry ice is used to ship infectious substances in Category B, the package shall be marked "Carbon dioxide, solid" or "Dry ice" - this is not considered further in these guidelines.

If liquid nitrogen is used as a refrigerant, special arrangements shall be made in advance with the carrier. Primary receptacles shall be capable of withstanding extremely low temperatures, and packaging and documentation requirements for liquid nitrogen shall be observed. In particular, the outermost packaging shall carry the hazard label for liquid nitrogen (see Figure 5). For air transport, the handling label for cryogenic liquids shall also be affixed (see Figure 6) - this is not considered further in these guidelines.

Training

The dangerous goods regulations require all personnel involved in transport to undergo appropriate training.

For the transport of Category A infectious substances, personnel must undergo training in accordance with the modal requirements. This can involve attendance at approved courses and passing examinations.

For the transport of Category B infectious substances there is a requirement that clear instructions on the use of the packaging are supplied to the user; this is regarded as sufficient “training” for the shipping of these substances. However, if such specimens are consigned with other dangerous goods (e.g. flammable liquids, radioactive materials, liquefied gases, etc.), then personnel must be trained in the proper procedures for their transport.

Training and awareness are important for all personnel involved in the transport of Category B infectious substances. Training of personnel, for example via consultation of guidance documents like this one, while not formally required by the modal regulations, is recommended and encouraged. Only through appropriate guidance and training can shippers ensure that the classification of the substance to be shipped is correct, and that proper packaging is selected and prepared. Carriers and other employers of transport workers should train their personnel in the appropriate procedures for recognizing and handling packages containing infectious substances and in how to address spills and protect themselves from exposure.

Recommendations for countries that have not adopted the United Nations system

The recommendations set out above apply wherever the United Nations system for the transport of infectious substances has been adopted. WHO encourages all countries to adopt this system, and recommends those that have not yet done so to follow its provisions. However, the principles described above are not intended to supersede national or local requirements.

Transport planning

It is the responsibility of the shipper to ensure the correct classification, packaging, labelling and documentation of all infectious substances destined for transport.

The efficient transport and transfer of infectious materials requires good coordination between the sender, the carrier and the receiver to ensure that the material is transported safely and arrives on time and in good condition. Such coordination depends upon well-established communications and a good working relationship between the three parties.

The carriage of any goods whether dangerous or not, is a commercial matter for a carrier. The dangerous goods rules described in these guidelines reflect governmental legal requirements. Indeed, different countries may have adopted State variations to the United Nations Model Regulations. In addition, a carrier that does not wish to carry particular goods is under no legal obligation to do so. Many carriers (airlines, hauliers and shipping lines) are “private carriers” and have the right to refuse to carry goods or add additional requirements. In recent years it has become clear that some carriers are indeed refusing to carry certain goods or are adding extra conditions. Provided such conditions do not conflict with the legal requirements, this type of action is not illegal.

The IATA Dangerous Goods Regulations list the main carrier restrictions in force among airlines. Some airlines will not carry dangerous goods at all, while others will carry only a very limited range

of goods. As carrier restrictions for the different modes of transport are not published centrally, harmonization between stakeholders is essential. The shipper (sender, consignor), carrier and the receiver (consignee) have specific responsibilities in ensuring successful transportation.

The shipper (sender, consignor)

- Makes advance arrangements with the receiver including investigating the need for import/export permits
- Makes advance arrangements with the carrier to ensure:
 - that the shipment will be accepted for appropriate transport
 - that the shipment (direct transport if possible) is undertaken by the most direct routing
- Prepares necessary documentation, including permits, dispatch and shipping documents
- Notifies the receiver of transportation arrangements once these have been made, well in advance of the expected arrival time.

The carrier

- Provides advice to the sender regarding the necessary shipping documents and instructions for their completion
- Provides advice to the sender about correct packaging
- Assists the sender in arranging the most direct routing and then confirms the routing
- Maintains and archives the documentation for shipment and transport.

The receiver (consignee)

- Obtains the necessary authorization(s) from national authorities for the importation of the material
- Provides the sender with the required import permit(s), letter(s) of authorization, or other document(s) required by the national authorities
- Arranges for the most timely and efficient collection on arrival
- Should acknowledge receipt to the sender.

Shipments should not be dispatched until:

- Advance arrangements have been made between the sender, carrier and receiver
- The receiver has confirmed with the national authorities that the material may be legally imported
- The receiver has confirmed that there will be no delay incurred in the delivery of the package to its destination.

Requirements for air mail

Infectious substances in Category A will not be accepted for shipment through postal services.

Infectious substances in Category B may be shipped by registered air mail, and the Universal Postal Union recommends the following procedure.

The basic triple packaging system is used with the same requirements as for other means of transport. The address label shall display the word "*Lettre*" or "Letter" and the green Customs Declaration

Label for Postal Mail is required for international mailing. "DIAGNOSTIC SPECIMENS", "CLINICAL SPECIMENS" or "BIOLOGICAL SUBSTANCE, CATEGORY B" shall be identified with the white diamond label with black letters "UN 3373" (see Figure 10).

Local/international restrictions may be in force. Prior contact should therefore be made with the national public operator to ascertain whether the packaged material will be accepted by the postal service in question.

Spill clean-up procedure

The appropriate response in the event of exposure to any infectious substance is to wash or disinfect the affected area as soon as possible, regardless of the agent. Even if an infectious substance comes into contact with non-intact skin, washing of the affected area with soap and water or with an antiseptic solution can reduce the risk of infection. Medical advice should be obtained any time there is a suspected exposure to infectious substances resulting from a damaged package. The following procedure for clean-up can be used for spills of all infectious substances including blood.

1. Wear gloves and protecting clothing, including face and eye protection if indicated.
2. Cover the spill with a cloth or paper towels to contain it.
3. Pour an appropriate disinfectant over the cloth or paper towels and the immediately surrounding area (5% bleach solutions are generally appropriate, but for spills on aircraft, quaternary ammonium disinfectants should be used).
4. Apply the disinfectant concentrically beginning at the outer margin of the spill area, working towards the centre.
5. After about 30 min, clear away the materials. If there is broken glass or other sharps are involved, use a dustpan or a piece of stiff cardboard to collect the materials and deposit them into a puncture-resistant container for disposal.
6. Clean and disinfect the area of the spillage (if necessary, repeat steps 2–5).
7. Dispose of contaminated materials into a leak-proof, puncture-resistant waste disposal container.
8. After successful disinfection, report the incident to the competent authority and inform them that the site has been decontaminated (see Incident reporting below).

Detailed information on disinfectants and their recommended use can be found in *Laboratory biosafety manual*, 3rd ed., Geneva, World Health Organization, 2004.

Incident reporting

No reports of infections resulting from transport-related exposures have been documented. There have been reports of the transmission of acute respiratory infections and tuberculosis associated with air travel, but these were attributed to direct person-to-person contact and not to packaging problems or shipping incidents.

Statistical data collected by a group of central laboratories showed the efficacy of packaging compliant with P650 and P620 in assuring that infectious substances are transported without leakage and loss of materials. For the 4.92 million primary containers shipped in 2003 to any of the worldwide regional offices of these central laboratories, just 106 breakages, 0.002% of the total number, were recorded. Moreover, the leakages that did occur were all contained by the absorbent material, and no damage to secondary containers or outer packagings was reported.

The various international modal regulations require the reporting of incidents to the relevant competent transport authorities in addition to the necessary health authorities. This applies to both categories of infectious substances, but particularly to those in Category A.

Annex 1

Additional information on the United Nations System for the Transport of Dangerous Goods

The United Nations dangerous goods web site provides comprehensive detail concerning the United Nations Recommendations on the Transport of Dangerous Goods. It also provides links to the modal agencies:

<http://www.unece.org/trans/danger/danger.htm>

The site below provides the full text of the United Nations Recommendations, which can be downloaded in PDF format. Readers wishing to see the text relating to the transport of infectious substances should download Part 2, Part 4 and Part 5 of the Recommendations:

http://www.unece.org/trans/danger/publi/unrec/rev13/13files_e.html

The site below provides the full text of the European Agreement concerning the International Carriage of Dangerous Goods by Road (ADR), which can be downloaded in PDF format. Readers wishing to study the text relating to the transport of infectious substances should download Part 2.2 (2.2.52 to 2.2.7), Part 4 Chapter 4.1 and Part 5:

<http://www.unece.org/trans/danger/publi/adr/adr2003/ContentsE.html>

Contracting parties to the various conventions for the transport of dangerous goods can be found on a number of web sites:

Air ICAO: http://www.icao.org/cgi/goto_m.pl?cgi/statesDB4.pl?en

Rail RID: <http://www.otif.org/>. RID is primarily for the countries of Europe, North Africa and the Middle East. There are a number of countries (mainly Eastern Europe and Asia that apply RID through the Organization for Cooperation of Railways (OSJD); details of RID membership can be found at http://www.otif.org/html/e/pres_cont_gouv_ferr.php

Road ADR: <http://www.unece.org/trans/danger/publi/adr/comp.htm> (lists competent authorities) and <http://www.unece.org/trans/danger/publi/adr/treaty.html> (lists contracting parties)

Sea IMO: <http://www.imo.org/home.asp>

Post UPU: <http://www.upu.int/> The Universal Postal Union develops recommendations on the transport of dangerous goods that are available at http://www.upu.int/acts/en/letter_post_manual.shtml

Annex 2

Examples of infectious substances included in Category A

The table provided below is an indicative list taken from the 13th edition of the United Nations Model Regulations. The air mode (ICAO) has anticipated the classification requirements that will be applicable for other modes in 2007. The relevant changes are indicated in the explanatory notes added to the table.

INDICATIVE EXAMPLES OF INFECTIOUS SUBSTANCES INCLUDED IN CATEGORY A IN ANY FORM UNLESS OTHERWISE INDICATED	
UN Number and Proper Shipping Name	Microorganism
UN 2814 Infectious substances affecting humans	<i>Bacillus anthracis</i> (cultures only)
	<i>Brucella abortus</i> (cultures only)
	<i>Brucella melitensis</i> (cultures only)
	<i>Brucella suis</i> (cultures only)
	<i>Burkholderia mallei</i> – <i>Pseudomonas mallei</i> – glanders (cultures only)
	<i>Burkholderia pseudomallei</i> – <i>Pseudomonas pseudomallei</i> (cultures only)
	<i>Chlamydia psittaci</i> – avian strains (cultures only)
	<i>Clostridium botulinum</i> (cultures only)
	<i>Coccidioides immitis</i> (cultures only)
	<i>Coxiella burnetii</i> (cultures only)
	Crimean-Congo haemorrhagic fever virus
	Dengue virus (cultures only)
	Eastern equine encephalitis virus (cultures only)
	<i>Escherichia coli</i> , verotoxigenic (cultures only)
	Ebola virus
	Flexal virus
	<i>Francisella tularensis</i> (cultures only)
	Guanarito virus
	Hantaan virus
	Hantaviruses causing haemorrhagic fever with renal syndrome
	Hendra virus
	Hepatitis B virus (cultures only)
	Herpes B virus (cultures only)
	Human immunodeficiency virus (cultures only)
	Highly pathogenic avian influenza virus (cultures only)
	Japanese Encephalitis virus (cultures only)
	Junin virus
	Kyasanur Forest disease virus
	Lassa virus
	Machupo virus
Marburg virus	
Monkeypox virus	
<i>Mycobacterium tuberculosis</i> (cultures only)	
Nipah virus	
Omsk haemorrhagic fever virus	

INDICATIVE EXAMPLES OF INFECTIOUS SUBSTANCES INCLUDED IN CATEGORY A IN ANY FORM UNLESS OTHERWISE INDICATED	
	Poliovirus (cultures only)
	Rabies virus (Note: "cultures only" added by the air mode from 2005)
	<i>Rickettsia prowazekii</i> (cultures only)
	<i>Rickettsia rickettsii</i> (cultures only)
	Rift Valley fever virus (Note: "cultures only" added by the air mode from 2005)
	Russian spring-summer encephalitis virus (cultures only)
	Sabia virus
	<i>Shigella dysenteriae type 1</i> (cultures only)
	Tick-borne encephalitis virus (cultures only)
	Variola virus
	Venezuelan equine encephalitis virus (Note: "cultures only" added by the air mode from 2005)
	West Nile virus (cultures only)
	Yellow fever virus (cultures only)
	<i>Yersinia pestis</i> (cultures only)
UN 2900 Infectious substances affecting animals only	African horse sickness virus (Note: deleted by the air mode from 2005)
	African swine fever virus (Note: "cultures only" added by the air mode from 2005)
	Avian paramyxovirus Type 1 – (Note: "Velogenic" added by the air mode from 2005) Newcastle disease virus (Note: "cultures only" added by the air mode in 2005)
	Bluetongue virus (Note: deleted by the air mode from 2005)
	Classical swine fever virus (Note: "cultures only" added by the air mode from 2005)
	Foot and mouth disease virus (Note: "cultures only" added by the air mode from 2005)
	Lumpy skin disease virus (Note: "cultures only" added by the air mode from 2005)
	<i>Mycoplasma mycoides – contagious bovine pleuropneumonia</i> (Note: "cultures only" added by the air mode from 2005)
	Peste des petits ruminants virus (Note: "cultures only" added by the air mode from 2005)
	Rinderpest virus (Note: "cultures only" added by the air mode from 2005)
	Sheep-pox virus (Note: "cultures only" added by the air mode from 2005)
	Goatpox virus (Note: "cultures only" added by the air mode from 2005)
	Swine vesicular disease virus (Note: "cultures only" added by the air mode from 2005)
	Vesicular stomatitis virus (Note: "cultures only" added by the air mode from 2005)

Annex 3

Packing Instruction P620

Infectious substances in Category A and designated as UN 2814 or UN 2900 may only be transported in packaging that meets the United Nations class 6.2 specifications and complies with Packing Instruction P620 (PI602 air mode), which is reproduced below. The various provisions mentioned are set out in the United Nations Model Regulations.


P620	PACKING INSTRUCTION	P620
This instruction applies to UN Nos. 2814 and 2900.		
The following packagings are authorized provided the special packing provisions of 4.1.8 are met: Packagings meeting the requirements of Chapter 6.3 and approved accordingly consisting of:		
(a) Inner packagings comprising:		
(i) watertight primary receptacle(s);		
(ii) a watertight secondary packaging;		
(iii) other than for solid infectious substances, an absorbent material in sufficient quantity to absorb the entire contents placed between the primary receptacle(s) and the secondary packaging; if multiple primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated so as to prevent contact between them;		
(b) A rigid outer packaging of adequate strength for its capacity, mass and intended use. The smallest external dimension shall be not less than 100 mm.		
Additional requirements:		
1. Inner packagings containing infectious substances shall not be consolidated with inner packagings containing unrelated types of goods. Complete packages may be overpacked in accordance with the provisions of 1.2.1 and 5.1.2; such an overpack may contain dry ice.		
2. Other than for exceptional consignments, e.g. whole organs which require special packaging, the following additional requirements shall apply:		
(a) Substances consigned at ambient temperatures or at a higher temperature: Primary receptacles shall be of glass, metal or plastics. Positive means of ensuring a leakproof seal shall be provided, e.g. a heat seal, a skirted stopper or a metal crimp seal. If screw caps are used, they shall be secured by positive means, e.g., tape, paraffin sealing tape or manufactured locking closure;		
(b) Substances consigned refrigerated or frozen: Ice, dry ice or other refrigerant shall be placed around the secondary packaging(s) or alternatively in an overpack with one or more complete packages marked in accordance with 6.3.1.1. Interior supports shall be provided to secure secondary packaging(s) or packages in position after the ice or dry ice has dissipated. If ice is used, the outer packaging or overpack shall be leakproof. If dry ice is used, the outer packaging or overpack shall permit the release of carbon dioxide gas. The primary receptacle and the secondary packaging shall maintain their integrity at the temperature of the refrigerant used;		
(c) Substances consigned in liquid nitrogen. Plastics primary receptacles capable of withstanding very low temperature shall be used. The secondary packaging shall also be capable of withstanding very low temperatures, and in most cases will need to be fitted over the primary receptacle individually. Provisions for the consignment of liquid nitrogen shall also be fulfilled. The primary receptacle and the secondary packaging shall maintain their integrity at the temperature of the liquid nitrogen;		
(d) Lyophilized substances may also be carried in primary receptacles that are flame-sealed glass ampoules or rubber-stoppered glass vials fitted with metal seals.		
3. Whatever the intended temperature of the consignment, the primary receptacle or the secondary packaging shall be capable of withstanding without leakage an internal pressure producing a pressure differential of not less than 95 kPa and temperatures in the range -40 °C to +55 °C.		

Annex 4

Packing Instruction P650

The text of United Nations Packing Instruction 650, in use for the transport of infectious substances in category B assigned to UN 3373 by all surface modes of transport is reproduced below. The shaded text on the right hand side indicates the ICAO variations to these instructions that apply to the transport by air from 2005. The text in bold in the right hand column indicates the changes that will be adopted by the other modes of transport from 2007 and can be used now without contravening current regulations. The various provisions mentioned are set out in the United Nations Model Regulations.

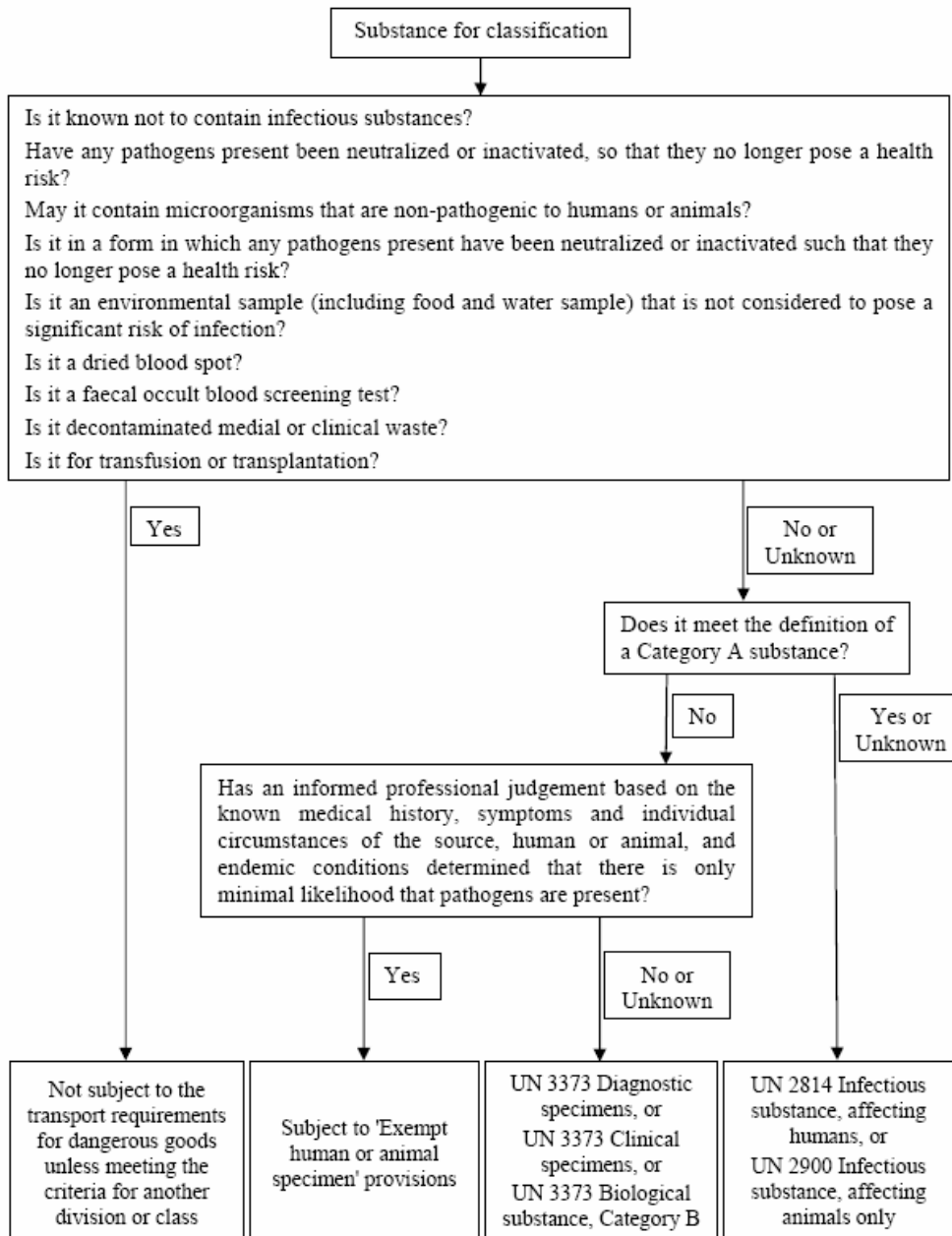
P650	PACKING INSTRUCTION	P650
This packing instruction applies to UN 3373.		
		Variations applying to air transport from 2005
(1)	The packaging shall be of good quality, strong enough to withstand the shocks and loadings normally encountered during transport, including transshipment between transport units and between transport units and warehouses as well as any removal from a pallet or overpack for subsequent manual or mechanical handling. Packagings shall be constructed and closed to prevent any loss of contents that might be caused under normal conditions of transport by vibration or by changes in temperature, humidity or pressure.	
(2)	The packaging shall consist of three components: <ul style="list-style-type: none"> (a) a primary receptacle, (b) a secondary packaging, and (c) an outer packaging 	The outer packaging must be rigid. Note: It is likely that from 2007 there will be requirement for the secondary or the outer packaging to be rigid.
(3)	Primary receptacles shall be packed in secondary packagings in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packagings shall be secured in outer packagings with suitable cushioning material. Any leakage of the contents shall not compromise the integrity of the cushioning material or of the outer packaging.	
(4)	For transport, the mark illustrated below shall be displayed on the external surface of the outer packaging on a background of a contrasting colour and shall be clearly visible and legible. The width of the line shall be at least 2 mm; the letters and numbers shall be at least 6 mm high.	For transport, the mark illustrated below shall be displayed on the external surface of the outer packaging on a background of a contrasting colour and must be clearly visible and legible. The mark must be in the form of a square set at an angle of 45° (diamond-shaped) with each side having a length of at least 50 mm, the width of the line must be at least 2 mm, and the letters and numbers must be at least 6 mm high. The proper shipping name "DIAGNOSTIC SPECIMENS" or "CLINICAL SPECIMENS" in letters at least 6 mm high must be marked on the outer package adjacent to the diamond-shaped mark. Note: From 2007 the terms "DIAGNOSTIC SPECIMENS" or "CLINICAL SPECIMENS" will be replaced by "BIOLOGICAL SUBSTANCE, CATEGORY B" and this name will have to appear on all packages for all modes of transport. ICAO, being the only organization currently requiring names on these packages, has agreed that this new name can be used immediately as an alternative.
		At least one surface of the outer packaging must have a

minimum dimension of 100 mm × 100 mm.	
	
(5)	The completed package shall be capable of successfully passing the drop test in 6.3.2.5 as specified in 6.3.2.3 and 6.3.2.4 of these Regulations except that the height of the drop shall not be less than 1.2 m.
(6)	For liquid substances
(a)	The primary receptacle(s) shall be leakproof; and must not contain more than 1 litre;
(b)	The secondary packaging shall be leakproof;
(c)	If multiple fragile primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated to prevent contact between them;
(d)	Absorbent material shall be placed between the primary receptacle(s) and the secondary packaging. The absorbent material shall be in quantity sufficient to absorb the entire contents of the primary receptacle(s) so that any release of the liquid substance will not compromise the integrity of the cushioning material or of the outer packaging;
(e)	The primary receptacle or the secondary packaging shall be capable of withstanding, without leakage, an internal pressure of 95 kPa (0.95 bar).
(f)	The outer package must not contain more than 4 litres. This quantity excludes ice, dry ice or liquid nitrogen when used to keep specimens cold.
(7)	For solid substances
(a)	The primary receptacle(s) shall be siftproof; and must not exceed the outer packaging mass limit;
(b)	The secondary packaging shall be siftproof;
(c)	If multiple fragile primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated to prevent contact between them.
(d)	Except for packages containing body parts, organs or whole bodies, the outer package must not contain more than 4 kg. This quantity excludes ice, dry ice or liquid nitrogen when used to keep specimens cold;
(e)	If there is any doubt as to whether or not residual liquid may be present in the primary receptacle during transport, then packaging suitable for liquids, including absorbent materials, must be used.
(8)	Refrigerated or frozen specimens: Ice, dry ice and liquid nitrogen
(a)	When dry ice or liquid nitrogen is used to keep specimens cold, all applicable requirements of these Regulations shall be met. When used, ice or dry ice shall be placed outside the secondary packaging or in the outer packaging or an overpack. Interior supports shall be provided to secure the secondary packaging in the original position after the ice or dry ice has dissipated. If ice is used, the outside

<p>packaging or overpack shall be leakproof. If carbon dioxide, solid (dry ice) is used, the packaging shall be designed and constructed to permit the release of carbon dioxide gas to prevent a build-up of pressure that could rupture the packagings and shall be marked "Carbon dioxide, solid" or "Dry ice".</p> <p>(b) The primary receptacle and the secondary packaging shall maintain their integrity at the temperature of the refrigerant used as well as the temperatures and the pressures which could result if refrigeration were lost.</p>	<p>When packages are placed in an overpack, the package markings required by this packing instruction shall either be clearly visible or be reproduced on the outside of the overpack.</p>
<p>(9) Infectious substances assigned to UN 3373 which are packed and marked in accordance with this packing instruction are not subject to any other requirement in these Regulations.</p>	<p>Infectious substances assigned to UN 3373 that are packed and marked in accordance with this packing instruction are not subject to any other requirement in these Instructions except for the following:</p> <p>(a) the proper shipping name, UN number and the name, address and telephone number of a person responsible must be provided on a written document (such as an air waybill) or on the package;</p> <p>(b) classification must be in accordance with provision 2;6.3.2 of the ICAO Technical Instructions;</p> <p>(c) the incident reporting requirements in provision 7;4.4 of the ICAO Technical Instructions must be met;</p> <p>(d) the inspection for damage or leakage requirements in provisions 7;3.1.3 and 7;3.1.4 of the ICAO Technical Instructions;</p> <p>(e) passengers and crew members are prohibited from transporting infectious substances either as, or in, carry-on baggage or checked baggage or on their person.</p>
<p>(10) Clear instructions on filling and closing such packages shall be provided by packaging manufacturers and subsequent distributors to the consignor or to the person who prepares the package (e.g. patient) to enable the package to be correctly prepared for transport.</p>	<p>Other dangerous goods must not be packed in the same packaging as Division 6.2 infectious substances unless they are necessary for maintaining the viability, stabilizing or preventing degradation or neutralizing the hazards of the infectious substances. A quantity of 30 ml or less of dangerous goods included in Classes 3 (flammable liquids), 8 (corrosives) or 9 (miscellaneous dangerous substances and articles) may be packed in each primary receptacle containing infectious substances. When these small quantities of dangerous goods are packed with infectious substances in accordance with this packing instruction no other requirements in these Instructions need to be met.</p> <p>Note: This provision is likely to be applied by all modes from 2007.</p>

Annex 5

Flowchart for the classification of infectious substances and patient specimens



Annex 11. Dry shippers

Dry shippers are large Dewar (vacuum) flasks that are designed for the safe shipment of specimens at liquid nitrogen temperatures without the risk of spilling liquid nitrogen. When prepared correctly a dry shipper does not contain any free liquid nitrogen.



Fig A11.1. A dry shipper and the protective bin in which it is shipped



Fig A11.2 A smaller dry shipper shown

Filling dry shippers

Always follow the manufacturer's instructions for filling. In general:

- Wear a face shield and insulated gloves of the type made for handling liquid nitrogen.
- Always work in well ventilated areas as high concentrations of nitrogen can cause suffocation.
- A significant amount of nitrogen gas will be generated as the cold liquid contacts the warm surfaces inside the shipper. Therefore always add the liquid nitrogen slowly.
- When the liquid reaches the neck of the dry shipper, stop filling. Replace the cap and set the dry shipper aside for the period specified by the manufacturer to allow the liquid nitrogen to saturate the absorbent.
- Repeat steps 1–3 until the liquid level no longer drops on standing. This may require as many as 15 repetitions.

Some manufacturers provide empty and full weights for their dry shippers. If the dry shipper will not reach the expected full weight there may be a problem with the absorbent's ability to hold the nitrogen. This may mean that the low temperature cannot be maintained for as long as is specified in the specifications of the shipper and there is then a risk that the specimens can be damaged. Under these circumstances contact the manufacturer or supplier of the equipment to determine if the dry shipper is safe to use.

Preparing "dry shippers" for transporting specimens

Remove all free liquid nitrogen from the "dry shipper" before transport.

1. Wear insulated gloves, a thermal apron and a face shield when emptying the dry shipper.
2. Empty the dry shipper by pouring the excess liquid nitrogen back into a large liquid nitrogen Dewar flask.
3. If this cannot be done, pour the liquid nitrogen out of the shipper in an appropriate area.
 - a. Do **not** pour liquid nitrogen onto the floor since it could splash onto your shoes or legs and cause severe burns.
 - b. Ensure that any area where liquid nitrogen is poured away is well ventilated.
4. Hold the dry shipper upside down until the liquid stops flowing.
5. Stand the dry shipper upright for the period specified by the manufacturer.
6. Repeat steps 2–4 as many times as necessary to remove any remaining liquid nitrogen.
7. Put your specimens into the dry shipper and replace the cap.
8. If the dry shipper into the protective case supplied by the manufacturer is designed to be shipped in such a protective device (FigA11.1) – not all of them are (Fig A11.2).

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.