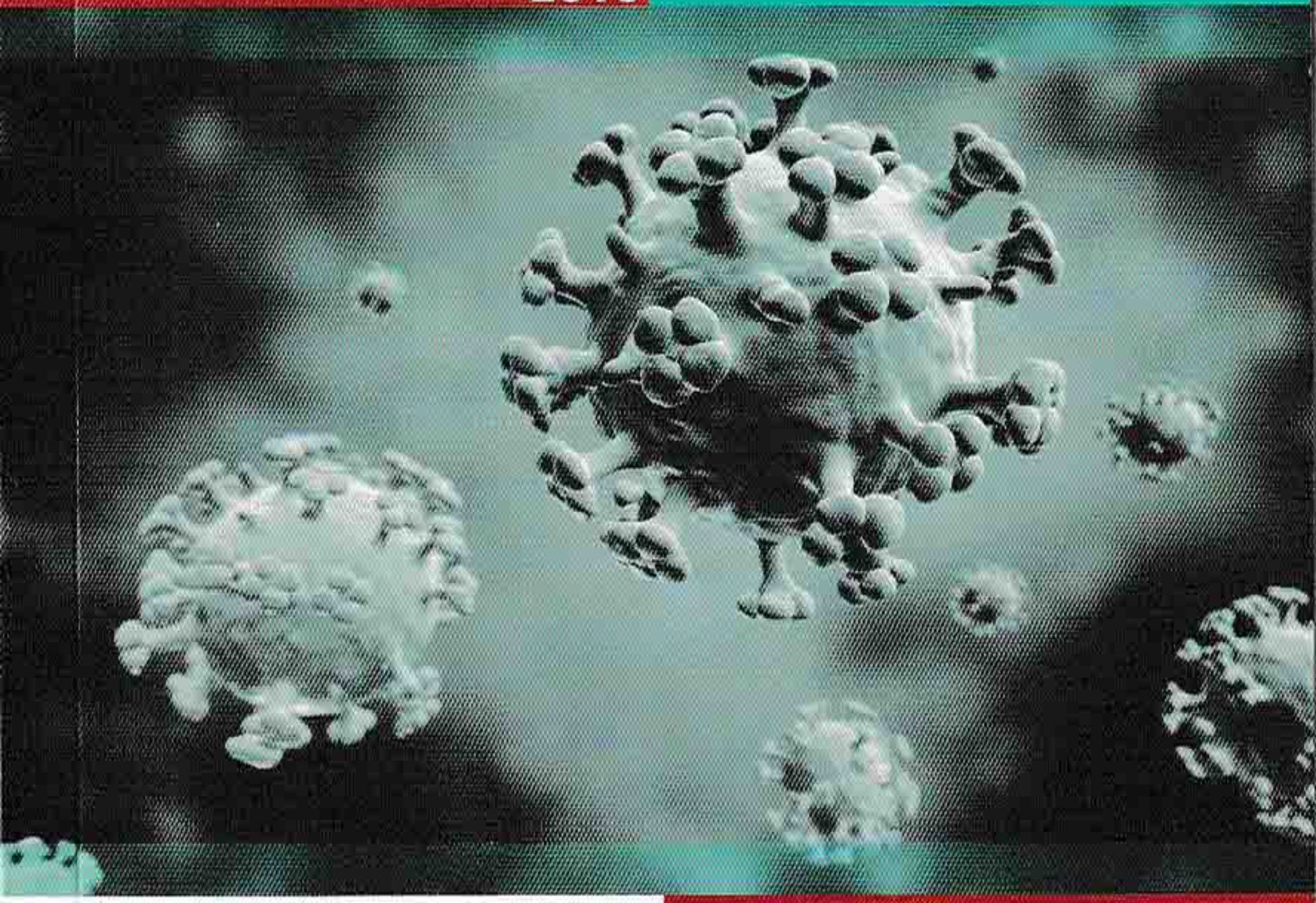
Standard Operating Procedures (SOPs) on Sample Collection, Storage and Transportation to National Influenza Centre (NIC)

2016



IEDCR

World Health Organization
Country Office for Bangladesh

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Institute of Epidemiology, Disease Control and Research (IEDCR)
& National Influenza Centre (NIC)
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Preface

The history of influenza shows that epidemics and especially pandemics extending across the globe and can be devastating. In addition, milder but more frequent influenza episodes cause thousands of deaths as well as considerable economic losses annually. Among the Influenza A, Influenza B and Influenza C, Influenza A which is continuously changing through antigenic drift and shift, poses the global threat. Rapid identification of influenza virus infection can assist healthcare providers in determining optimal strategies for preventing and/or treating influenza. Rapid diagnosis of influenza illness occurring early in the season can be used to prompt members of target groups to receive vaccine before illness becomes widespread in the community. As because influenza viruses undergo constant antigenic change, both virologic and epidemiological surveillance are necessary to identify influenza new virus variants, to monitor their health impact in populations, and to provide data necessary for selection of influenza vaccine strains each year. Knowledge regarding the circulating virus type/subtype can also assist healthcare providers in making treatment decisions. With the increased use of antiviral drugs, virologic surveillance is also important to determine the level of drug-resistance among circulating influenza viruses. Finally, disease surveillance allows for identification of high-risk persons, determination of the effectiveness and strategies for vaccine and antiviral therapy each year. For this, proper sample collection, storage and transportation are essential. The developed SOPs will guide the field team for proper collection, storage and transportation of specimen for influenza during routine surveillance or during any outbreak.

Standard Operating Procedures (SOPs) on Sample Collection, Storage and Transportation to National Influenza Centre (NIC)

1. Introduction

These guidelines are intended for use by health professionals and laboratory staff to ensure safe collection, handling and transport of human specimens for diagnosis of influenza with pandemic potential. The guidelines were compiled with technical assistance from WHO Bangladesh office and they will be updated, as necessary, as more information becomes available, and are to be used in conjunction with usual laboratory standards

2. Background

An influenza pandemic is a likely event to causea large scale public health emergency.

According to WHO pandemic influenza preparedness (PIP) framework and in concert with Bangladesh pandemic response plan, all health care providers should be alert for patients with respiratory illness that could be pandemic influenza. For all suspected patients, respiratory samples for virus detection and acute and convalescent serum samples should be collected.

The overall approach to diagnosis and management will be affected by the WHO/Ministry of Health pandemic alert status. During the early phases of influenza outbreaks, influenza A diagnostic tests with the maximum sensitivity and specificity and a turnaround time of less than 24 hours will be required to ensure accurate identification and to assist a rapid public health response.

The WHO recommended strategy for initial testing of each specimen is to diagnose influenza A virus infection rapidly and exclude other common viral respiratory infections. Investigations for other potential causes of the illness as deemed appropriate by the attending physicians will require other general clinical tests such as biochemical, serological and haematological assays. These Standard Operating Procedures (SOPs) therefore cover both testing for influenza and general diagnostic laboratory testing.[1]

[1] WHO. 2005. WHO laboratory biosafety guidelines for handling specimens suspected of containing avian influenza A virus. Geneva: World Health Organization.
URL: http://www.who.int/csr/disease/avian_influenza/guidelines/handlingspecimens/en/index.html.

The influenza virus is predominantly spread by large droplets and may be spread by direct and indirect contact. Comprehensive biosafety procedures are required in each laboratory, especially where aerosol-producing procedures are being carried out on respiratory samples.

The principle of biosafety protection against influenza A is to establish multiple layers of protection. This principle applies as follows:

1. Personal protection: Laboratory staff should wear personal protective equipment (PPE).
2. Aerosol and droplet precaution: A Biological Safety Cabinet should be used for all technical procedures that may generate aerosols or droplets (eg, vortexing, opening and pipetting specimen tubes).
3. Contact precaution: Decontamination should be performed for all technical procedures that may result in contact contamination.

Operational Definition for NISB

- Influenza Like Illness (ILI)
  ILI: An acute respiratory tract infection with Fever ≥38°C, and Cough with onset within last 10 days
- Severe Acute Respiratory Illness (SARI)
  SARI: An acute respiratory tract infection with Fever ≥38°C, and Cough with onset within last 10 days requiring hospital admissiion.

3. General Procedures

3.1 Specimens

3.1.1 Preferred specimens

Collect upper respiratory tract samples. The specimens of choice are:

1. Nasopharyngeal swab (NPS)
2. Throat swab (TS)

During the early phases of a pandemic, when accurate diagnosis is crucial, take TWO separate samples.

NPSs pose a lower risk to staff during collection than do nasopharyngeal aspirates or nasal washes, both of which may generate aerosols and must be performed in a controlled environment – such as a negative pressure room. Nasal swabs are not recommended as they provide lower yields of virus.

- A NPS can be combined with TS in a single Virus Transport Medium (VTM) tube if necessary.
- If VTM is unavailable, use 1 ml sterile saline, and immerse the swab in the fluid.
- Use VTM-swab tubes that are approved by the receiving laboratory.

3.1.2 Other specimens [2]

Depending on the nature of the outbreak, it may be appropriate to collect other specimens. Anterior nasal swabs are less sensitive. Invasive procedures (such as bronchoalveolar lavage or lung biopsy) may also be performed for the diagnosis of virus infection. The optimal sampling strategy will only be available once the illness caused by PI virus has been defined.

3.1.3 When samples should be collected

It is preferable to take respiratory samples for virus isolation, or for the detection of viral nucleic acids or antigens, during the first three days after the onset of clinical symptoms. However, they may be taken up to a week after the illness onset, or even later in severely ill or immune-compromised patients.

[2] Postmortem samples are not covered in these guidelines.

3.1.4 Blood for Serology

Take an acute phase serum sample (7–10 ml whole blood) as soon as possible after the onset of clinical symptoms and no later than seven days after onset. Collect a convalescent-phase serum sample 14 days later.

3.2 Safety

Persons collecting respiratory samples must be properly trained. The key points are:

- Keep yourself safe.
- Wear personal protective equipment (PPE) and know how to correctly and safely put it on and remove it.
- Keep others safe.
- Label specimen containers before entering the specimen collection room. Labelling should include patient name, ID number (if known), age and date of collection.
- Do not take paperwork, including request forms, into the specimen collection room. On the request form, highlight the words suspected pandemic influenza infection.
- Record contact details for patient and requester. Laboratory staff needs to know who to notify for critical results.
- Alert laboratory staff that samples are being taken, to allow time for preparation.
- Keep sample containers separate from all other samples.
- Integrate testing with patient care.
- Don't let fear of PI interfere with diagnosis and management.

4. Specimen Collection for Influenza Surveillance in Human

4.1 Purposes

4.1.1 To collect appropriate specimens for detection of circulating influenza strains, agent specific antigen and/or antibody

4.1.2 To ensure biosafety and infection control measures during specimen collection

4.2 Applicability

4.2.1 Influenza surveillance

4.2.2 Outbreak investigation of influenza and other respiratory infections in human

4.3 Responsible personnel and their responsibilities:

4.3.1 Designated doctors (Surveillance Physician)

4.3.1.1 Select & enrol patients according to protocol

4.3.1.2 Duly fill-up questionnaires

4.3.1.3 Regularly report to local focal person & national focal person

4.3.1.4 Ensure maintenance of patient (ILI & SARI) records

4.3.1.5 Report & suggest any necessary event involving surveillance activities in hospital.

4.3.2 Qualified pulmonologist

4.3.2.1 Perform Bronchoalveolar lavage (BAL)

4.3.3 Medical technologist (lab)

4.3.3.1 Collection of specimen

4.3.3.2 Help surveillance physician to identify ILI patients

4.3.3.3 Assist surveillance physician to enroll ILI patients

4.3.3.4 Maintain ILI register

4.3.4 Trained Nursing Staff

4.3.4.1 Help surveillance physician to identify SARI patients

4.3.4.2 Assist surveillance physician to enroll SARI patients

4.3.4.3 Assist medical technologist to collect nasal swab & throat swab

4.3.4.4 Maintain SARI register

4.3.5 Trained surveillance assistant (TSA)

4.3.5.1 Coordinate case selection, sample collection, storage and transport at the surveillance site
4.3.5.2 Maintain liaison with the surveillance site and the NIC

4.4 Types of Specimen

4.4.1 Influenza like illness (ILI) cases:

4.4.1.1 Nasal swab
4.4.1.2 Throat swab
4.4.1.3 Nasopharyngeal swab
4.4.1.4 Serum (At first visit and after 14 days)

4.4.2 Severe acute respiratory Illness (SARI)

4.4.2.1 Sputum
4.4.2.2 Nasopharyngeal swab
4.4.2.3 Nasal swab
4.4.2.4 Throat swab
4.4.2.5 Nasopharyngeal wash
4.4.2.6 Nasopharyngeal aspirate
4.4.2.7 Serum (At first visit and after 14 days)
4.4.2.8 Tracheal aspirate
4.4.2.9 Bronchoalveolar lavage (BAL) fluid
4.4.2.10 Body fluid (Pleural fluid, CSF) as per sequel / complications

4.5 Procedure

4.5.1 Nasal swab collection

4.5.1.1 Materials
4.5.1.1.1 Hand sanitizer/ soap and water
4.5.1.1.2 Disposable multilayered surgical Mask
4.5.1.1.3 Lab coat
4.5.1.1.4 Powder free Gloves
4.5.1.1.5 Dacron swab in polythene blister
4.5.1.1.6 Universal Viral Transport Media (VTM)
4.5.1.1.7 Cryo marker
4.5.1.1.8 Labelling Sticker
4.5.1.1.9 Tissue paper

4.5.1.2 Procedure

4.5.1.2.1 Confirm identity of the patient

4.5.1.2.2 Explain the procedures (to the patients and/or attendants)

4.5.1.2.3 Obtain consent (from the patients and/or attendants)

4.5.1.2.4 Wash hands with soap and running water.

4.5.1.2.5 Wear lab coat, mask and gloves.

4.5.1.2.6 Take out the VTM tube and swab sticks from package and check expiry date

4.5.1.2.7 Label the VTM with specimen type, the patient's name, age, ID number and date of collection according to requisition slip.

4.5.1.2.8 Loosen the screw cap of the VTM tube.

4.5.1.2.9 Request the patient to sit on a chair .Keep a hand over the forehead of the patients and request to tilt head slightly backwards.

4.5.1.2.10 Insert the swab at least 2 cm into the nasal cavity along the upper part of the nostril.

4.5.1.2.10: Collection of nasal swab

4.5.1.2.11 Rotate the swab against nasal mucosa for 2 to 3 times.

4.5.1.2.12 Repeat the same procedure with the same swab in other nostril.

4.5.1.2.13 Immediately dip full dacron part of the swab into VTM.

4.5.1.2.14 Press and rotate the swab against the wall of the VTM tube.

4.5.1.2.15: Breaking the stick

4.5.1.2.15 Break the stick at the level of opening of the VTM tube.

4.5.1.2.16 Close the vial loosely by screw cap and prepare for collecting throat swab.

4.5.2 Throat swab

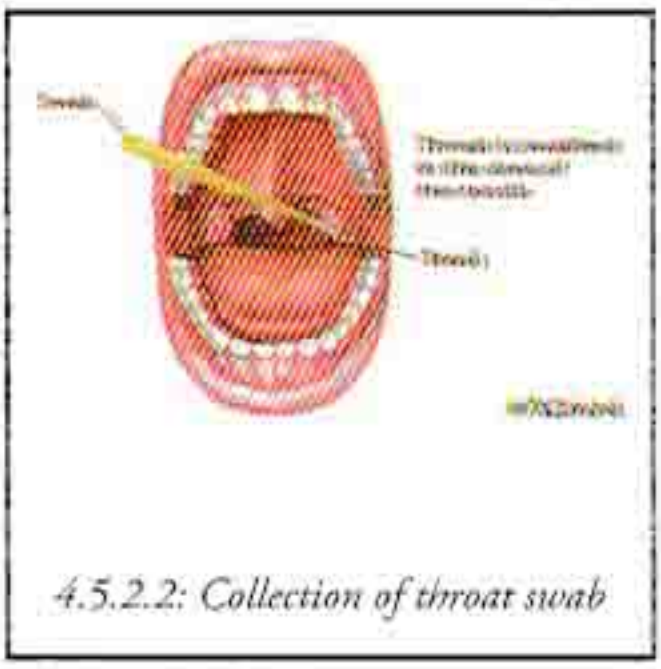
4.5.2.1 Materials

4.5.2.1.1 Hand sanitizer
4.5.2.1.2 Lab coat
4.5.2.1.3 Disposable multilayered surgical Mask
4.5.2.1.4 Powder free Gloves
4.5.2.1.5 Dacron swab in polythene blister
4.5.2.1.6 Universal Viral Transport Media (VTM)
4.5.2.1.7 Cryo marker
4.5.2.1.8 Labeling Sticker
4.5.2.1.9 Tissue paper
4.5.2.1.10 Disposable tongue depressor

4.5.1.2.16: Closing the vial

4.5.2.2 Procedure

4.5.2.2.1 Request the patient to open mouth widely.
4.5.2.2.2 Gently press the tongue by tongue depressor.
4.5.2.2.3 Insert the 2nd sterile swab stick to rub the tonsilar areas and posterior pharynx.



4.5.2.2: Collection of throat swab

N.B: Take care not to touch the tongue.

4.5.2.2.4 Press and rotate the swab against the wall of the VTM tube
4.5.2.2.5 Break the stick at the level of mouth of the VTM tube
4.5.2.2.6 Close the cap tightly to avoid leakage
4.5.2.2.7 Check labelling information
4.5.2.2.8 Store specimen properly (SOP-Specimen storage)

4.5.2.2.5: Breaking the stick

4.5.2.2.6: Closing the vial cap

4.5.3 Nasopharyngeal swab

4.5.3.1 Materials

4.5.3.1.1. Hand sanitizer/ soap and water
4.5.3.1.2. Disposable multilayered surgical Mask
4.5.3.1.3. Lab coat
4.5.3.1.4. Powder free Gloves
4.5.3.1.5. Dacron swab in polythene blister (flexible, fine-shafted)
4.5.3.1.6. Universal Viral Transport Media (VTM)
4.5.3.1.7. Cryo marker
4.5.3.1.8. Labeling Sticker
4.5.3.1.9. Tissue paper

4.5.3.2 Procedure

4.5.3.2.1 Confirm identity of the patient
4.5.3.2.2 Explain the procedures (to the patients and/or attendants)
4.5.3.2.3 Obtain consent (from the patients and/or attendants)
4.5.3.2.4 Wash hands with soap and running water.
4.5.3.2.5 Wear lab coat, mask and gloves.
4.5.3.2.6 Take out the VTM tube and swab sticks from package and check expiry date
4.5.3.2.7 Label the VTM with specimen type, the patient's name, age, ID number and date of collection according to requisition slip.
4.5.3.2.8 Loosen the screw cap of the VTM tube.
4.5.3.2.9 Request the patient to sit on a chair Keep a hand over the forehead of the patients and request to tilt head slightly backwards.
4.5.3.2.10 Insert the swab into the nostril and back to the nasopharynx and left in place for a few seconds
4.5.3.2.11 Rotate the swab against nasopharynx mucosa and withdraw
4.5.3.2.12 Immediately dip full dacron part of the swab into VTM.
4.5.3.2.13 Press and rotate the swab against the wall of the VTM tube.
4.5.3.2.14 Break the stick at the level of opening of the VTM tube.
4.5.3.2.15 Close the cap tightly
4.5.3.2.16 Transfer the VTM in dryshipper

4.5.4 Blood collection

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 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).

In general, Standard precautions should always be practiced 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 and perform hand hygiene every time gloves are removed.

4.5.4.1 Materials

4.5.4.1.1 Lab coat
4.5.4.1.2 Mask
4.5.4.1.3 Powder free Gloves
4.5.4.1.4 Alcohol pad/70% isopropyl alcohol
4.5.4.1.5 Tourniquet,
4.5.4.1.6 Needle – syringe,
4.5.4.1.7 Medical Adhesive tape
4.5.4.1.8 Vacutainer tube (6ml),

4.5.4.1.9 Centrifuge machine,
4.5.4.1.10 Centrifuge tube (1.5ml) / Cryovial,
4.5.4.1.11 Transfer pipettes (Plastic dropper)
4.5.4.1.12 Hand sanitizer

4.5.4.2 Procedure

4.5.4.2.1 Provide explanation of the procedure to the patients and/or attendants

4.5.44.2.2 Obtain informed consent from the patients and/or attendants

4.5.4.2.3 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

4.5.4.2.4 Wash hands with soap and running water thoroughly.

4.5.4.2.5 Wear apron and gloves.

4.5.4.2.6 Palpate the area at the bend of the elbow to locate a good size straight and visible vein without applying a tourniquet.

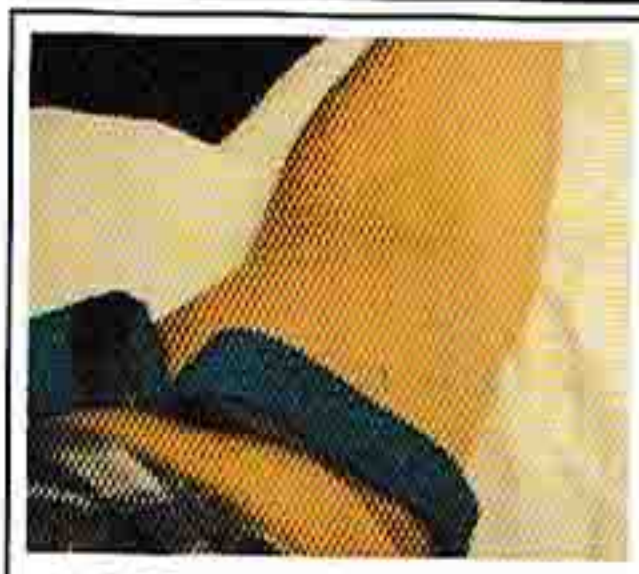
4.5.4.2.7 Apply a tourniquet around the arm and tie approximately 4–5 finger widths above the selected site.

4.5.4.2.8 Ask the patient to form a fist so that the veins are more prominent.

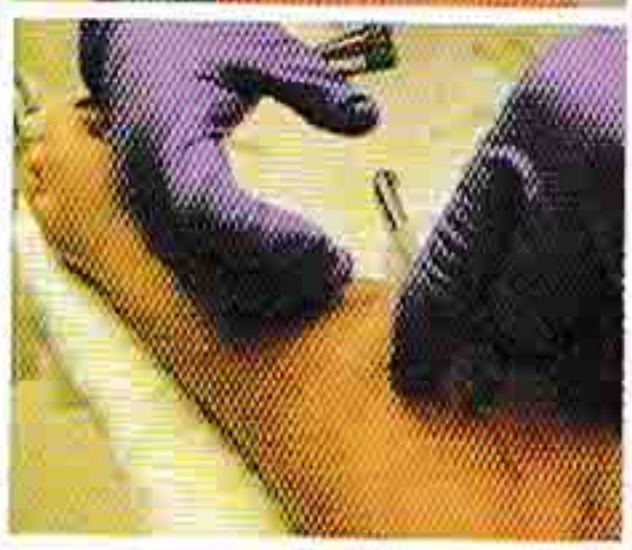
4.5.4.2.9 Rub the area with 70% isopropyl alcohol/ alcohol pad soaked swab starting from the center rotating to periphery up to 2 cm diameter.

4.5.4.2.10 Let the disinfectant evaporate (wait 30 seconds so that rubbed alcohol dries up).

4.5.3.2.6: Palpating the vain



4.5.3.2.7: Applying tourniquet



4.5.3.2.7: Applying tourniquet

4.5.4.2.11 Do not re-palpate the vein.

4.5.4.2.12 Draw 5 ml blood from adult and 3 ml from children in vacutainer tube with a 5 cc syringe.

4.5.4.2.13 Release the tourniquet and put an adhesive bandage on the punctured site.

4.5.3.2.11: Releasing tourniquet

4.5.4.2.14 Keep the vacutainer tube in standing position into the tube holding rack for 30 minutes.

4.5.4.2.15 Centrifuge the vacutainer at 3000 rpm for 5 minutes

4.5.4.2.16 Transfer the serum with a plastic dropper into the 1.5ml centrifuge tube/cryovial.

4.5.4.2.17 Check labelling

4.5.4.2.18 Store properly (SOP- Specimen storage).

4.5.5 Sputum

4.5.5.1 Materials

4.5.5.1.1 Sterile wide mouth screw cap disposable plastic container.

4.5.5.1.2 Water for gargling

4.5.5.1.3 Labelling Sticker

4.5.5.1.4 Hand sanitizer

4.5.5.1.5 Lab coat

4.5.5.1.6 Mask

4.5.5.1.7 Powder free Gloves

4.5.5.1.8 Tissue paper

4.5.5.1.9 Universal VTM

4.5.5.2 Procedure

4.5.5.2.1 Check identity and take consent (from the patients and/or attendants)

4.5.5.2.2 Label the container and give it to the patient

4.5.5.2.3 Send the patients to the designated open space for sputum collection

4.5.5.2.4 Instruct the patients regarding the followings-

- Gargle with water adequately before giving sputum;
- Take a deep breath;
- Cough and expectorate sputum deeply from inside the chest;
- Place the rim of the specimen container under the lower lip;
- Collect all of the expectorated sputum;
- Close the lid of the container and place it on the table;

4.5.5.2.5 Wash hands with soap and running water.

4.5.5.2.6 Wear lab coat, mask and gloves.

4.5.5.2.7 Dip the swab stick in to the sputum and transfer the swab immediately in to the VTM

4.5.5.2.8 Close the VTM tightly

4.5.5.2.9 Store the VTM properly (SOP-Specimen storage)

4.5.6 Bronchoalveolar lavage (BAL)

4.5.6.1 Materials

4.5.6.1.1 Flexible bronchoscope

4.5.6.1.2 Sterile collection trap

4.5.6.1.3 Suction tubing

4.5.6.1.4 Sterile saline

4.5.6.1.5 Vacuum source

4.5.6.1.6 50cc Syringe

4.5.6.1.7 Optional 3 way stop-cock

4.5.6.1.8 Lidocaine 1-2%

4.5.6.1.9 N95 mask for operator and assistant

4.5.6.1.10 Universal Viral transport media (VTM)

4.5.6.1.11 50ml falcon tube

4.5.6.2 Procedure

4.5.6.2.1 Obtain informed consent.

4.5.6.2.2 Review radiographs to determine ideal site of alveolar lavage.

4.5.6.2.3 (In diffuse infiltrates, the right middle lobe (RML) or the lingula in the supine patient is preferred).

4.5.6.2.4 Prepare bronchoscope, collection trap, and tubing.

4.5.6.2.5 Prepare supplemental oxygen and monitoring equipment.
4.5.6.2.6 (ECG, pulse-oximetry, BP cuff).
4.5.6.2.7 Premedicate with bronchodilators and warm the saline solution
4.5.6.2.8 Position patient, preferably in supine position when approaching RML or lingula.
4.5.6.2.9 Apply monitors and supplemental oxygen.
4.5.6.2.10 Plan to perform the BAL preceding any other planned bronchoscopic procedure to avoid specimen contamination.
4.5.6.2.11 Avoid suctioning prior to obtaining BAL specimen.
4.5.6.2.12 Thoroughly rinse the suction channel with saline prior to the BAL.
4.5.6.2.13 Use the minimum amount of 2% lidocaine topically
4.5.6.2.14 Advance bronchoscope until wedged in a desired sub segmental bronchus at the desired location.
4.5.6.2.15 Infuse 20mL of saline with a syringe, observing the flow of saline at the distal tip of the bronchoscope.
4.5.6.2.16 Maintaining wedge position, apply gentle suction (50-80mmHg), collecting the lavage specimen in the collection trap.
4.5.6.2.17 Repeat steps 13 and 14, up to 5 times as needed (total 100-120 mL), to obtain an adequate specimen (40-60 mL - usually 40-70% recovery of total instillate).
4.5.6.2.18 Observe for flow of bubbles returning from the alveolar space.
4.5.6.2.19 Gentle re-orientation of bronchoscope tip may allow better return of fluid.

N.B.

- Distal airways may collapse at higher negative suction pressures.
- Reduction in pressure or intermittent suctioning may help with distal airway collapse.
- Instructing the patient to inhale and exhale deeply may also help improve return of specimen.
- Higher aliquots and higher total volume can occasionally be used (up to 300 mL).

4.5.6.2.20 Process BAL specimen as soon as possible.
4.5.6.2.21 Observe Patient for a minimum of 1 hour after the procedure, with continued monitoring.

N.B:

- If an outpatient procedure, the patient should be accompanied by an attendant.
- BAL should be planned to be performed prior to any other bronchoscopic procedure.

4.5.7 Tracheal aspirate

Collect specimen only from patients with endotracheal tube in situ.

4.5.7.1 Materials

4.5.7.1.1 Mucus trap (Luken's trap)
4.5.7.1.2 Large volume syringes (50cc) to aspirate tracheal fluid.
4.5.7.1.3 N95 mask for operator and assistant
4.5.7.1.4 Universal Viral transport media (VTM)

4.5.7.2 Procedure

4.5.7.2.1 Attach a sterile catheter to a Lukens trap and carefully pass the catheter through the endotracheal tube
4.5.7.2.2 Apply suction to aspirate the sample into the Lukens trap.
4.5.7.2.3 Place the aspirate into one VTM tube
4.5.7.2.4 Store the VTM properly

4.5.8 Nasopharyngeal wash

4.5.8.1 Materials

4.5.8.1.1 Hand sanitizer
4.5.8.1.2 Gloves
4.5.8.1.3 Mask
4.5.8.1.4 Lab coat
4.5.8.1.5 5 ml syringe
4.5.8.1.6 Blunt cannula (18-22 gauge butterfly) without needle
4.5.8.1.7 Sterile normal saline (0.9% NaCl)
4.5.8.1.8 Sterile plastic container
4.5.8.1.9 Universal Viral Transport Media (VTM)
4.5.8.1.10 Labeling Sticker
4.5.8.1.11 Tissue paper
4.5.8.1.12 Cryo-marker

4.5.8.2 Procedure

4.5.8.2.1 Wash hands
4.5.8.2.2 Wear gloves and mask
4.5.8.2.3 Label the VTM vial as per requisition form
4.5.8.2.4 Draw 0.9% saline up to 3 ml into syringe
4.5.8.2.5 Ask the patient to sit in the front with head leaning forward while occluding one nostril

N.B: In case of children,

- Ask the attendant to sit in front with child in upright position in his/her lap
- Instruct attendant to hold arms and legs of the child firmly.
- Lean child's head forward (by nurse) while occluding one nostril

4.5.8.2.6 Place sterile container close to the nose
4.5.8.2.7 Flush 2-3 ml saline into nostril
4.5.8.2.8 Drain saline into sterile container
4.5.8.2.9 Transfer the contents in VTM
4.5.8.2.10 Check VTM with patient name and ID
4.5.8.2.11 Assess respiratory rate
4.5.8.2.12 Store the VTM properly

4.5.9 Nasopharyngeal aspirate

4.5.9.1 Materials

4.5.9.1.1 Hand sanitizer
4.5.9.1.2 Lab coat
4.5.9.1.3 Mask
4.5.9.1.4 Gloves
4.5.9.1.5 Portable suction pump
4.5.9.1.6 Sterile suction catheter
4.5.9.1.7 Mucus Trap (Luken'strap)
4.5.9.1.8 Universal Viral Transport medium (VTM)

4.5.9.2 Procedure

4.5.9.2.1 Wear lab coat, gloves, and mask
4.5.9.2.2 Label the VTM tube properly
4.5.9.2.3 Collect nasopharyngeal secretions by aspiration through a catheter connected to a mucus trapand fitted to a vacuum source.
4.5.9.2.4 Insert the catheter for 5-6 cm into the nasal cavity parallel to the palate.
4.5.9.2.5 Apply the vacuum andwithdraw the catheter slowly with a rotating motion.
4.5.9.2.6 Collect mucus from the other nasal cavity with the same catheter in a similar manner.
4.5.9.2.7 Flush the catheter with 3 ml of transport medium after collection of specimen from both nasal cavities.,
4.5.9.2.8 Close the VTM tube tightly
4.5.9.2.9 Store properly (SOP-Specimen storage)

4.6 Infection control and waste management

4.6.1 Infection control

4.6.1.1 Wear lab coat, mask and gloves before handling any specimen.
4.6.1.2 Wipe the work bench with 70% alcohol
4.6.1.3 Wash hand with soap and running water

4.6.2 Waste disposal

4.6.2.1 Dispose gloves, mask in biohazard bag
4.6.2.2 Close the biohazard bag and keep for final disposal after autoclaving.

5. Specimen Storage for Influenza Surveillance in Human

5.1 Purposes

5.1.1 To ensure safe storage of specimen for the prevention of contamination, putrefaction and leakage.

5.1.2 To ensure biosafety, biosecurity and infection control

5.2 Applicability

5.2.1 National Influenza surveillance

5.2.2 Outbreak investigation of influenza and other respiratory infections in human

5.3 Responsible personnel and their responsibilities:

5.3.1 Designated doctors

5.3.1.1 Ensure biosafety and biosecurity of the specimen

5.3.2 Medical technologist (lab)

5.3.2.1 Ensure appropriate storage of the specimens at appropriate temperature.

5.3.3 Trained surveillance assistant (TSA)

5.3.3.1 Overall coordination for storage at the surveillance site

5.3.3.2 Maintain a liaison with the surveillance site and the NIC

5.4 Procedure at field sites

5.4.1 Materials

5.4.1.1 Lab grade refrigerator (2-8°C)

5.4.1.2 Dry Shipper

5.4.1.3 Hand sanitizer: 70% Alcohol

5.4.1.4 Gloves

5.4.1.5 Mask

5.4.1.6 Lab Goggles

5.4.2 Procedure

5.4.2.1 Where dry shipper available-

5.4.2.1.1 Wear double gloves

5.4.2.1.2 Check for labelling of the specimens

5.4.2.1.3 Wear goggles

5.4.2.1.4 Open the lid of the dryshipper

N.B: Keep your face away from opening

5.4.2.1.5 Bring out desired number of canisters

5.4.2.1.6 Close the lid of dry shipper immediately

5.4.2.1.7 Put the specimen tube/s into the canister

5.4.2.1.8 Keep the canister back in dry shipper.

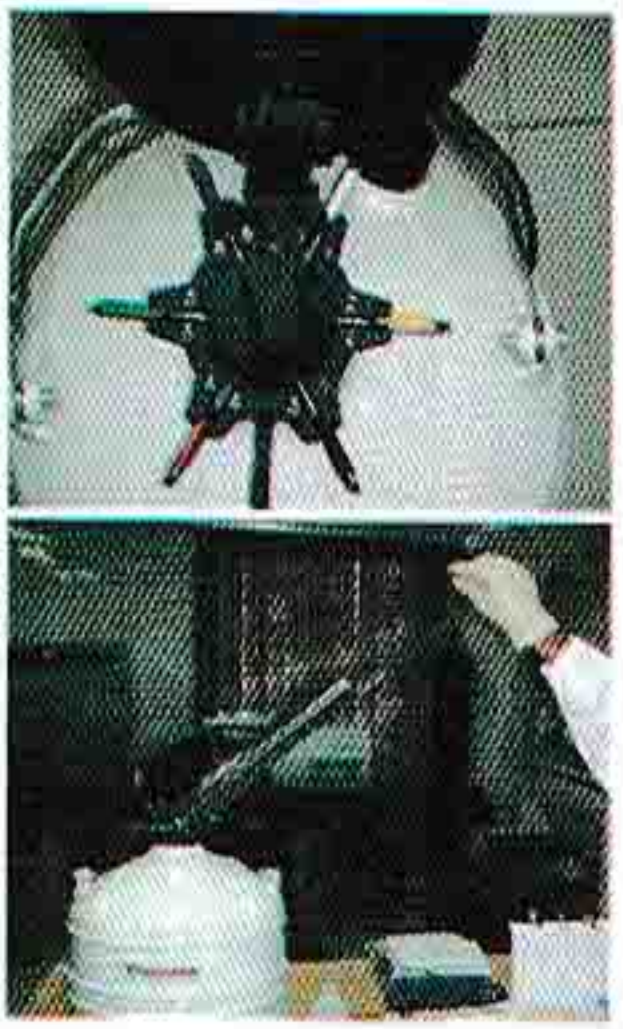
5.4.2.1.9 Close the lid of dry-shipper immediately.

5.4.2.1.10 Remove gloves and maintain hand hygiene.

5.4.2.1.4: Opening dry shipper

N.B:

- Do not store specimen in dry shipper more than 2 wks.
- Dry shipper to be handled by trained personnel.
- To avoid frequent opening of dry shipper gather all specimen containers before opening of the dry shipper



2.4.2.1.5: Canister collection

5.4.2.2 Where dry shipper not available

5.4.2.2.1 Wear gloves

5.4.2.2.2 Wear mask

5.4.2.2.3 Check for labelling of the specimens

5.4.2.2.4 Store specimen tube/s in a refrigerator at 2-8°C for up to 48 hours.

2.4.2.1.7: Putting specimen in canister

5.5 Sample Storing Procedure at NIC, IEDCR

5.5.1 Materials needed:

5.5.1.1 Lab grade refrigerator (4°C)
5.5.1.2 Ultra low temperature freezer (-80°C)
5.5.1.3 Hand sanitizer: 70% Alcohol
5.5.1.4 Gloves
5.5.1.5 Mask
5.5.1.6 Full barrier PPE (During handling outbreakspecimen)
5.5.1.7 Microcentrifuge tube
5.5.1.8 Micropipette and tips
5.5.1.9 Cryo -marker
5.5.1.10 Plastic tray with Icepack
5.5.1.11 Biosafety cabinet (BSC-2)

5.5.2 Procedure

5.5.2.1 For specimens transported by dry shipper-
5.5.2.1.1 Check the list of the specimens with log
5.5.2.1.2 Wear appropriate PPE
5.5.2.1.3 Wear double gloves
5.5.2.1.4 Wear goggles
5.5.2.1.5 Take a tray with 3-4 icepacks over it
5.5.2.1.6 Open the lid of the dryshipper (N:B Keep your face away from opening)
5.5.2.1.7 Check for specimen rejection criteria
5.5.2.1.8 Bring out the canisters one by one from the dry shipper.
5.5.2.1.9 Empty all the canisters over the tray containing ice packs
5.5.2.1.10 Wait for 30 min
5.5.2.1.11 Bring the specimens to room temperature
5.5.2.1.12 Take all specimens to BSC-2
5.5.2.1.13 Vortex specimen for 1 min
5.5.2.1.14 Centrifuge specimens at 6000g for 1 min at 4°C
5.5.2.1.15 Remove swab sticks from VTM after squeezing against the tube wall
5.5.2.1.16 Label 1 microcentrifuge tubes and 3 Storage vials for each specimen
5.5.2.1.17 Transfer the specimens from primary container to microcentrifuge tubes and store vials.
5.5.2.1.18 Keep 1 microcentrifuge tubes at 4°C for RNA extraction
5.5.2.1.19 Keep the 3storage vials at -80°C with proper documentation

5.5.2.2 For specimens transported by cool box-

5.5.2.2.1 Wear appropriate PPE
5.5.2.2.2 Open the cool box
5.5.2.2.3 Check for specimen rejection criteria
5.5.2.2.4 Check the list of the specimens with log
5.5.2.2.5 Take all specimens tube to BSC-2
5.5.2.2.6 Vortex specimen for 1 min
5.5.2.2.7 Centrifuge specimens at 6000g for 1 min at 4°C
5.5.2.2.8 Remove swab sticks from VTM after squeezing against the tube wall
5.5.2.2.9 Label 1 microcentrifuge tubes and 3 Storage vials for one specimen
5.5.2.2.10 Transfer the specimens from primary container to microcentrifuge tubes and store vials.
5.5.2.2.11 Keep 1 microcentrifuge tubes at 4°C for RNA extraction
5.5.2.2.12 Keep the 3storage vials at -80°C with proper documentation

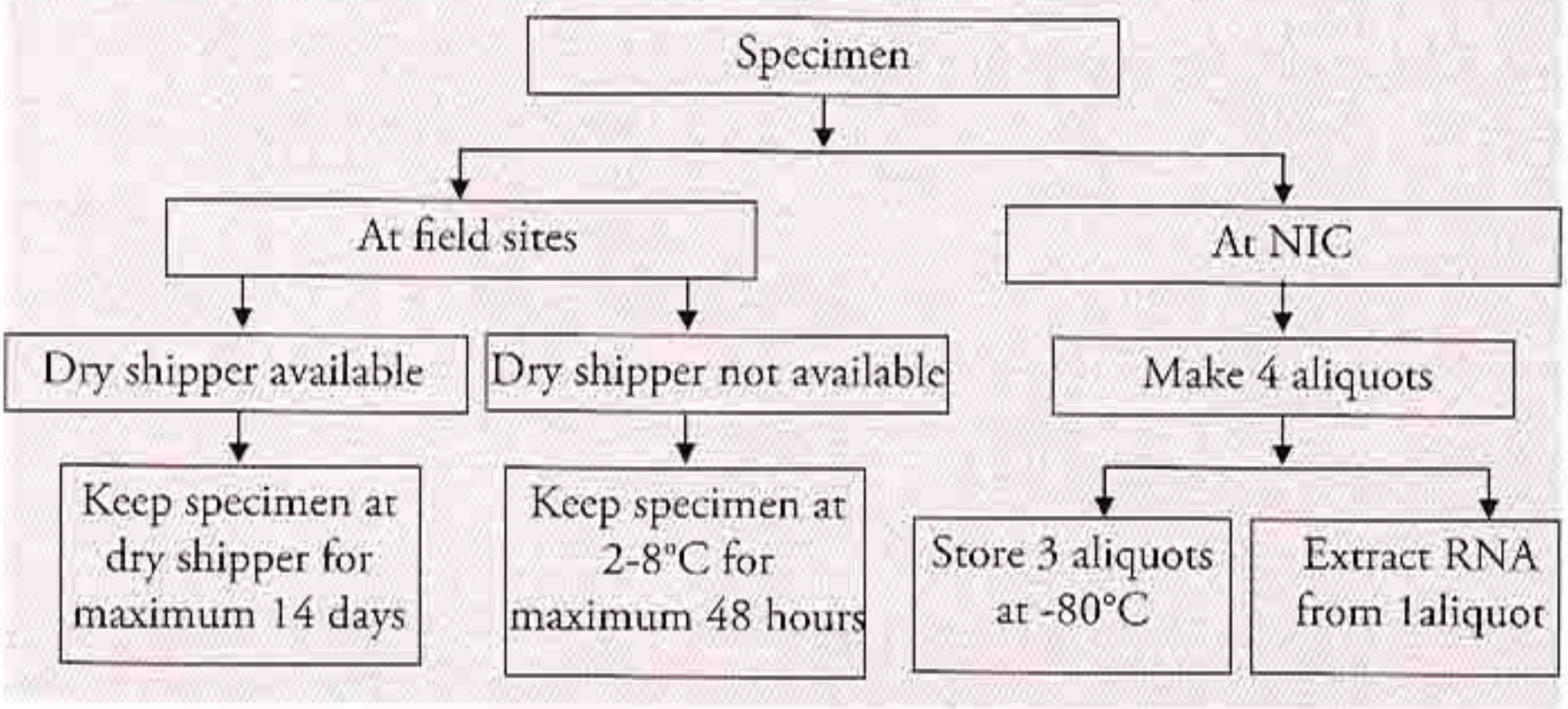
5.6 Infection control and waste management

5.6.1 Infection control

5.6.1.1 Wear lab coat, mask and gloves before handling any specimen container
5.6.1.2 Wipe the work bench with 70% alcohol
5.6.1.3 Wash hand with soap and water

5.6.2 Waste disposal

5.6.2.1 Dispose gloves, mask in biohazard bag
5.6.2.2 Close the biohazard bag and keep for final disposal after autoclaving.



6. Specimen Transport for Influenza Surveillance in Human

6.1 Purpose

6.1.1 To ensure safe and timely transportation of clinical specimen maintaining appropriate temperature

6.1.2 To ensure biosafetyand biosecurityduring transportation

6.2 Applicability

6.2.1 National Influenza surveillance

6.2.2 Outbreak investigation of respiratory infectionsin human

6.3 Responsible personnel and their responsibilities

6.3.1 Medical technologist (lab)

6.3.1.1 Ensure transportation of the specimens at appropriate temperature

6.3.1.2 Ensure all necessary documents are transported along with the specimens

6.3.1.3 Inform NIC before transportation

6.3.2 Trained surveillance assistant (TSA)

6.3.2.1 Overall coordination for transportation from the surveillance sites

6.3.2.2 Ensure all necessary documents are transported along with specimens

6.3.2.3 Inform NIC before transportation

6.3.2.4 Maintain a liaison with the surveillance site and the NIC

6.3.3 Lab attendant/MLSS

6.3.3.1 Ensure safe transportation timely

6.3.3.2 Hand over the specimens along with necessary documents to designated person at NIC

6.4 Transport of surveillance specimen

6.4.1 Materials

6.4.1.1 Dry Shipper
6.4.1.2 Cryomarker
6.4.1.3 Hand sanitizer: 70% Alcohol
6.4.1.4 Gloves

6.4.2 Procedure

6.4.2.1 Take out the dry shipper from the outer plastic container.
6.4.2.2 Label with mailing address (To and From)
6.4.2.3 Ship the dry shipper by lab attendant/MLSS
6.4.2.4 Include an itemized list of specimens with identification numbers and lab instructions.

2.4.2.1.7: Putting specimen in canister

Transport within 2 weeks from date of dry shipper refill

6.5 Transport of outbreak specimen

During any outbreak, handle specimen more cautiously as it may contain new viral strains

6.5.1 Materials

6.5.1.1 Lab coat
6.5.1.2 Gloves
6.5.1.3 Disposable mask/N95 mask
6.5.1.4 Tissue paper
6.5.1.5 Cool box
6.5.1.6 Ice packs / Dry Shipper
6.5.1.7 Cryo marker for labellingaddress
6.5.1.8 Hand sanitizer : 70% Alcohol
6.5.1.9 Disinfectants: 0.5% Na- hypochlorite solution

6.5.2 Procedure

6.5.2.1 Contact with responsible person of NIC: Virology Laboratory, IEDCR before transportation.
6.5.2.2 Wear lab coat, gloves and mask
6.5.2.3 Take out the ice packs from freezer and keep it at room temperature for 20 min for conditioning
6.5.2.4 Take out the specimen from refrigerator/ freezer
6.5.2.5 Place the specimen containers (VTM) in to a specimen rack
6.5.2.6 Wrap the specimenrack with sufficient amount of absorbent paper e.g. tissue paper
6.5.2.7 Place the ice packs inside the cool box besides the walls.
6.5.2.8 Put the specimen rack inside the cool box.
6.5.2.9 Close the cool box and Label with receiver and sender address
6.5.2.10 Send the cool box by lab attendant/ MLSS
6.5.2.11 Include an itemized list of specimens with identification numbers and lab instructions
6.5.2.12 Wash hands thoroughly with soap and water

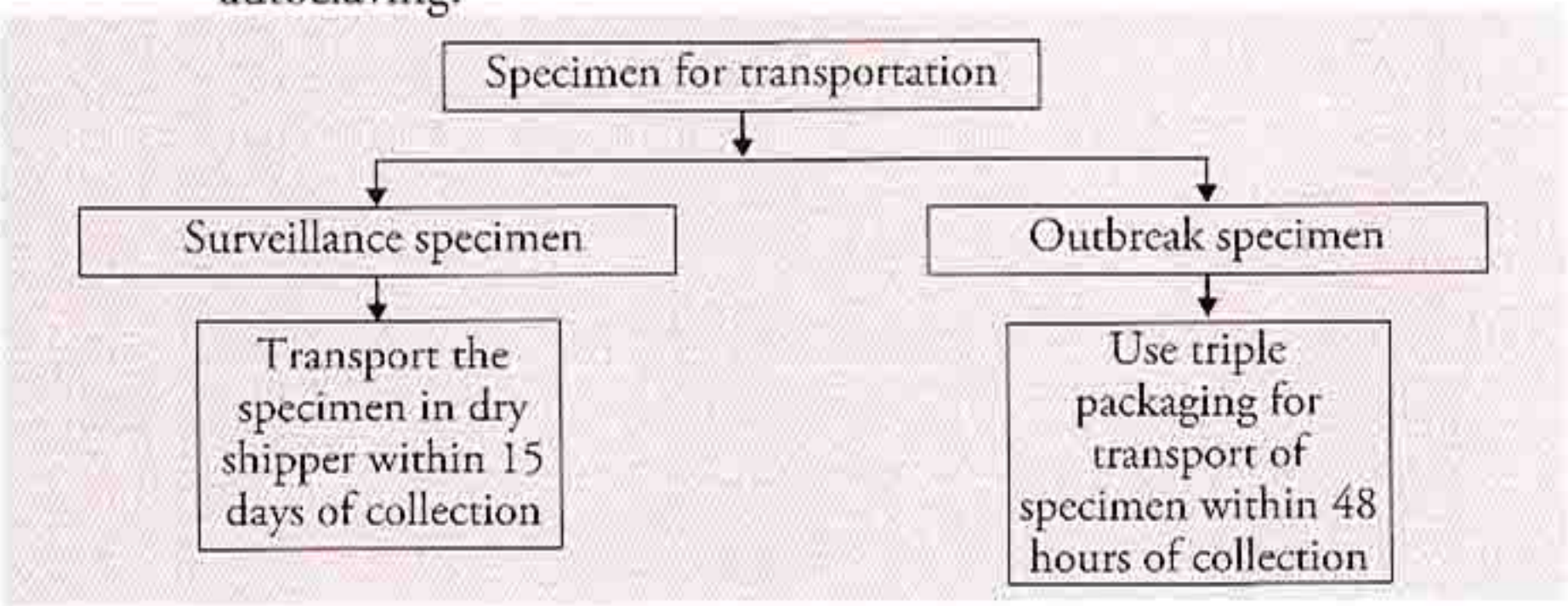
6.6 Infection control and waste disposal

6.6.1 Infection control

6.6.1.1 Wear lab coat, mask and gloves before handling any specimen container
6.6.1.2 Wipe outside of the cool box with 0.5% hypochlorite solution
6.6.1.3 Wipe the work bench with 70% alcohol
6.6.1.4 Wash hand with soap and water

6.6.2 Waste disposal

6.6.2.1 Dispose gloves, mask in biohazard bag
6.6.2.2 Close the biohazard bag and keep for final disposal after autoclaving.



Annexure I

During collection of specimens the following should kept in mind

I. Blood specimen

b. Collect convalescent phase sera specimens after fourteen (14) days of onset of symptoms.

II.Respiratory specimen

a. For ILI patients, collect both the nasal and throat swab.
b. For SARI patients, collect nasal and throat swab with sputum. Other specimen stated above may collect in special situation.
c. In case of mechanically ventilated patients, the desired specimens from the respiratory tract are

- Bronchoalveolar lavage and endotracheal aspirate (Only in specialized hospital)

d. Collect specimens from different sites of respiratory tract to increase the chance of virus isolation and identification.
e. Collect multiple specimens on multiple days (e.g. collect respiratory specimen on every day for first week if patient is in hospital

Annexur II

Consultative Group (Laboratory Experts):

1. Prof. Saniya Tahmina, Dept. of Microbiology, Sir Salimullah Medical College
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3. Prof. Shahina Tabassum PhD, Professor and Chairman, Dept. of Virology, BSMMU
4. Prof. Md. Shariful Alam Zilani PhD, Dept. of Microbiology, Ibrahim Medical College
5. Prof. Sabina Shahnaz, Dept. of Microbiology, Holy Family Red Crescent Medical College
6. Prof. Dr. Munir Hossain, Dept. of Microbiology, Dhaka National Medical College
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