सत्यमेव जयते
Government of India

Guidelines for Collection, Storage and transportation of Human Clinical samples for Laboratory Diagnosis of Influenza

National Centre for Disease Control
22- Sham Nath Marg New Delhi -110054

DIRECTORATE GENERAL OF HEALTH SERVICES MINISTRY OF HEALTH AND FAMILY WELFARE GOVT OF INDIA

Laboratory confirmation of influenza

A ***confirmed case*** of influenza virus infection is defined as a person with an acute febrile respiratory illness with laboratory confirmed influenza virus infection by one or more of the following tests:

- **Real Time PCR**
- **Viral culture(only in the reference laboratory)**
- **Four-fold rise in influenza virus specific neutralizing antibodies(only in the reference laboratory)**

Clinical sample to be collected:

- A variety of Respiratory specimens can be collected including throat swab (oropharyngeal swab), Nasal swab, nasopharyngeal swabs, Bronchoalveolar lavage, tracheal aspirates, nasopharyngeal or oropharyngeal aspirates as washes.
- In case of lower respiratory tract infection (pneumonia), and intubated patients, Bronchoalveolar lavage or tracheal aspirates should be collected.
- Swab specimens should be collected only on swabs with a synthetic tip (such as polyester/Dacron/rayon/flocked Nylon swab) and mounted on aluminum or plastic shaft. **Swabs with cotton and wooden shafts are not recommended**. **Specimens collected with swabs made of calcium alginate are not acceptabl**e.
- Bronchoalveolar lavage or tracheal aspirates should be collected in a sterile screw capped container in a sufficient quantity of 3-5 ml. Alternatively, the sample collected in mucus extractor can also be sent.
- Sputum is not a preferred sample for influenza testing.

Time of Sample Collection

- As soon as possible preferably within 48 hours of onset of symptoms
- Preferably before antiviral medications are administered

Bio-safety measures for sample collection

- Before initiating collection of sample appropriate PPE should be worn by the sample collector

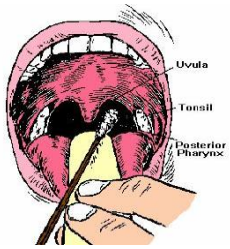
Personal Protective Equipment

- Masks (N-95)/ triple layered surgical masks
- Gloves
- Protective eye wear (goggles) (In case there is possibility of splash)
- Protective clothing (gown or apron)

Collection, Storage and Transport of Samples:

A. Methods of Collection

- Throat swab and Nasal swab
- Nasopharyngeal swab



Throat Swab

- Easiest sample to be collected
- Have the patient open his/her mouth wide open.
- The patient should try to resist gagging and closing the mouth during sample collection
- The posterior pharyngeal wall and tonsils should be swabbed

Nasal Swab:

- Insert swab into nostril along the lower floor of the nose till one reaches below the anterior turbinate.
- Leave the swab in place for a few seconds.
- Slowly remove swab while slightly rotating it. Use a different swab for the other nostril.
- Put tip of swab into vial containing VTM, breaking applicator's stick.

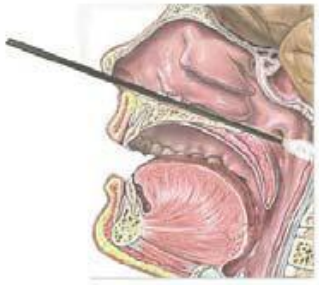
Both Nasal and Throat swabs can be collected into the same VTM to increase the viral yield.

Nasopharyngeal Swab:

This can be collected by two methods:

- Through the nasal route and
- Through oral route

Nasopharyngeal swab through Nasal route:



- Insert a **thin flexible swab** into nostril and right upto nasopharynx.
- Leave the swab in place for a few seconds.
- Slowly remove swab while slightly rotating it.
- Use a different swab for the other nostril.
- Put swab with tip downwards into vial containing VTM, breaking applicator's stick.
- Both the swabs can be put in same VTM vial

Nasopharyngeal swab through Oral route:

- Insert a **thin flexible swab** through mouth over the tongue and turn the swab upwards behind the soft palate to reach the nasopharynx.
- Leave the swab in place for a few seconds.

- Slowly remove swab and put the swab with tip downwards into vial containing VTM, breaking the extra portion of the swab stick.

B. Labelling the Samples

- Preferably use pre-printed barcode* labels.
- In case of non-availability, the following label can be pasted on the specimen container.

Label

Patient ID No. :

Patient's Name:

Hospital Name:

Date of sample collection:

- The label material should be made of leucoplast available in the hospital.
- **ONLY** ball point pen to be used for labeling (don't use ink /gel pen).
- The sample should always be accompanied with clinical proforma duly filled by the physician.

C. Storage of samples:

- Sample should be sent to the laboratory as soon as possible at 4 -8°C but not later than 48 hours
- In case of delay beyond 48 hours, the specimens must be stored at -70 °C.
- Avoid repeated freezing and thawing of the samples

D. Transportation of samples

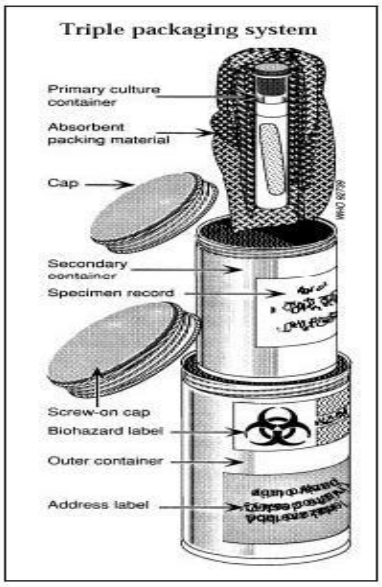
Transportation of sample from OPD/Ward/Casuality/ICU to the laboratory within the same institute

- Tightly closed and appropriately labeled VTM vial containing the sample should be transported to lab in a closed box (preferably vaccine carrier) with absorbent material (cotton /tissue paper) wrapped around the vial immediately after collection.
- Sample should be preferably transported in cold chain (4-8◦C).
- The sample should never be exposed to temperature more than 25◦C

Transportation of sample from the Hospital/Institution to the laboratory located elsewhere

- All samples should be transported after proper packaging using the standard triple packaging system https://www.who.int/csr/emc97_3.pdf

• Absorbent cotton, tissue paper, waste newspaper for wrapping primary container. Secondary container to hold primary container i.e. bigger tube or sealed plastic bag. Insulated ice box



with icepack, sample proforma fastened onto the secondary container.

- Sample should accompany the clinical details as per proforma enclosed in Annexure I
- While transportation cold chain should be maintained.

Waste Disposal: should be done as per guidelines of your hospital

PATIENT PROFORMA FOR INFLUENZA TESTING

Patient's Name ………………………………….CR/OPD No.................................

Age …………………..…Sex ………………….. Tel. No. ……….................................

Address ……………………………………………………………….…Block…………………..

District ………………………………………. State ....

Occupation ……………………………….Date of onset of illness ………..……………

Name of hospital …………………………….……………………………………………….

District ………………………………………. State ....

Name of Doctor/Health personal ……………………………………………………………

Tel. : ……………………………………E mail ID ………………………………………………

Clinical Signs & symptoms (encircle all that are present in the patient):

Fever axilla > (38^0C/ 100.4F)/ Oral > (38.5^0C/101.3F)/ Cough/ Sore throat/ Nasal catarrh/ Shortness of breath/ Difficulty in breathing/ Hemoptysis/ Cyanosis/ Hypotension/ Somnolence/ Convulsions (in children)/ Refusal to accept feeds (in children)/ Irritability (in children)

Pre-existing medical conditions (encircle all that are present in the patient):

Chronic pulmonary conditions/ chronic cardiovascular conditions/ chronic neurological conditions that impair breathing or clearance of respiratory secretions/ chronic metabolic diseases, specify…………………………………. / renal dysfunction/ haemoglobinopathies/ immunosuppressed/ immunocompromised/ Pregnancy – (If Yes, Mention Trimester)…………/children 6 months – 18 years on chronic aspirin therapy/ hypertension/ obesity/ others……………………………………………………..…

Sample Collection:

Data of sample collection …………………………………………………………………

Sample collected: throat swab/nasopharyngeal swab/other …………………………

No. of samples collected …………………………………

History of Vaccination: Yes/No, If Yes then, date of vaccination: …………………………………………..

Treatment History:

Antiviral Treatment taken: Yes No

Drug	Date Initiated	Date Discontinued	Dosage (If known)
Oseltamivir			
Other (Specify)			

Investigations Done: Yes No

Chest X-Ray findings ……………………………………………………………………

Outcome ……………………………………………………………………………………

Signature

Name:

Designation:

For Laboratory Use only:

Name of the laboratory: ……

Date of sample received: ……

Date of result: ……

Lab result of influenza testing……..

Signature of Lab in charge

Name:

Designation: