

Collecting and Preserving Appropriate Influenza Samples from Humans for Laboratory Confirmation



Objective of Samples Collection

1. Perform a timely laboratory diagnosis for all influenza viruses
 - Isolation of virus (culture)

Laboratory diagnosis of influenza virus

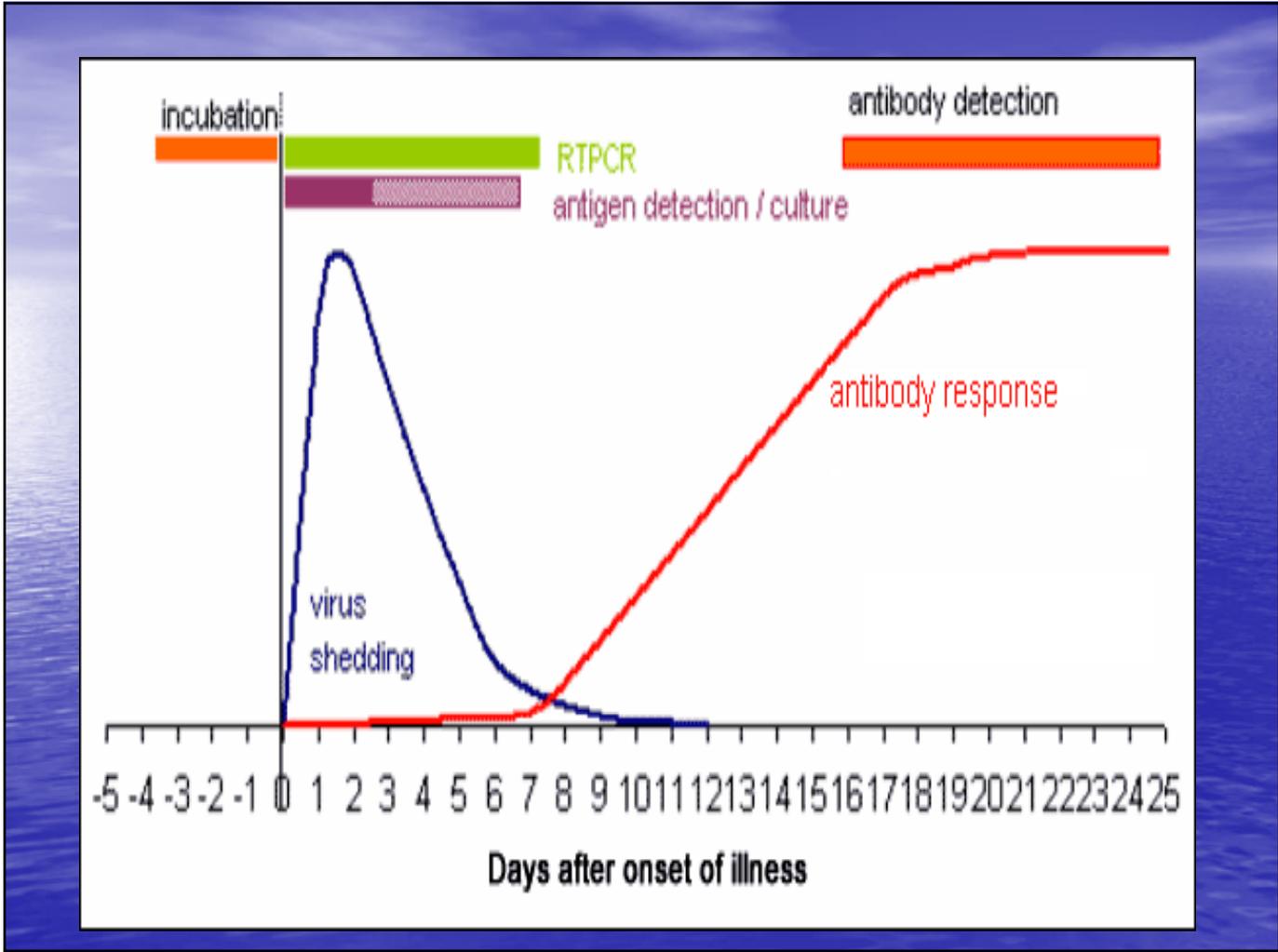
- Depends on
 - - collection of high quality of specimens
 - - rapid transport to the laboratory
 - - appropriate storage before laboratory testing

Types of Samples

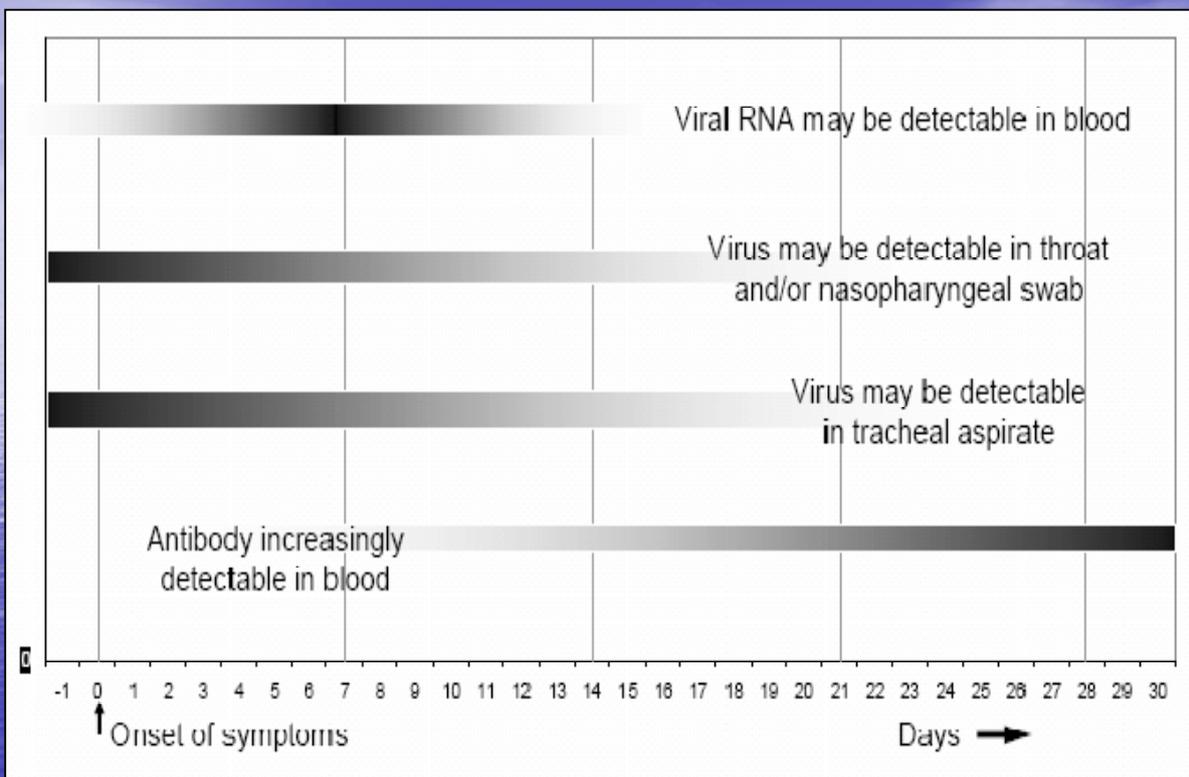
- Upper respiratory tract
 - Posterior pharyngeal (throat) swabs
 - Nasopharyngeal swabs or aspirate
- Lower respiratory tract (for intubated patients)
 - Tracheal aspirate
 - Bronchoalveolar lavage
- Blood
 - Sera (acute or convalescent)

Samples Collection Time

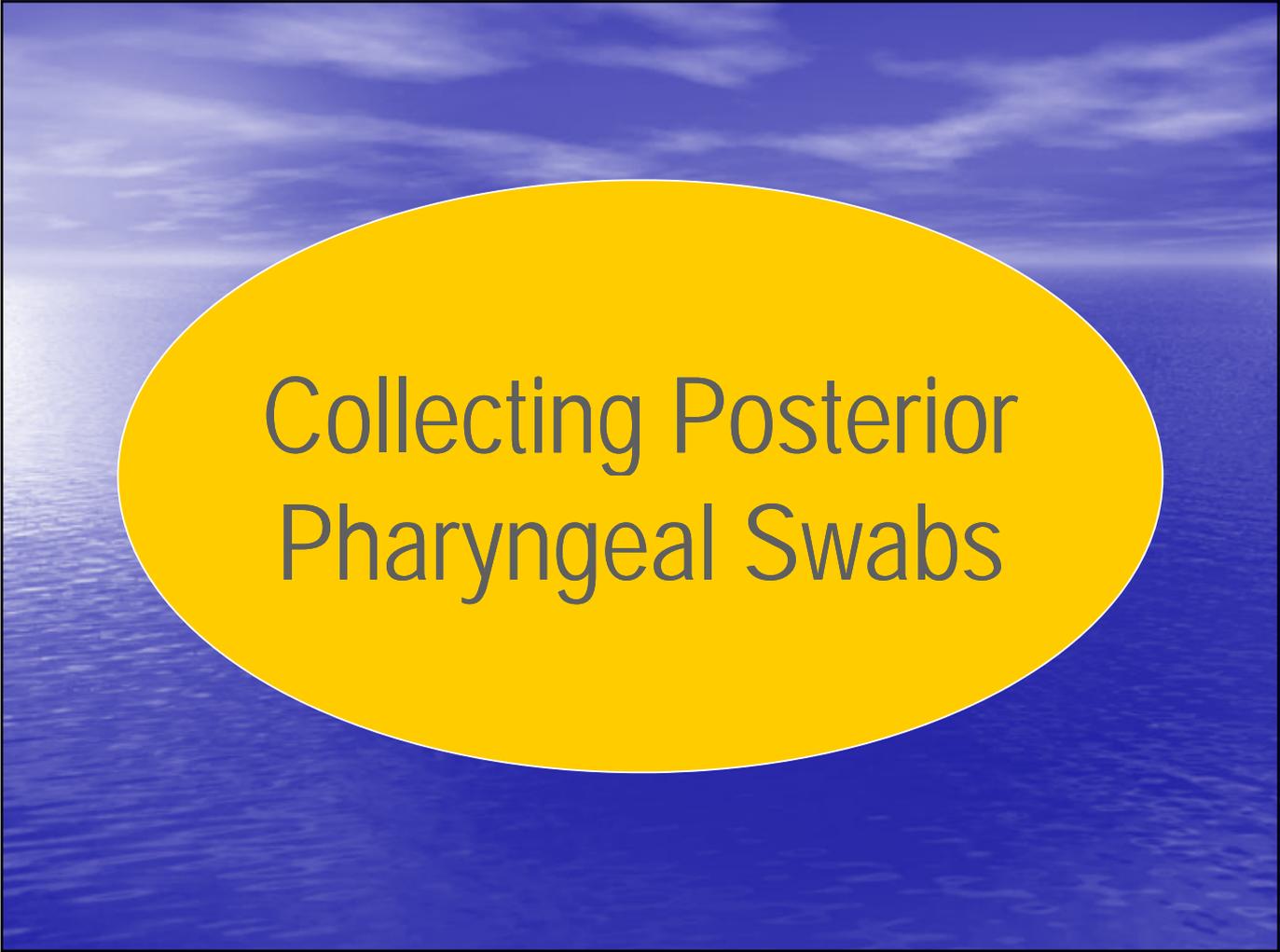
- **Throat & naso-pharyngeal swabs**
 - Acute phase to detect the virus - before or within 3 days of onset of symptoms
- **Serum sample**
 - Acute phase sample = 7 days at least after symptoms
 - Convalescent sample = 3 to 4 weeks after symptoms
- **Whole blood (plasma)**
 - Viral RNA detection = 7 to 9 days after symptoms development



Samples Collection Time



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



Collecting Posterior
Pharyngeal Swabs

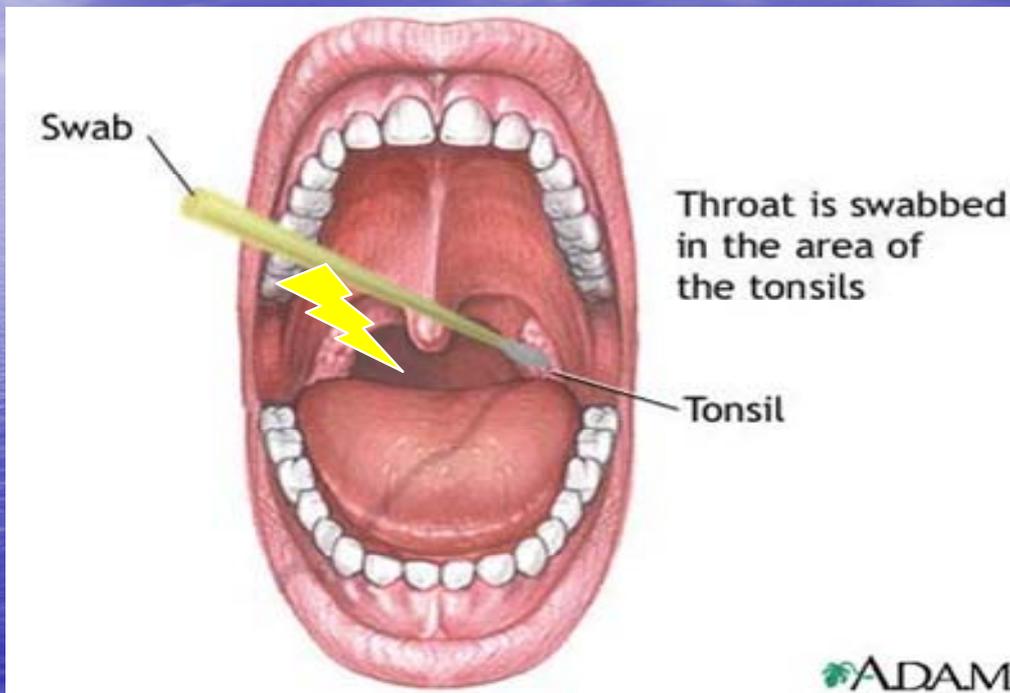
Collection Procedures for Posterior Pharyngeal Swabs

1. Properly restrain the patient (if needed);
2. Unwrap a Dacron swab from the stem-end of the packaging and be careful not to touch the swab tip;
3. Hold the tongue out of the way with a tongue depressor;
4. Remove swab and insert the entire tip of the swab into the throat. Use gentle 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;

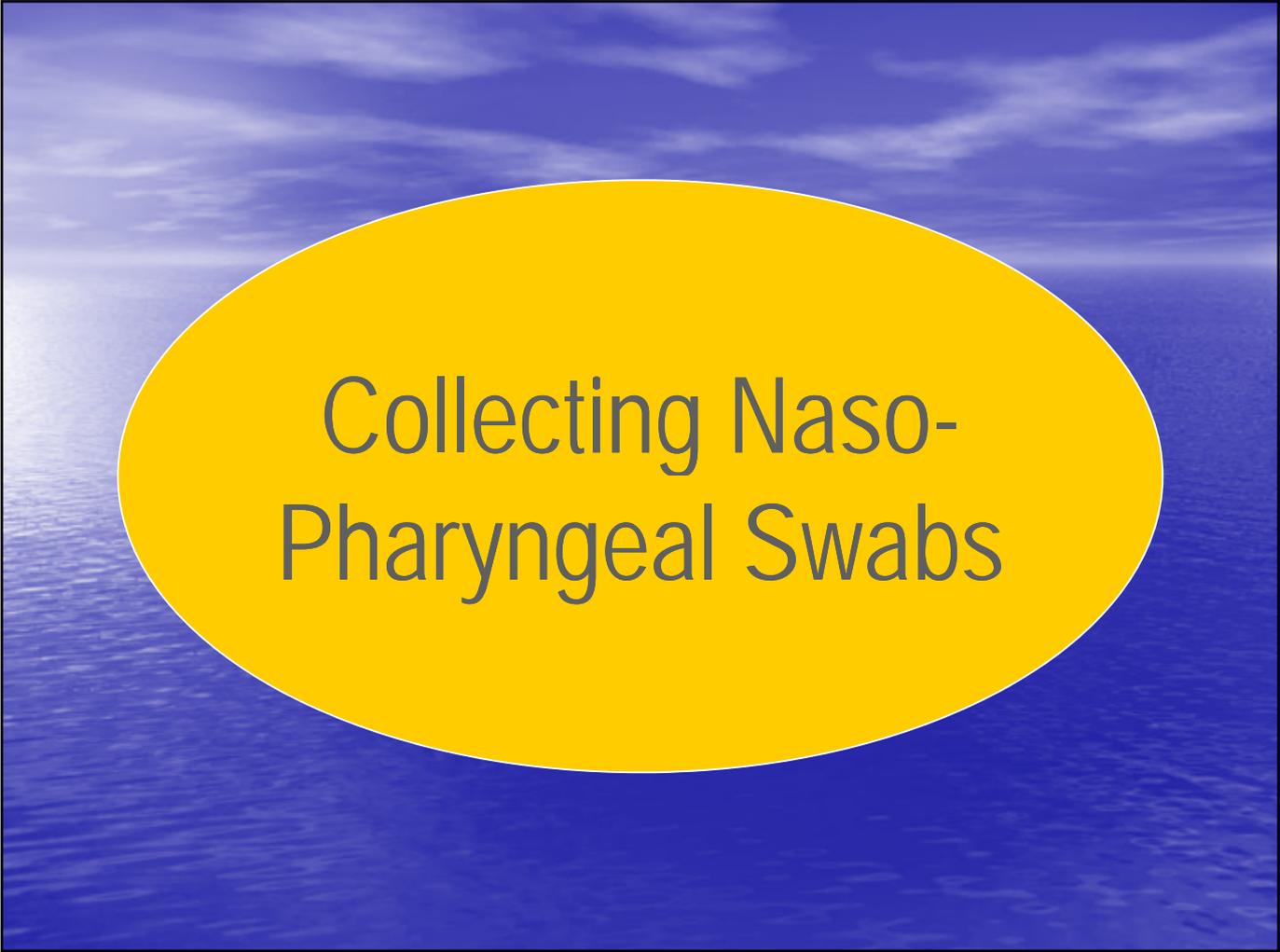
Collection Procedures for Posterior Pharyngeal Swabs (... continue ...)

5. Open the vial and place the swab tip in the viral transport media approximately $\frac{3}{4}$ of the way toward the bottom of the vial; squeeze swab gently on wall of vial
6. Cut or snap the stem of the swab so that the swab remains in the vial and the cap can be screwed on tightly. The entire swab end and a portion of the stem should be left in the vial;
7. Wipe scissor with 70% alcohol if they were used to cut the swab stem;
8. Label the tube with appropriate information

Collection Procedures for Posterior Pharyngeal Swab (... continue ...)



Sampling area for the posterior pharyngeal swab



Collecting Naso-
Pharyngeal Swabs

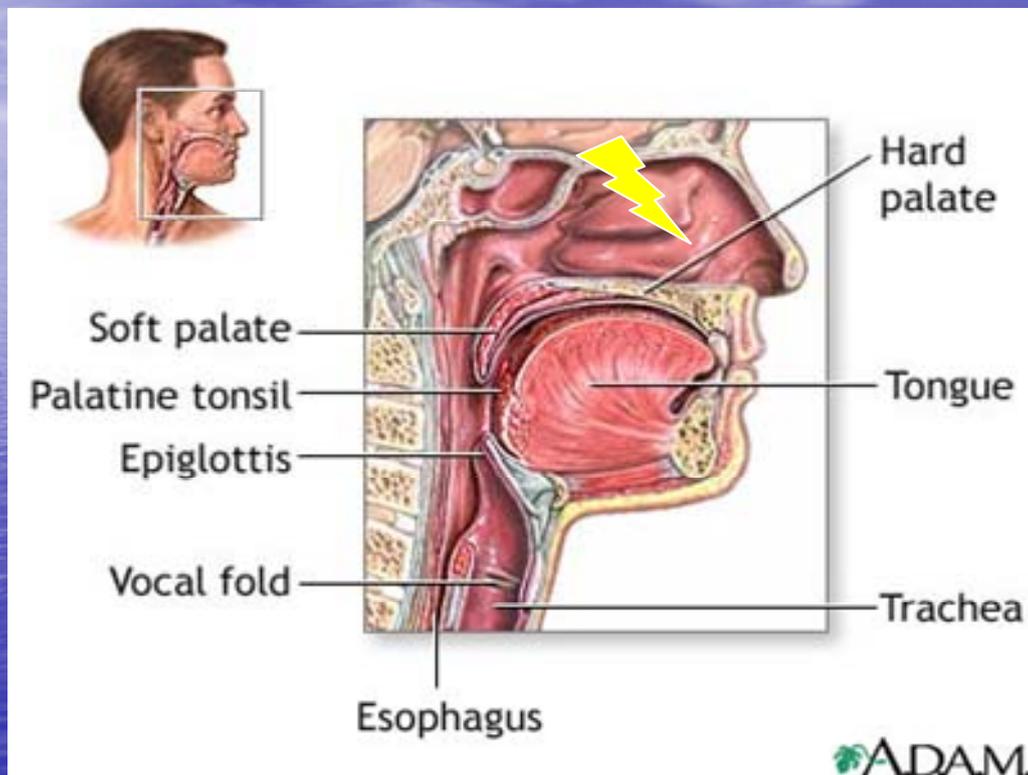
Collection Procedures for Naso-Pharyngeal Swabs

1. Properly restrain the patient (if needed);
2. Insert a flexible, fine-shafted polyester swab into the nostril and back to the naso-pharynx. The swab should be slid straight into the nostril with the patient's head held slightly back. The swab is inserted following the base of the nostril toward 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;
3. Leave the swab in place for a few seconds;
4. Withdraw slowly with a rotating motion;

Collection Procedures for Naso-Pharyngeal Swabs (... continue ...)

5. Open the vial and place the swab tip in the viral transport media approximately $\frac{3}{4}$ of the way toward the bottom of the vial; Squeeze swab gently on wall of the vial
6. Cut or snap the stem of the swab so that the swab remains in the vial and the cap can be screwed on tightly. The entire swab end and a portion of the stem should be left in the vial;
7. A second swab should be used for the other nostril and put into the same vial.
8. Wipe scissor with 70% alcohol if they were used to cut the swab stem;
9. Label the tube with appropriate information

Collection Procedures for Naso-Pharyngeal Swabs (... continue ...)



Sampling area for the Naso-Pharyngeal Swab

Storage Conditions

- Posterior- & Naso-Pharyngeal Swabs should be preserved in the appropriate VTM and kept at 4 ° C pending transport to the laboratory.
- Swab in VTM can be kept for a maximum of 4 days at 4 ° C or otherwise it should be frozen at -70 ° C or in Liquid Nitrogen (suitable for both virus isolation and PCR).

Diagnosis of influenza

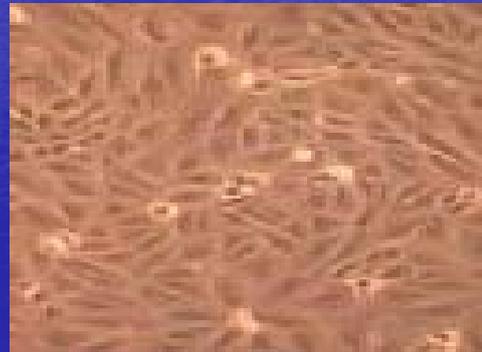
- Detection of live virus
- Detection of viral genetic material- nucleic acid
- Detection of immune antibody

Laboratory methods for detection of influenza virus

- Immunofluorescence assay
- Polymerase chain reaction and real-time PCR assay
- Viral culture

Viral culture

- Gold standard
- Necessary for vaccine strain selection/ production and important for strain surveillance



Viral culture

- MDCK(Madin Darby canine kidney) cells are preferred cell line for culture of influenza virus
- Virus obtained for identification and for further antigenic and genetic characterization
- Provides results in 4-10 days

