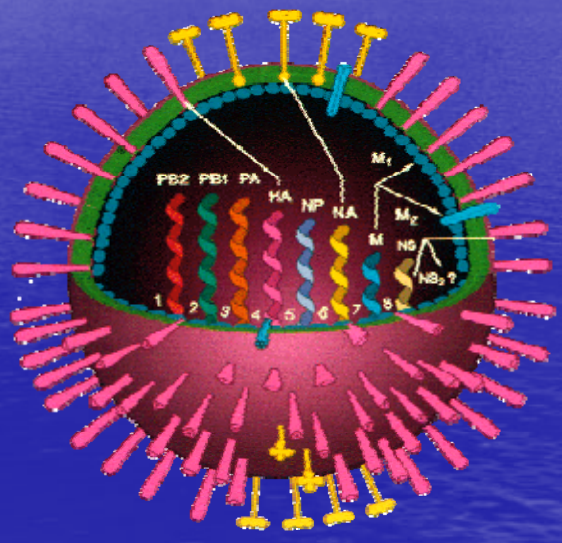
# Influenza Viruses ( Family Orthomyxovirus)

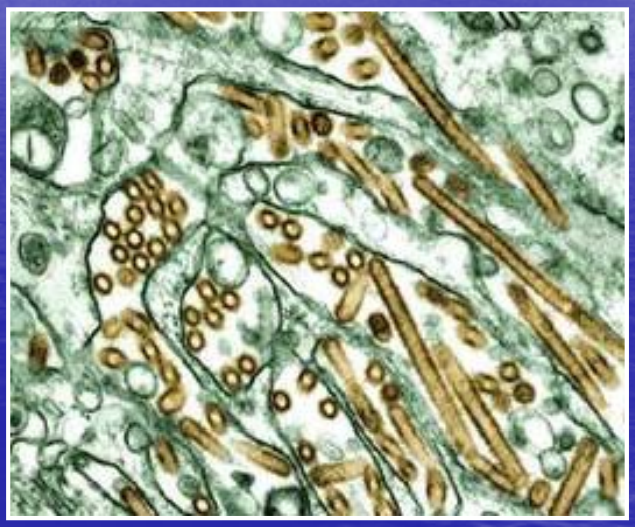
- Characterized by ability to change
  - Continually → yearly epidemics
  - Drastically → sporadic pandemics




- Negative single-stranded RNA virus
- 8 gene segments code for 10 proteins

# Influenza Viruses (... continue ...)

- Types A, B, and C
  - Only types A and B cause significant disease in humans
  - Types B and C limited to humans
  - Type A viruses
    - More virulent
    - Affect many species

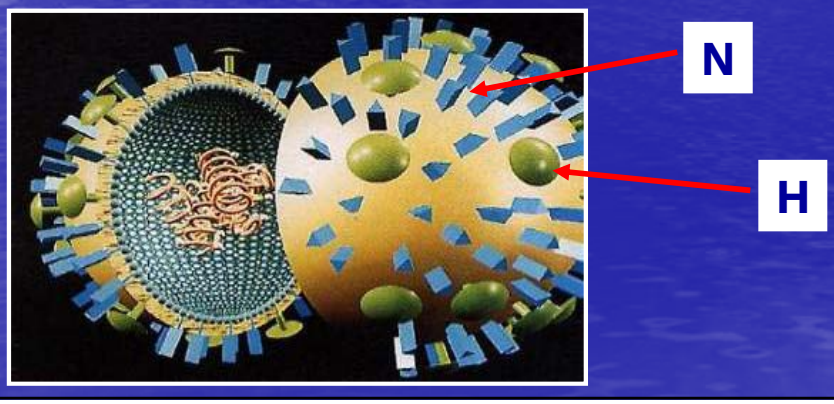


## Influenza A Viruses

- Cause epidemics and pandemics
- Infect multiple species
  - Humans
  - Birds (wild birds, domestic poultry)
  - Other animals: pigs, horses, dogs, marine mammals (seals, whales), ferrets, tigers
- Subtypes based on surface glycoproteins

# Influenza A Viruses (… continue …)

- Influenza A viruses categorized by subtype
  - Classified according to two surface proteins
    - Hemagglutinin (H) – 16 known
      - Site of attachment to host cells
      - Antibody to HA is protective
    - Neuraminidase (N) – 9 known
      - Helps release virions from cells
      - Antibody to NA can help modify disease severity



## Antigenic "Drift"

*Minor* antigenic changes to the hemagglutinin protein

- Point mutation in viral RNA
- Continuous process
- Cause of seasonal epidemics
- Immunity may be strain specific
- Vaccines must be updated each year

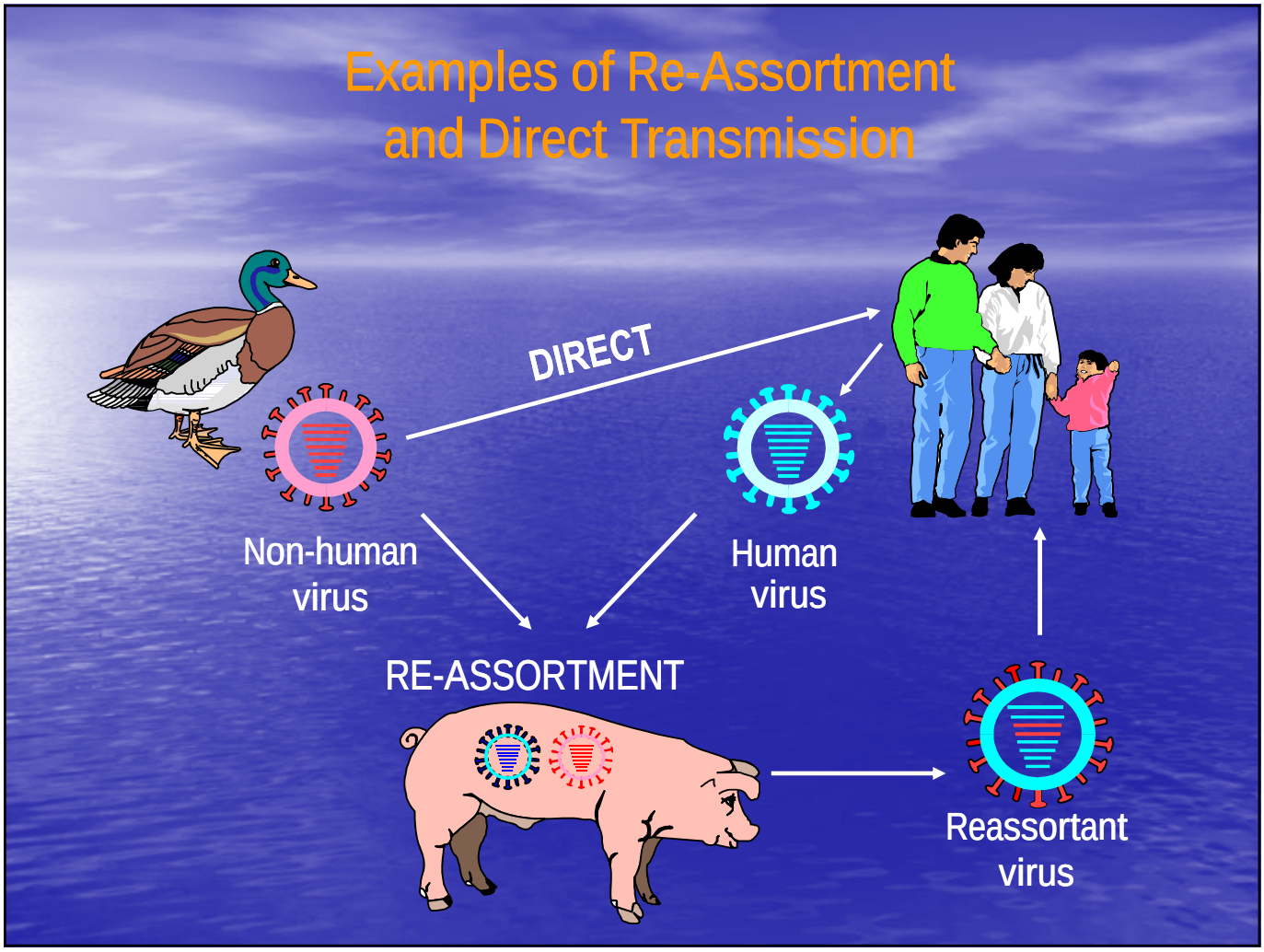
# Antigenic "Shift"

*Major* antigenic changes leading to emergence of a new human influenza A virus subtype (e.g. new HA subtype) through:

- Genetic reassortment (human and animal viruses)
- Direct animal (poultry) to human transmission

A pandemic can occur if:

- Efficient and Sustained virus transmission occurs among humans


Examples of Re-Assortment
and Direct Transmission
DIRECT
Non-human
virus
Human
virus
RE-ASSORTMENT
Reassortant
virus

## Estimated Mortality from Influenza Pandemics

- 1918-19 (H1N1) Entirely Avian origin
  ~ 40 million deaths worldwide
- 1957-58 (H2N2) Avian-Human reassortant
  ~ 1-2 million deaths worldwide
- 1968-69 (H3N2) Avian-Human reassortant
  ~700,000 deaths worldwide

# WHO Phases of a Pandemic

## *Inter-pandemic Period*

Phase 1: No new Influenza virus subtypes in humans

Phase 2: No new virus subtypes in humans; animal subtype poses a risk of human disease

# WHO Phases of a Pandemic
# (… continue …)

## *Pandemic Alert Period*

Phase 3: Human infection with novel virus; no instances of human-to-human spread

Phase 4: Small, localized clusters of human-to-human spread *(current status)*

Phase 5: Larger clusters, still localized; virus adapting to humans

# WHO Phases of a Pandemic (... continue ...)

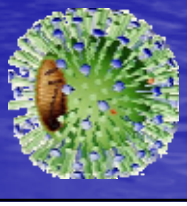
## *Pandemic Period*

Phase 6: Increased and sustained transmission in the general population.

## *Post Pandemic Period*

Recovery Phase

# 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

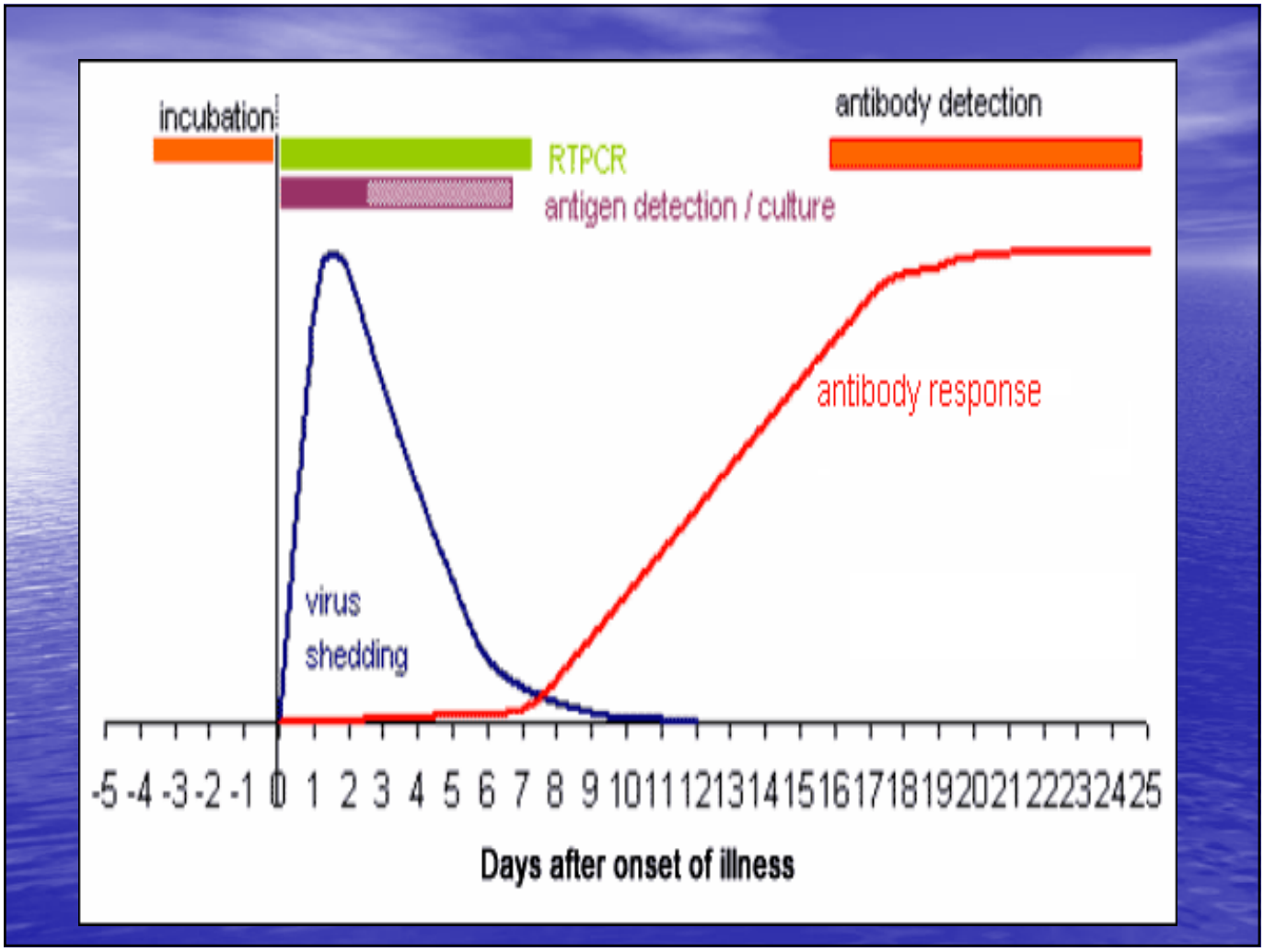
- Depends on
- - collection of high quality of specimens
- - rapid transport to the laboratory
- - appropriate storage before laboratory testing

# Types of Samples

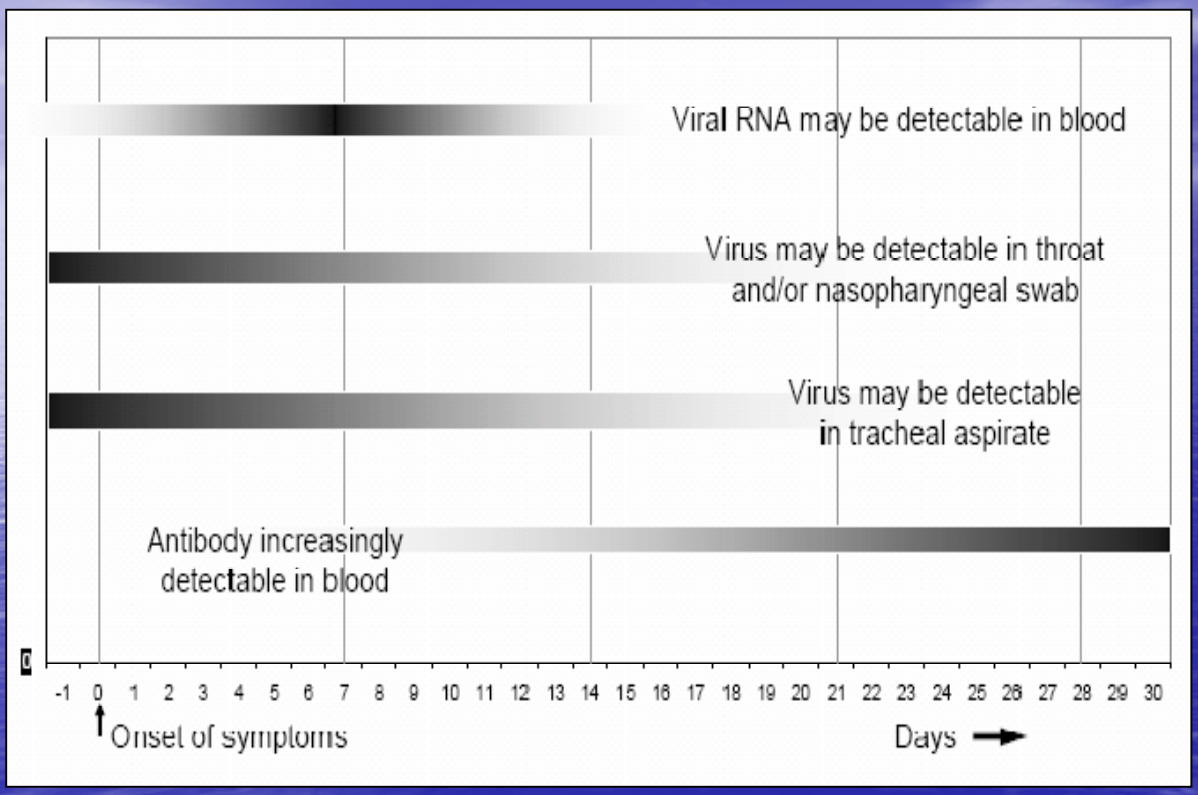
- Upper respiratory tract
  - Posterior pharyngeal (throat) swabs
  - Nasopharyngeal swabs or aspirate
- Lower respiratory tract (for intubated patients)
  - Tracheal aspirate
  - Bronchoalveolar lavage
- Blood
  - Sera (acute and convalescent)

# Samples Collection Time

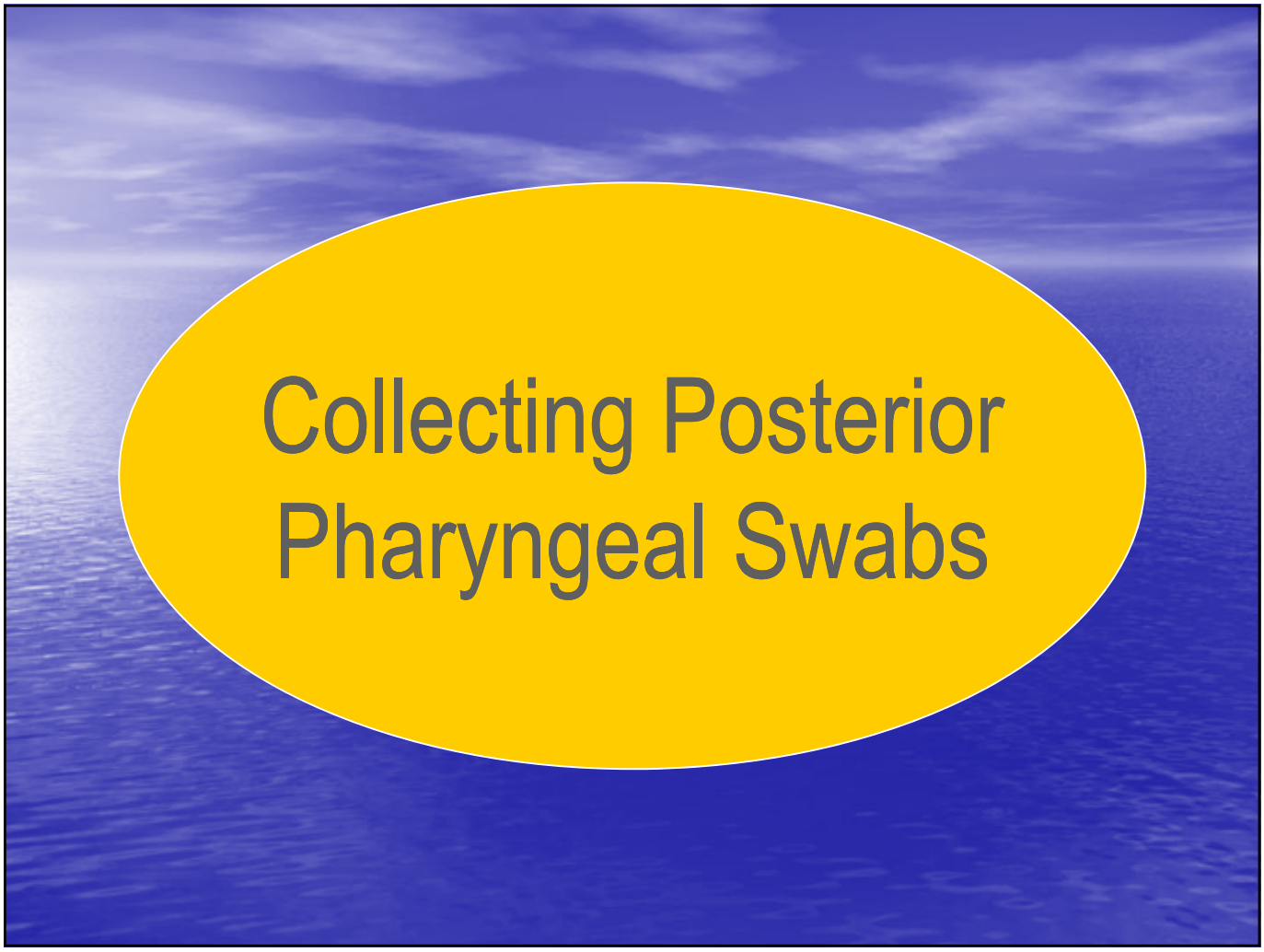
- Throat & naso-pharyngeal swabs
  - Acute phase to detect the virus - before or within 3 days of onset of symptoms
- Serum sample
  - Acute phase sample = 7 days at least after symptoms
  - Convalescent sample = 3 to 4 weeks after symptoms
- Whole blood (plasma)
  - Viral RNA detection = 7 to 9 days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


incubation
antibody detection
RTPCR
antigen detection / culture
antibody response
virus
shedding
-5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Days after onset of illness

# Samples Collection Time




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


Collecting Posterior
Pharyngeal Swabs

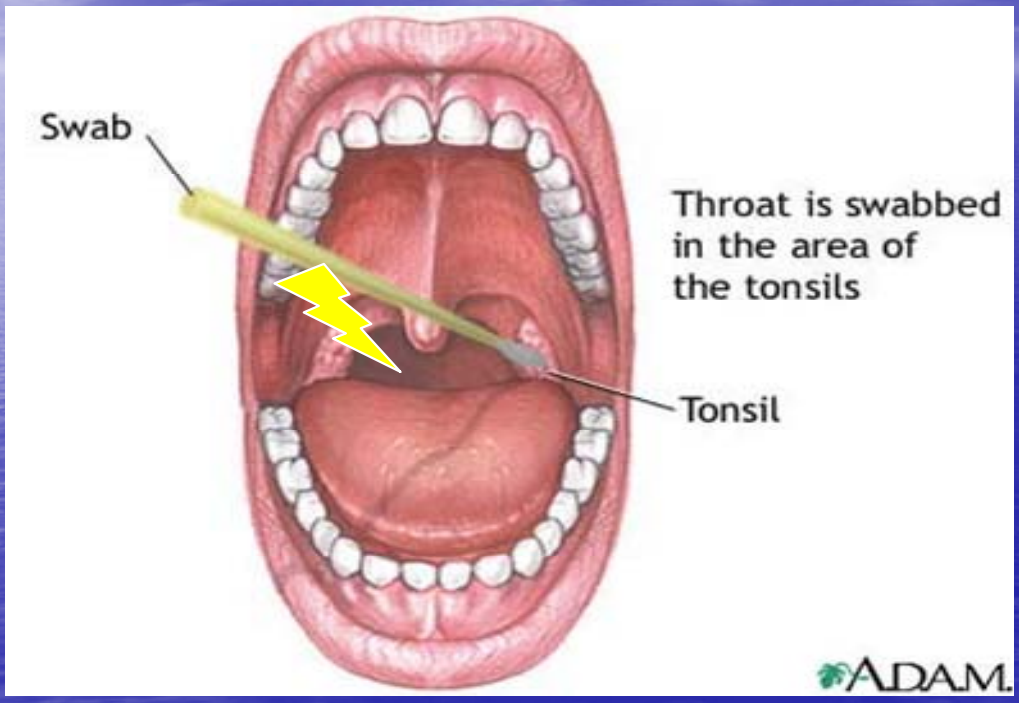
# Collection Procedures for Posterior Pharyngeal Swabs

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 ¾ 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

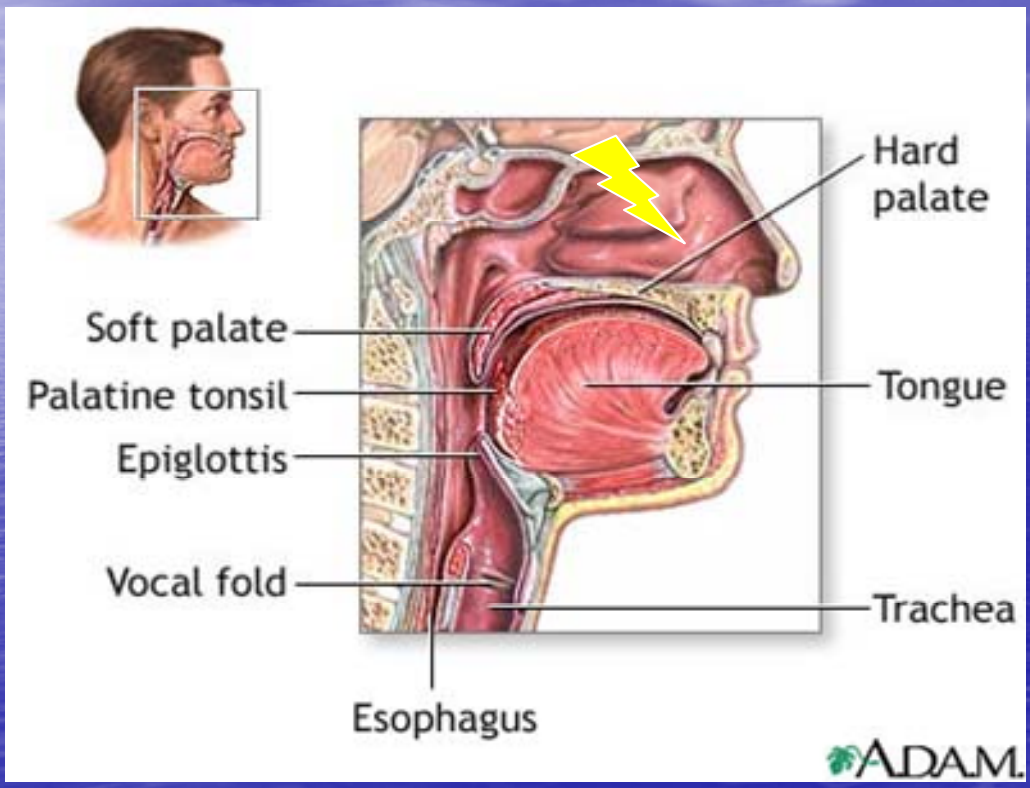
## Collection Procedures for Naso-Pharyngeal Swabs

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 ¾ 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

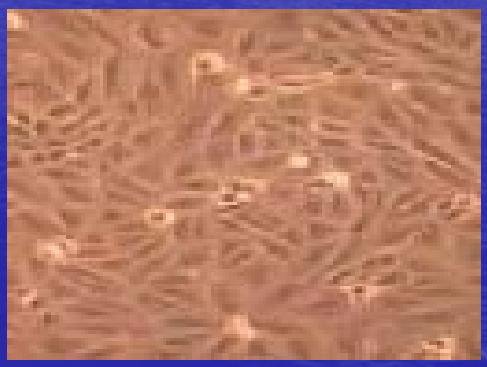
- Detection of live virus
- Detection of viral genetic material- nucleic acid
- Detection of immune antibody

# Laboratory methods for detection of influenza virus

- Immunofluorescense 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

## Viral culture

5. Inoculate specimen as soon as possible in MDCK cell line and incubate at 37 °c
6. Perform heamadsorption test on day 1,3,5,7and 21
7. If above test is positive perform a heamagglutination test
8. Type virus using WHO antisera

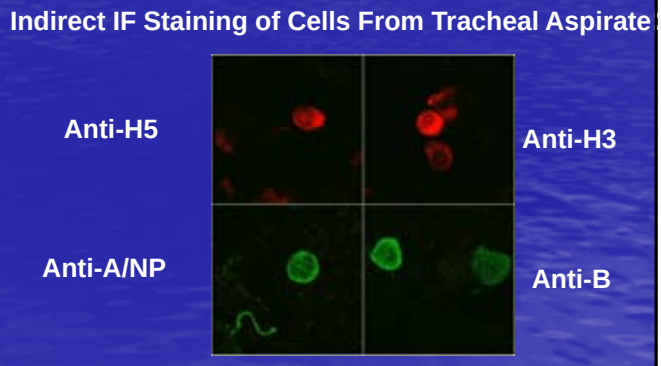
# Antigenic tests

**Immunofluorescence (IFA)**

- Typing/subtyping
- Direct testing of cells grown from original specimens
- Monoclonal antibodies;

-WHO Influenza reagent kit

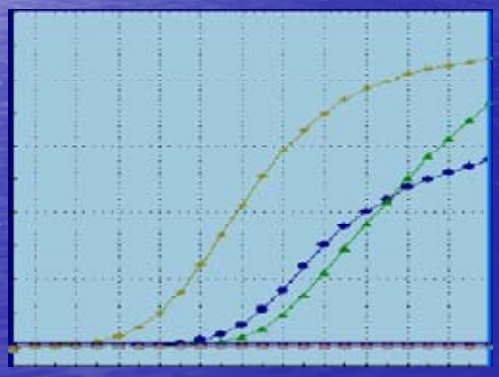
-Commercially available



# RT PCR typing and subtyping

- Test original samples or grown virus
- Higher sensitivity and specificity

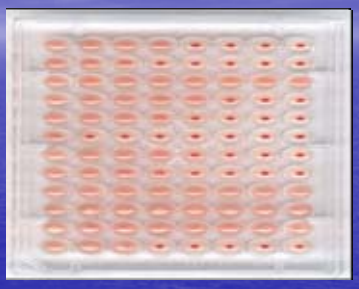
Real time PCR




RT PCR – agarose gel

## Serological tests

- **Hemaglutination inhibition assay**
- **Microneutralization assay**



-Detect presence of immune antibody

-Seroconversion (>4fold) should be observed

-Retrospective confirmation of infection

-sera must be collected at optimum time to detect antibody response

# Laboratory characterisation of influenza

## Diagnosis

- Confirmatory diagnostic testing by rRT-PCR
- Virus isolation
- Antigenic, genetic and antiviral resistance characterization
- Serology testing (HAI and Microneutralization)

## Surveillance

- Virus isolation
- Antigenic, genetic and antiviral resistance characterization
- Testing by rRT-PCR (if necessary)

# International Influenza surveillance goals

- Identify and characterize circulating viruses
  - WHO Collaborating Center for Influenza

- Establish disease burden in countries where data are lacking

- Support early identification of novel influenza viruses and disease
  - Avian influenza cases in humans
  - Onset of pandemic influenza

## Surveillance

Surveillance enables WHO to recommend influenza vaccines strains twice annually

- live-attenuated influenza vaccine
- inactivated vaccine

# Uses of data: laboratory surveillance

- Informs annual vaccine strain selection
- Assess match between vaccine and circulating strains
- Antiviral resistance assessment
- Assess immune response in humans to seasonal and potential pandemic viruses

# *Thank You!!!*

*Pursem Vidula Nalini*
*Clinical Scientist*
*Virology Department*