Laboratory Diagnosis of Influenza

General overview of diagnostic methods in virology

1. Direct Examination

Electron Microscopy (morphology)
Light microscopy (histological appearance)
Immunofluorescence (antigen detection)
Molecular techniques (detection of viral genomes)

2. Indirect Examination

Cell Culture methods (plaque formation, cytopathic effect)
Embryonated eggs (haemagglutination, inclusion bodies)
Laboratory animals (disease or death confirmation by neutralization)

3. Serology

Classical Techniques

*Complement fixation tests
*Haemagglutination inhibition tests
*Neutralization tests
*Single Radial Haemolysis

Advanced Techniques

*Immunoassays (RIA, ELISA)
* Western Blot

Orthomyxoviridae

- Order : Mononegavirales
- medium sized(80-120 nm), pleomorphic, enveloped
- Genome: linear, segmented (6-8), (-)sense, single-stranded RNA, 10-13.6 kb
- It has six genera, including genera:-

✓ *Influenzavirus A*

✓ *Influenzavirus B*

✓ *Influenzavirus C*

- cause respiratory illness with systemic symptoms.

Acquaintance

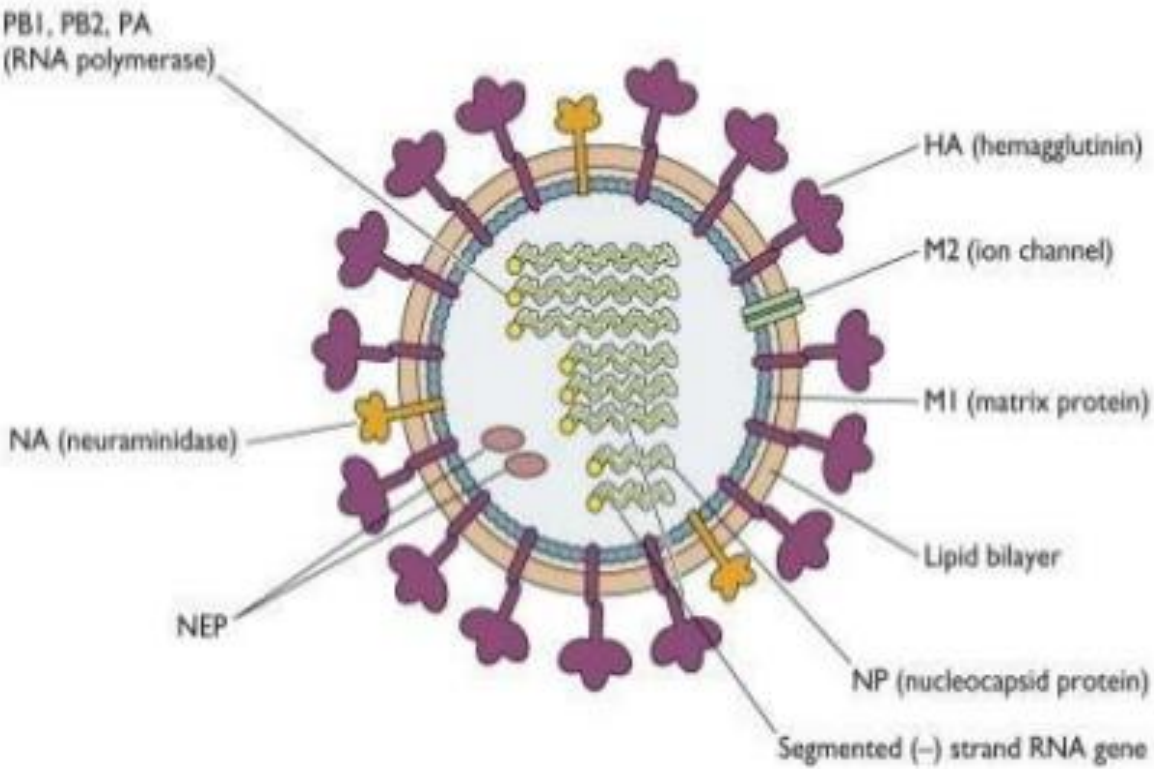
- Influenza virus is a negative-sense single-stranded RNA virus under the orthomyxoviridae family. It causes Influenza disease commonly known as 'Flu'.
- Influenza is a highly contagious viral infection of the respiratory passages of birds and mammals including us can occur in epidemics.
- It is the most familiar virus in this present world.

Continued....

- The outer layer is the lipid membrane, spikes are composed of glycoproteins: Hemagglutanin (HA) and Neuraminidase (NA).
- There present M2 protein embedded in lipid membrane and M1 protein beneath the lipid membrane.
- The complete genome of Influenza is segmented into 8 fragments but 7 fragments in case of Influenza C. There total 11 genes are located in the genome encoded 11 proteins.
- The genomic size is about 13.5 bp.

* *HA and NA proteins determine the subtype of influenza virus.*

Morphology



❖Influenza type A,B, and C are similar in structure. Those are roughly spherical of 80-120 nm in diameter but type-C may occur as filamentous.

Continued....

NA= Neuraminidase

HA= Hemagglutinin

NP= Neucleoprotein

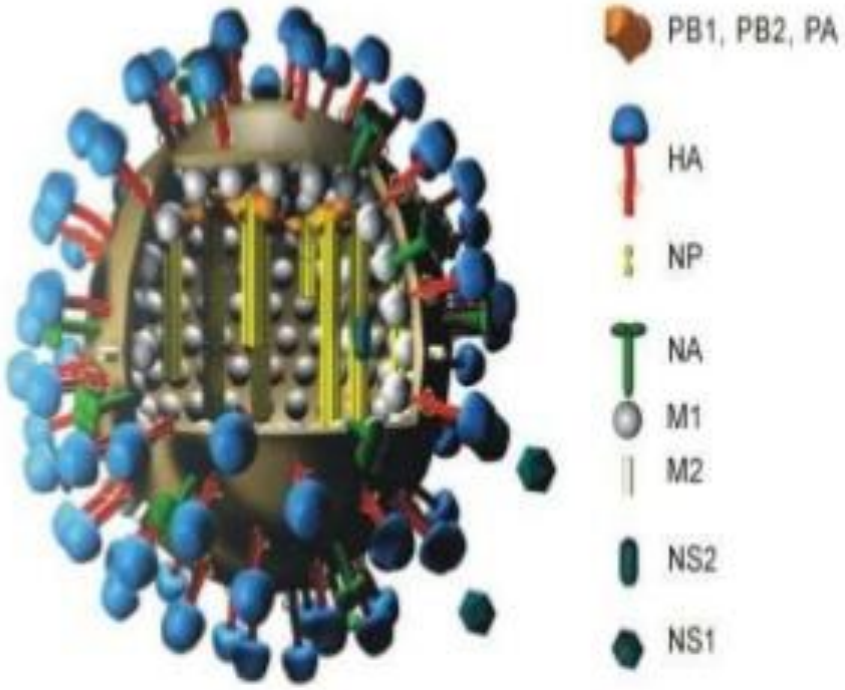
NS1= Non-structural protein

NS2(NEP)=mediates the export of RNP-complexes from nucleus.

PB1, PB1-F2, PB2, PA = subunits of viral RNA polymerase and are all crucial for viral transcription and replication.

M1= Matrix proteins form capside.

M2= Proton-selective Ion-channel



Variation

- There are total 25 serotypes of Influenza: 16 HA and 9 NA varieties.

Influenza A: most virulent human pathogen among influenza viruses, wide host-range, highest serotypes; cause severe disease including: Bird-flu(H5N1), Swine-flu(H1N1), become pandemics!

Influenza B : mostly found in human and only two serotypes present. Influenza B don't cause pandemics due to limited host range.

Influenza C : It is milder and causes less severe disease. Don't become endemic and no subtype present.

Hemagglutinin

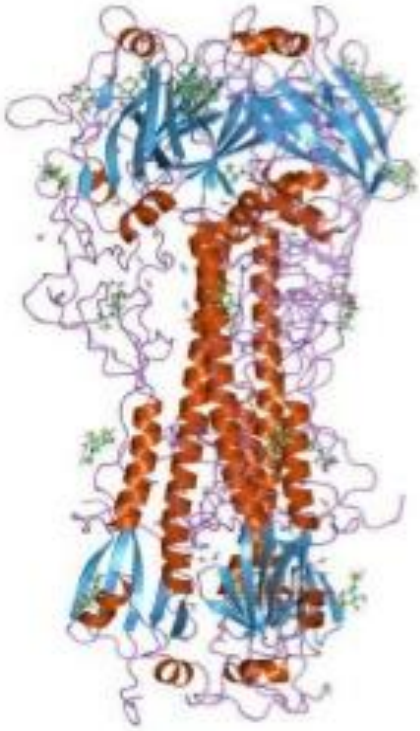
-Influenza virus surface glycoprotein, cylinder-shaped, consists of 549 amino acids.

-It has a central alpha-helix coil and three spherical heads contain sialic acid binding sites

-works in viral attachment with sialic acid receptor and in fusion of viral envelope with endosome.

-There are at least 18 different HA antigens. H18 discovered in 2013.

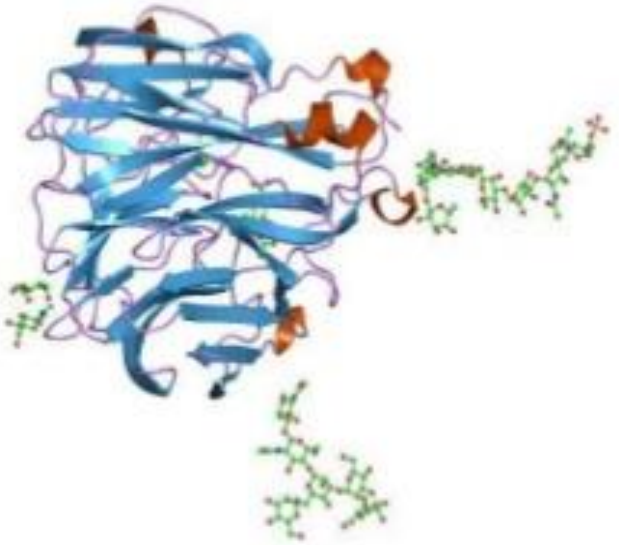
-Potential target for antiviral drugs



Neuraminidase

-Influenza virus surface glycoprotein

- It has 9 different NA antigens



- enzymatically cleaves the sialic acid groups from host cells, thus promote entry and replication.

- promotes the release of progeny viruses

- Target of antiviral drugs: Zanamvir, Oseltamivir

Pathophysiology

- Influenza virus can infect both upper and lower respiratory tracts. Sialic acid on epithelial cells are the receptors.
- The typical incubation period of influenza is 24 hours to 4 days with average: 2 days.
- Children's are more readily affected than adults.

Easily spread
Rarely fatal

Spreads slowly
Often fatal

Transmission

Influenza can be spread in three main ways:

Direct transmission: an infected person frequently touch their nose, mouth or conjunctiva.

Virus-laden aerosols: coughing, sneezing, speaking,normal breathing all produce aerosols. A good sneeze can generate up to 20,000 aerosols. Even a single droplet may cause infection. Droplet-nuclei (1-4 μm) suspended in the air for long period and spreads the disease much.

Indirect transmission: through contaminated objects :called fomites, such as toys, doorknobs, light switches etc.

Antigenic Shift

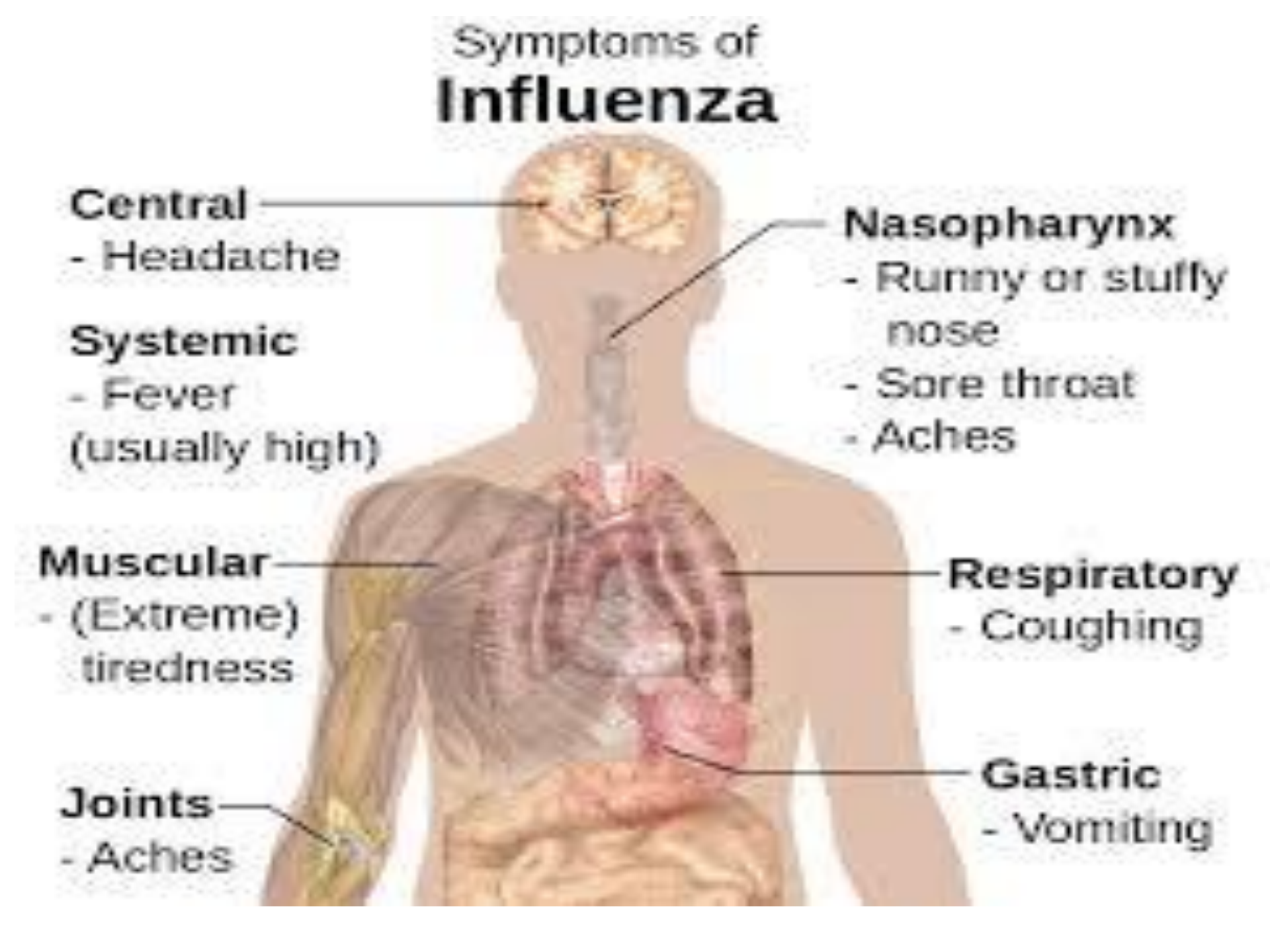
When more than one type of influenza virus infects a single cell the separation of viral genome into eight separated segments allows mixing or reassortment of new viral RNAs that induces new strains of influenza virus. This process called antigenic-shift. It occurs only in influenza Type A.

- caused by exchange of genetic materials (RNA)
- new strains generate
- results in pandemics
- eg. Asian flu by H2N2 subtype in 1958-1959.
- only in influenza type A

Antigenic Drift

Due to the absence of proofreading activity, the RNA-dependent RNA polymerase copies the viral genome making error roughly in every 10 thousand nucleotides (which is the approximate length of the influenza vRNA). Hence, the majority of newly assembled influenza viruses are mutants, thus the antibody-binding sites become changed. This is called antigenic-drift.

- caused by point mutation
- change in HA and NA
- results in epidemics
- eg. outbreak of influenza A H3N2 (2003-2004)
- occurs in all type A, B and C


Symptoms of
Influenza
Central
- Headache
Systemic
- Fever
(usually high)
Muscular
- (Extreme)
tiredness
Joints
- Aches
Nasopharynx
- Runny or stuffy
nose
- Sore throat
- Aches
Respiratory
- Coughing
Gastric
- Vomiting

COLD

- LOSS OF APPETITE
- SORE THROAT
- SNEEZING
- COUGH
- VOMITING

FLU

- MUSCLE PAIN
- HIGH FEVER
- HEADACHE
- FATIGUE
- CHILLS

Avian flu

- Avian influenza known as bird-flu. All known viruses that cause influenza in birds belong to the species *influenza A virus*.
- Most highly pathogenic strain H5N1 had been spreading throughout Asia since 2003 and reached to Europe In 2005.
- There are many subtypes of AIV: H5N1, H7N3, H7N7 H7N9, and H9N2

Swine flu

*Swine flu also known as 'Pig influenza'. It is caused by Swine influenza viruses including Influenza C and Influenza A subtypes: H1N1, H1N2, H2N1, H3N1, H3N2, and H2N3.

* Direct transmission of an influenza virus from pigs to humans is occasionally possible.

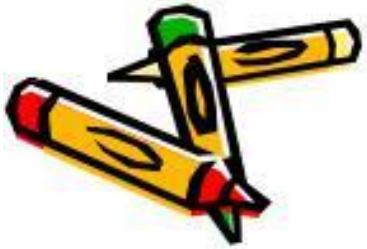
* In 2009, a swine-origin H1N1 virus strain commonly referred to as "swine flu" caused the '2009 flu pandemic'.

Laboratory Diagnosis of Influenza Virus infections

- **Direct detection of viral antigens in infected cells**
  Immuofluorescent staining of OP or NP samples

- **Isolation:**
  - **Inoculation into** the amniotic cavity of the chick embryo. detect HA then confirm & type by HAI
  - Primary Monkey Kidney cell lines
    - Detect HA & confirm and type by HAI of culture supernatant
    - Haemadsorption affinity of Tissue culture cells confirm and type by Haemadsorption Inhibition

  - **Serology:**
    - HAI
    - Elisa
    - Complement fixation



Electron-Micrograph

Laboratory Diagnosis

Diagnosis of influenza relies on:

- isolation of the virus;
- identification of viral antigen or viral nucleic acid in the patient's cells, or
- demonstration of a specific immunologic response.
- *Other tests* are*:* ELISA and RIA. Paired acute and convalescent sera are necessary, because normal individuals usually have influenza antibodies. A fourfold or greater increased in titer must occur to indicate influenza infection.

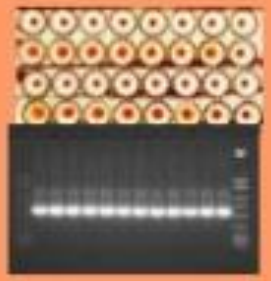
ROUTINE INFLUENZA DIAGNOSTICS & ANALYSIS

Patient clinical details:
Influenza like illness, temperature, cough malaise

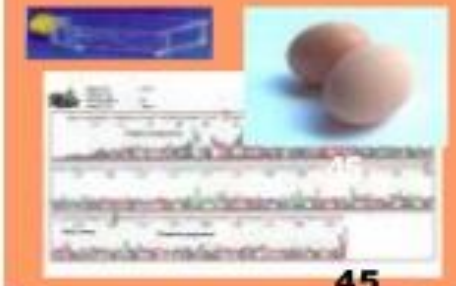
Patient sample: throat swab, aspirate (nasopharyngeal /bronchoalveolar lavage)

Rapid test*
Directigen Flu A+B
Binax Now A/B
Capilia Flu A,B

Lab assay
Direct IFA
RT-PCR
HI assay



Further analysis
Virus Culture
Extensive HI
Sequence
HA & NA



Laboratory Diagnosis

Rapid Influenza Diagnostic Test

- Antigen detection test that detect influenza viral nucleoprotein antigen
- Can provide results within
  30 minutes
- Sensitivity 40-69%