Medical Virology

INTRODUCTION TO MEDICAL VIROLOGY

Evolutionary origin of viruses

There are 3 theories exist:

1.Regressive theory

- **Viruses are degenerate forms of intracellular parasites**

  e.g. Leprosy bacillus, rickettsiae & chlamydia

- **Lack their own rRNAs or protein synthesis machinery**

  Controversy on how RNA virus evolved???

2. Progressive theory

- **Viruses originated from cellular RNA & DNA components**
- **Normal cellular nucleic acids that gained the ability to replicate autonomously & hence to evolve**
- **DNA viruses came from plasmids or transposable elements**

  ►They then evolved coat proteins & transmissibility

- **Retroviruses derived from retro-transposons & RNA virus from mRNA**

3. Co - evolution theory

- **Viruses coevolved with life (free living cells)**
- **All of the above theories could be correct ?**
- **No convincing reason to think that RNA viruses have evolved in the same way as DNA viruses**
- **How viruses become independent genetic entities?**

General characteristics of viruses

- **Smallest infectious agents: 20 - 400 nm in diameter**
- **Too small to be seen by light microscope**
- **Genome consists of only either RNA or DNA**
- **Obligate intracellular parasite (replicate only in living cells)**
- **Self replicating**

  Direct host cell machinery to synthesize new virion

- **Have protein coat : interacts with immune responses**
- **May or may not contain lipid envelope**
- **Neither eukaryotic nor prokaryotic cells**

Viral structures:

- **Capsid:** protective protein coat that enclose NA genome
- **Nucleo-capsid :** Capsid plus NA
- **Structural units :** Basic protein building blocks of the coat

- **Capsomers**
  - Viral morphological units
  - Seen in electron microscope
  - Represent clusters of polypeptides
  - Number vary from one virus group to the other

- **Envelope**

  - Acquired during viral maturation by a budding process

  - Consists of lipid bilayer & glycoprotein

  Functions:

  - Serves as binding site to host cell receptors

  - have antigenicity characteristics (due to glycoproteins)

  - confers viral genome instability

  e.g. Enveloped viruses are highly sensitive to heat & liquid solvents

- **Lipid**

  - Derived from host cell membrane

  - surrounds some viral particles

- **Glycoprotein**

  - **Exposed on the surface of the envelop**

  - **Highly antigenic property**

  - **Undergo antigenic change**

- **Spikes**

  - **Virus encoded glycoprotein protruding from lipid bilayer**

  - **Serve for viral attachment & entry to host cell**

- **Virion**

  - **Complete & infective viral particle**

  - **Transfers viral NA from cell to cell**

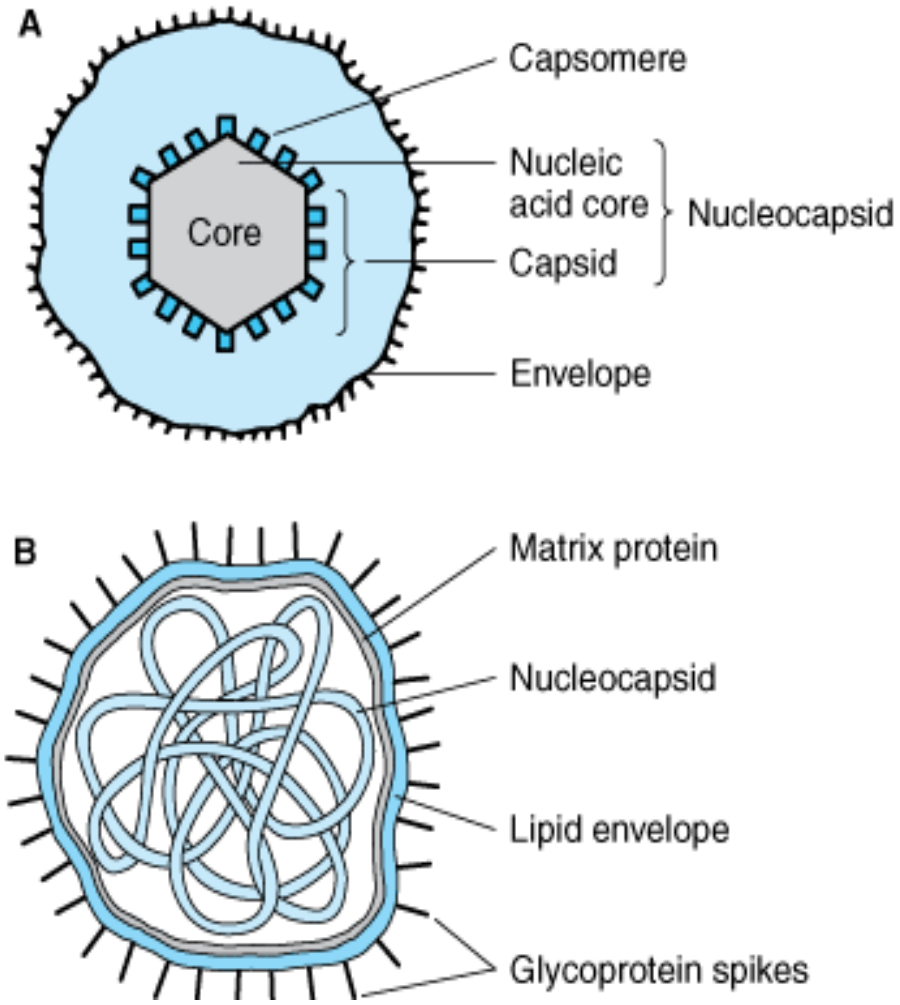
- **Simple viron (naked virus)**

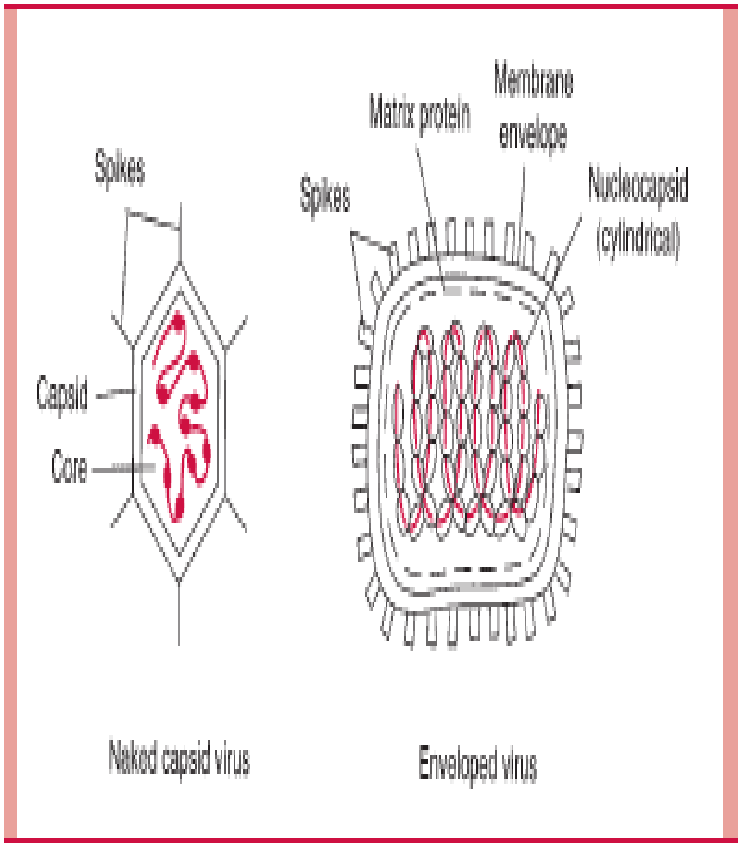
  - contain NA & capsid

- **Complex viron (enveloped virus)**

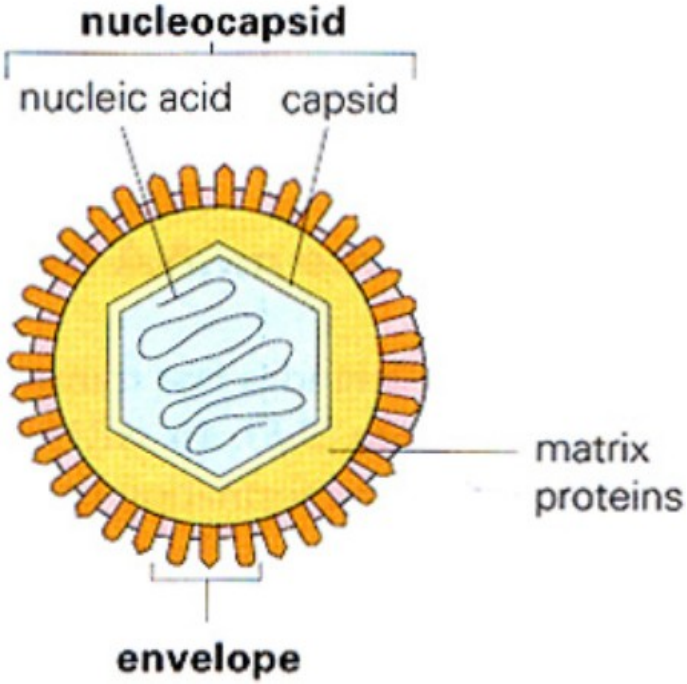
  - contain NA, capsid & envelope

- **+ssRNA: same as viral mRNA**
- **-ssRNA: as a template transcribe complimentary mRNA**

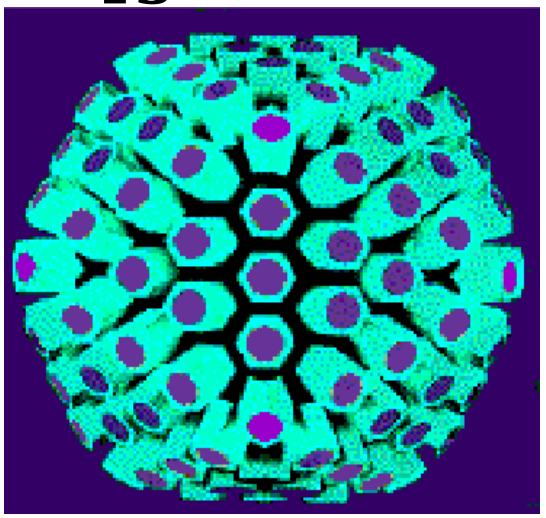




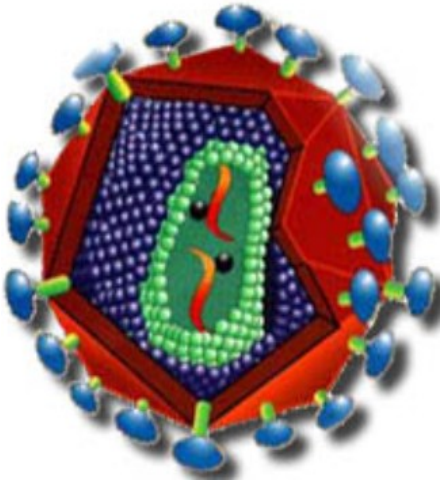
Diag. 1. components of complete virus particle : A) Enveloped virus with icosahedral symmetry. B) Virus with helical symmetry.
(Medical Microbiology, 24th ed; 2000)



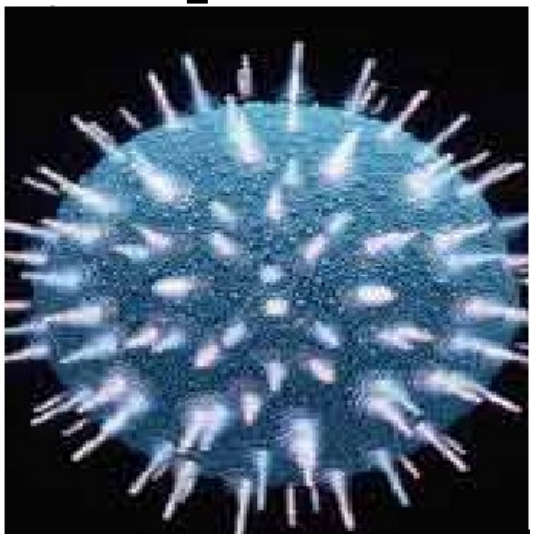
Capsomers



Spikes



Spikes



Host Range

- **Broad and / or limited**

  e.g: Influenza virus, Rotavirus

- **Can infect:**

  - unicellular organisms

  e.g. Bacteria….bacterio phages

  - higher plants & animals

Factors regulating susceptibility & resistance to infections

1.Age

e.g.: - HSV & Rotavirus: Severely affect newborn infants

- HBV & HIV: Affect all age group

- Child hood diseases (measles, mumps)

Susceptibility to viral infections depend on:

- **Ability of host cells to support viral multiplication**
- **Ability to withstand loss of cells or aging**
- **Capacity for tissue regeneration**
- **Ability to mount effective immune response**
- **Status of susceptible host (chronic diseases)**

2. Nutritional status

- **Role in multiplication rate /disease progression**
- **Response to Rx or Prognosis Eg: HIV/AIDS**

3.Genetic factors

Regulate susceptibility & resistance to infection

e.g: Mx gene of mice controls susceptibility to infection with Influenza virus

►**Humans possess an analogous gene**

4. Immunological status of a host

e.g: Chronic diseases (Diabetes, cancer, etc)

5. Suitable receptors on the surface of host cells

e.g: CD4 cells

6. Availability of cellular machinery for virus replication

e.g: Enzymes, such as proteases, integrase

7. Fitness of infecting virus

- **Ability to produce complete virion that can infect a new cell**
- **Potential to spread the infection**

Examples of Viral Receptors

Virus	Receptor	Cellular Function
Influenza A	Sialic acid	Glycoprotein
Reoviruses	Sialic acid	Glycoprotein
	EGF receptor	Signaling
Adenoviruses	Integrins	Binding to extracellular matrix
Epstein–Barr	CR2	Complement receptor
Herpes simplex	Heparan sulfate	Glycoprotein
Human herpes 7	CD4	Immunoglobulin superfamily
HIV	CD4	Immunoglobulin superfamily
	CXCR4 and CCR5	Chemokine receptors
Human coronavirus	Aminopeptidase N	Protease
Human rhinoviruses	ICAM-1	Immunoglobulin superfamily
Measles	CD46	Complement regulation
Poliovirus	PVR	Immunoglobulin superfamily
Rabies	Acetylcholine receptor	Signaling
SV40	MHC I	Immunoglobulin superfamily
Vaccinia	EGF receptor	Signaling

Source: *Sherris Medical Microbiology 4th ed, 2004*

I. Classification of Viruses

1. Type of Nucleic Acid

- DNA or RNA (ss /ds)
- Strategy of replication

2. Size & morphology

- Type of symmetry (Icosahedral, Helical . Complex)
- Number of capsomers
- Presence or absence of envelope

3. Presence of specific enzymes

E.g: - RNA and DNA polymerase

- Neuraminidase

- Reverse transcriptase

4. Host tissue/cell tropism

E.g: Hepatitis viruses, HIV, etc

- **II. Unconventional viruses**

Are sub viruses: infectious agents smaller than normal viruses

1. Viroid & virusoid

- **Contain RNA only**
- **Associated with plant disease only**

2. Prions

- **Infectious particles encoded by gene of normal host cells**
- **Composed of proteins only**
- **Cause of certain ‘slow virus disease called transmissible apongiform encephalopathies’ in animals & humans**

- **Some conventional viruses are useful :**
  - **phage typing of bacteria**
  - **sources of enzymes**
  - **production of pesticides**
  - **preparation of antibiotics**
  - **preparation of anti cancer agents**
  - **gene vectors in genetic engineering**

Steps in Virus Replication Cycle

Six steps

1. Adsorption/attachment

- **Proteins on the surface of a virion (virion attachment proteins, VAP) attach to specific receptors on host cells**

e.g: HIV (gp^{120})….. Helper T lymphocytes (CD4 proteins)

- **Receptors on the virus capside or envelope irreversibly binds to cellular receptors on the cell member**

► Limit the host spp. & cells infected

2. Penetration

- **Virus enter the cells**

- **Ways of viral entry into the cells:**

  - Endocytosis ……for entry of naked viruses

  - Simple fusion (coat of enveloped viruses fuse with host cell member)

  e.g: Enveloped viruses

- **Viral NA is released**

  (Release the virus nucleo- capsid into host cytoplasm)

3.Uncoating

- **Viral capsid is broken by viral or cellular enzyme**
- **Viral NA transported to within the host cell**
  - ► **Transcribed to form new progeny virions**

4. Biosynthesis (gene expression & genome replication)

- **Step engaged in synthesis of viral proteins & viral NA**
- **Synthesis of mRNA from viral DNA or directly from some RNA viruses & codes for viral proteins such as:**
  - **Capsid / envelope: encode structural proteins/ building blocks of virion**

- **Enzymes encode for: - DNA / RNA polymerase**

  - Other replication enzymes

- **NA replication produces new viral genomes**

dsDNA viruses:

- **Except Poxviruses, dsDNA viruses replicate in the nucleus using host cell dependent RNA polymerase to synthesize their mRNA**
- **RNA viruses mainly in cytoplasm**

RNA viruses : 3 kinds

1. +ssRNA

+ssRNA virus itself can direct as viral mRNA

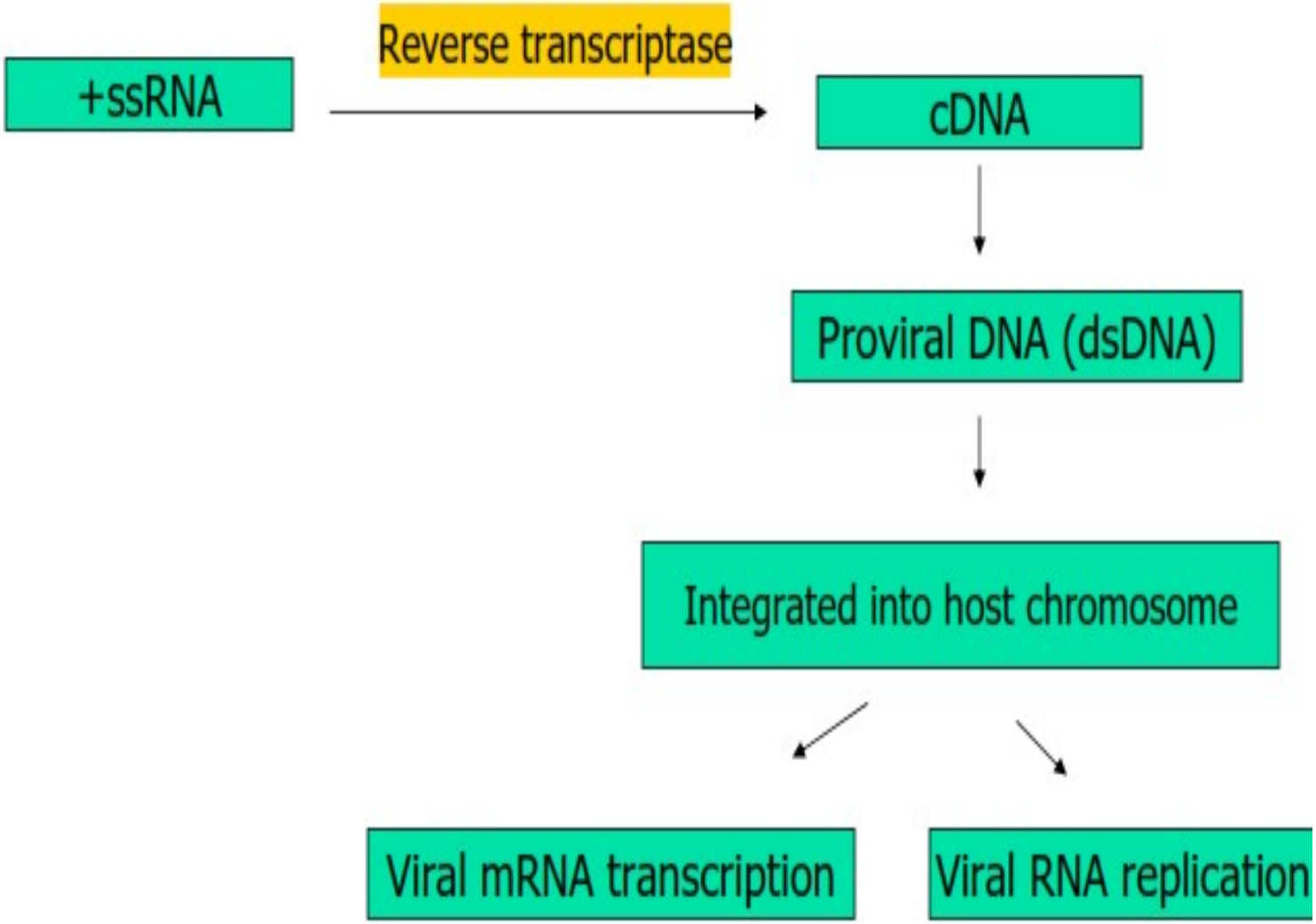
2. -ssRNA

Use the virion RNA polymerase to synthesize viral mRNA

3. Retro viruses

- **Use virion reverse transcriptase to synthesize a DNA copy of the viral RNA genome**
- **Use the host cell RNA polymerase to synthesize viral mRNA**

(3) Retroviruses:



5. Assembly

- **Assembly of viral nucelo - capsid may take place in:**

Nucleus……DNA viruses

Cytoplasm……RNA viruses

At cell surface……Avian Influenza virus

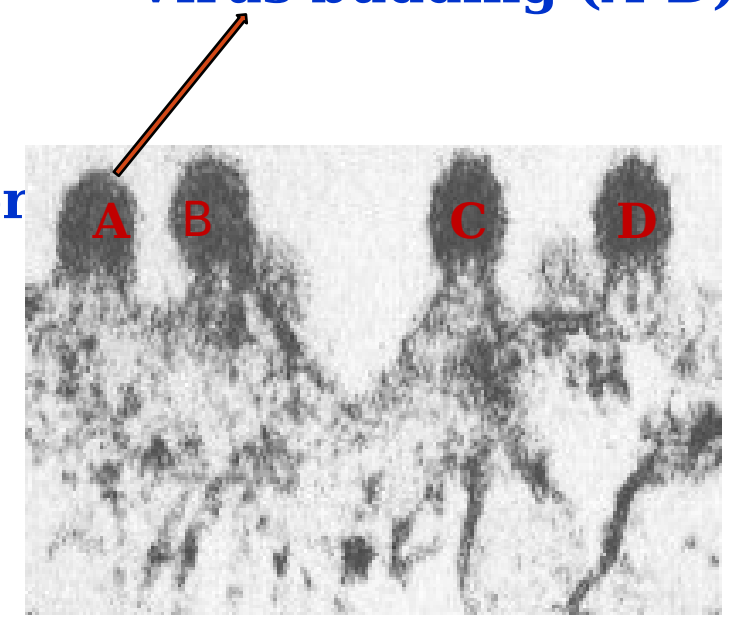
N.B: Accumulation of virions at sites of assembly may form inclusion bodies

- **Visible in stained cells with light microscope**

6. Release

- **Release of new intact infectious virions**
- **May occur by:**
  - **Budding through cell membr**

    e.g: Enveloped viruses

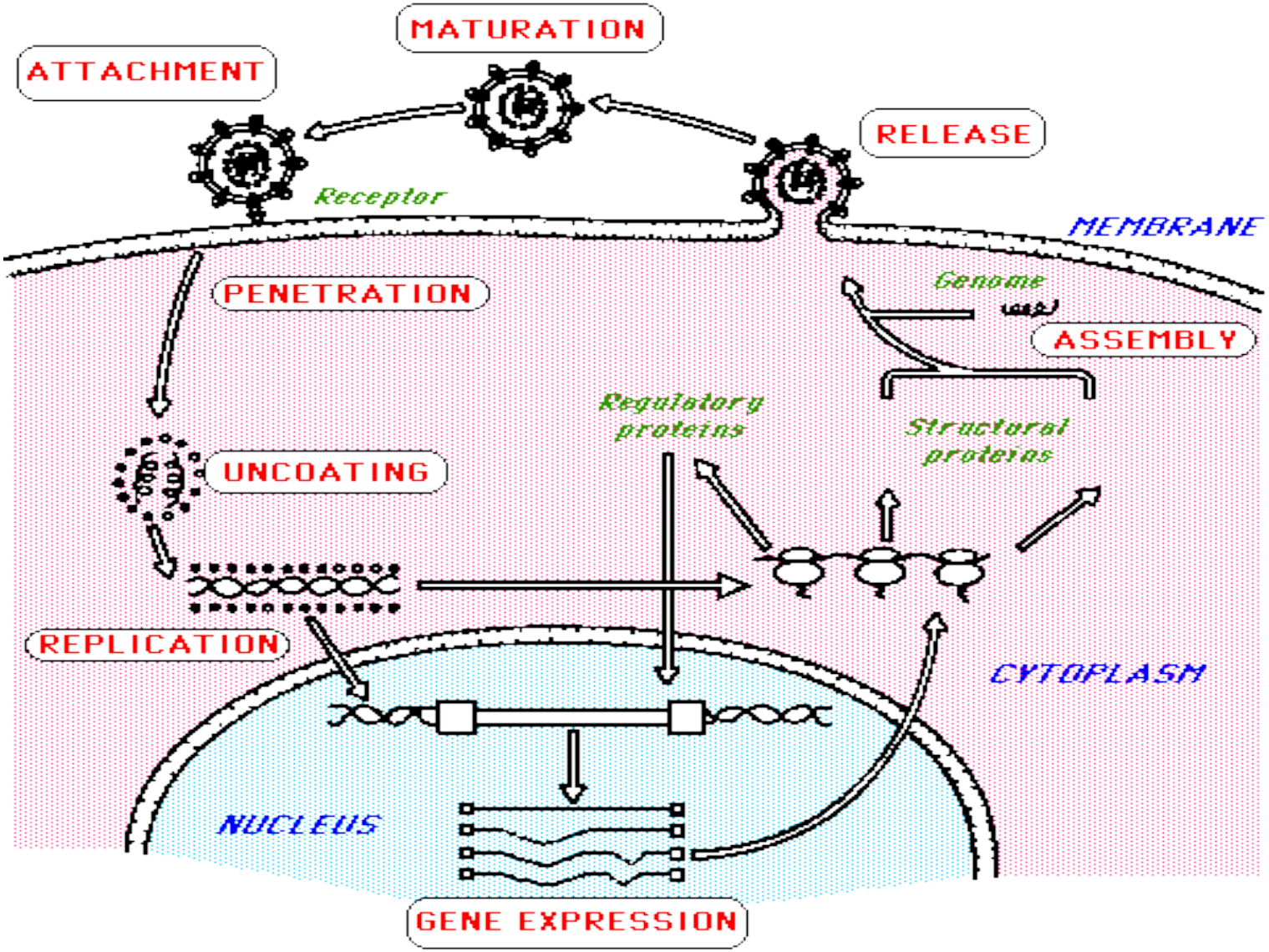


  - **Lysis of infected host cells**

    e.g: Naked viruses

(Diagram)

Viral Replication Cycle



Unusual multiplication

1. Abortive infection

Virus infects a cell but, can not complete the full replication cycle (can not produce a virion)

2. Defective virus

- **Lacks one or more functional proteins (genes) required for replication**
- **Can not replicate with out a helper virus that provide missing genes e.g: HDV requires helps from HBV**

3. Interference

Infection of cell by a virus results in that cell becoming resistant to infection by other viruses

Viral Genome

- **Hereditary determining genetic material : DNA ; or RNA**
- **Mutation in DNA / RNA viruses occurs through :**
  - **Substitution of base (s)**
  - **Addition of base(s)**
  - **Deletion of base(s)**
- **Origin of mutation**
  - **Spontaneous mutations**
  - **Mutations induced by physical / chemical means**

Example of mutants:

- Temp. sensitive mutants (ts)

- Drug resistant mutant

- **Temp. sensitive mutants (ts):**

- Permissive Temp. 28 -35^{0}C

- Non permissive Temp. 36-40 ^{0}C

Example of ts mutant is attenuated mutant (vaccine mutant)

Recombination & Reassortment

- **Recombination**

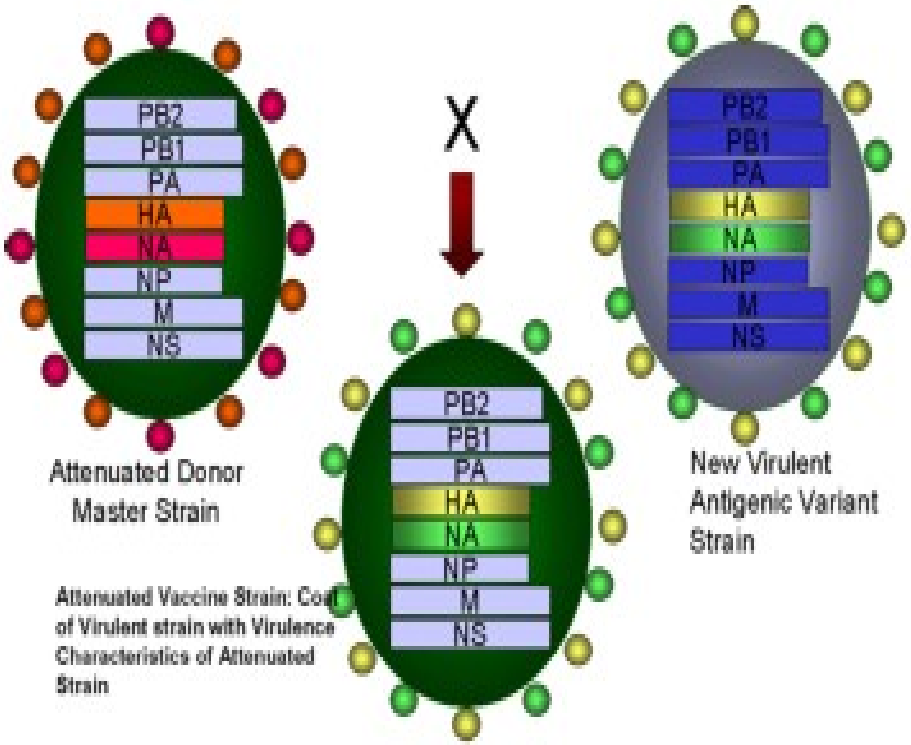
Exchange of genes b/n two related viruses which infect the same cell

e.g: active virus-active virus / active virus - inactive virus

- **Reassortmet**

Exchange of genes b/n two segmented viruses which infect

the same host cell



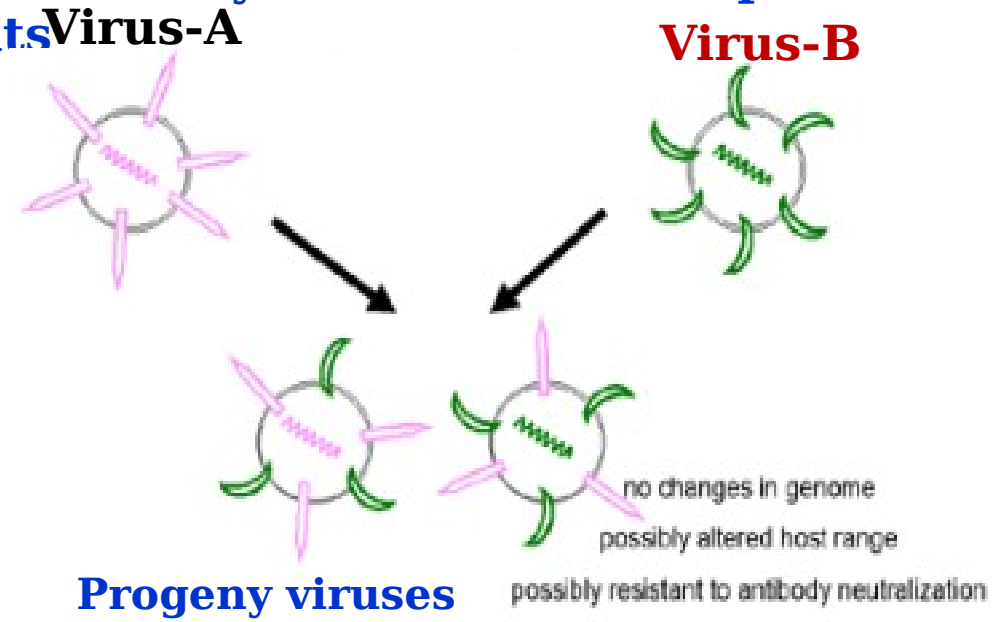
- **Gene integration**

Insertion of viral genetic element (s) into host cell genome

e.g: tumor viruses

- **Phenotypic mixing**

If two different viruses infect a cell (mixed infection), progeny viruses may contain coat components derived from both parents.



Effects of physical & chemical agents on virus

- **Inactivation**

Viruses lose their infectiousness when they exposed to physical/chemical agents

➡ **Viruses can not produce complete & infectious virions**

- **Resistance**
  - **Low Temp. (-196^{0}C)**
  - **pH (5-9)**
  - **Antibiotics**
- **Sensitive**
  - **Temp. (>50 ^{0}C) ; UV /radiation**
  - **lipo-solvents (e.g: enveloped viruses)**

Viral infection vs host macromolecular synthesis

- **Inhibit host RNA, DNA or protein synthesis**

  ► **Affect regulation of expression of host cell gene**

- **Cause cytopatic Effect (CPE) , which is detectable morphological changes in infected host cell**

  ► **CPE may encompass:**

  -Cell rounding

  - swelling or shrinking

  - inclusion bodies

  - cell death

- **Many viruses induce apoptosis in infected cells:**
  - **Important host cell defense against a virus**
  - **Cell death before the completion of the viral replication may limit:**

    - no. of viral progeny

    - spread of infection

- **However, some viruses delay or prevent apoptosis**

  ►**Create a chance to replicate freely**

SPECIFIC VIROLOGY

MAJOR DISEASES	IMPORTANT VIRUSES
AIDS	**HIV-1**
Hepatitis	**HAV, HBV, HCV, HDV, HEV, CMV, EBV**
Cancer	**HPV,HCV, EBV, HBV, HHV-8, HTLV-I**
Encephalitis	**HSV,togaviruses, flaviviruses, rabies, picornaviruses**
Meningitis	**Enteroviruses, mumps, HSV-2, VZV**
Gastro intestinal tract	**Rotavirus, caliciviruses, adenoviruses**
Common cold	**Rhinoviruses, coronaviruses, adenoviruses**
Lower respiratory tract	**Influenza, parainfluenza, ReSV, adenoviruses, SARS-CoV**
Congenital & Neonatal Infections	**CMV, Parvovirus B19, rubella, HSV, HIV**

GASTROENTERITIS

- **In recent years, improvements in:**
  - **hygiene**
  - **sanitation and**
  - **water supply in developing countries has resulted in a drop in the proportion of cases due to bacterial causes**
- **As a result the majority of diarrhoeal disease hospitalizations are due to viruses**

- **Clinical features**

- **Infection transmits through the faecal-oral route**
- **Viral gastro-enteritis has a short incubation period (about 24 hrs)**

Symptoms of viral gastro-enteritis:

- **sudden onset of vomiting**
- **profuse watery (secretory) diarrhea (self limiting within 2-3 days)**
- **Morbidity & mortality is due to dehydration**
- **Young infants (>5 yrs) are at greatest risk**

- **Pathogenesis**

- **Viruses infect enterocytes of the upper small bowel, causing inflammation & reduce function of villi to absorb fluid**

- **Beside, viruses such as rotavirus produce enterotoxins that promote fluid & electrolyte loss from enterocytes**

- **Virus is cleared & symptoms resolve with the development of a local immune response.**

- **Specific secretory IgA Abs confer immunity & prevent symptomatic re-infections with antigenically related viruses**

- **The 4 commonest viral causes of diarrhea are:**
  - **Rotaviruses**
  - **Caliciviruses (Norovirus and Sapovirus)**
  - **Adenoviruses**
  - **Astroviruses**

Virology of Diarrheal Viruses

	Rotavirus	Norovirus	Adenovirus	Astrovirus
Family	Reoviridae	Caliciviridae	Adenoviridae	Astroviridae
Genome type	dsRNA	ssRNA	dsDNA	ssRNA
Genome organisation	Segmented	Linear	Linear	Linear
Enveloped	No	No	No	No
Morphology	Spherical with wheel-like surface structure	Small with star-like surface structure	Icosahedral	Small, with star-like surface structure

Adeno Virus

- **Family: Adenoviridae**
- **Undeveloped icosahedral ds DNA viruses,**
- **approximately 80 nm in diameter**
- **There are 41 human adenoviruses which are divided into 6 sub-genera, A-F**
- **Common stereotypes: 1 -8, 11, 21, 35, 37, 40 & 41**
- **Types 40 & 41 are main enteric pathogens !**

Classification of human adenoviruses & their associated diseases

Sub group	Viruses	Target organ/disease
A	**12, 18, 31**	**GIT**
B	**3, 7, 11, 21**	**Pharynx, lungs, conjunctiva, Urinary tract**
C	**1, 2, 5, 6**	**Pharynx**
D	**8, 9, 19**	**Eye (keratoconjunctivitis)**
E	**4**	**Upper respiratory tract, eye**
F	**40, 41**	**GIT**

Epidemiology

- **Infections occur throughout the year**
- **Highly resistant to inactivation**
- **viable virus may remain on environmental surfaces**
- **Nosocomial transmission of adenovirus leading to outbreaks is common in paediatric ICUs**
- **Transmission is through respiratory droplets, fomites & ingestion**

Commonly obsereved in :

- **Military camps**
- **swimming pool users**
- **residential institutions**
- **hospitals**
- **day care centers**

Incubation period: 2 - 14 days

Adeno viruses are responsible for a range of clinical conditions involving:

1. Respiratory system

- **Upper respiratory infections**
  - **rhinitis**
  - **pharyngitis**
  - **tonsillitis**

- **Lower respiratory infections**
  - **bronchitis**
  - **acute respiratory disease**
  - **pneumonia**

2. Eye **Acute follicular conjunctivitis & kerato conjunctivitis**

3. Gastrointestinal

- **Gastroenteritis ; mesenteric adenitis ; hepatitis ; appendicitis**
- **Diarrhea tends to last longer than other viruses that cause Gastroenteritis E.g. Rotavirus**
  - **May cause fatal disease in immuno - compromised patients**
- **Most infections are asymptomatic**
- **those that do manifest clinically are usually acute & self-limiting.**
- **Some subtypes may be harboured asymptomatically for years**

Lab. diagnosis

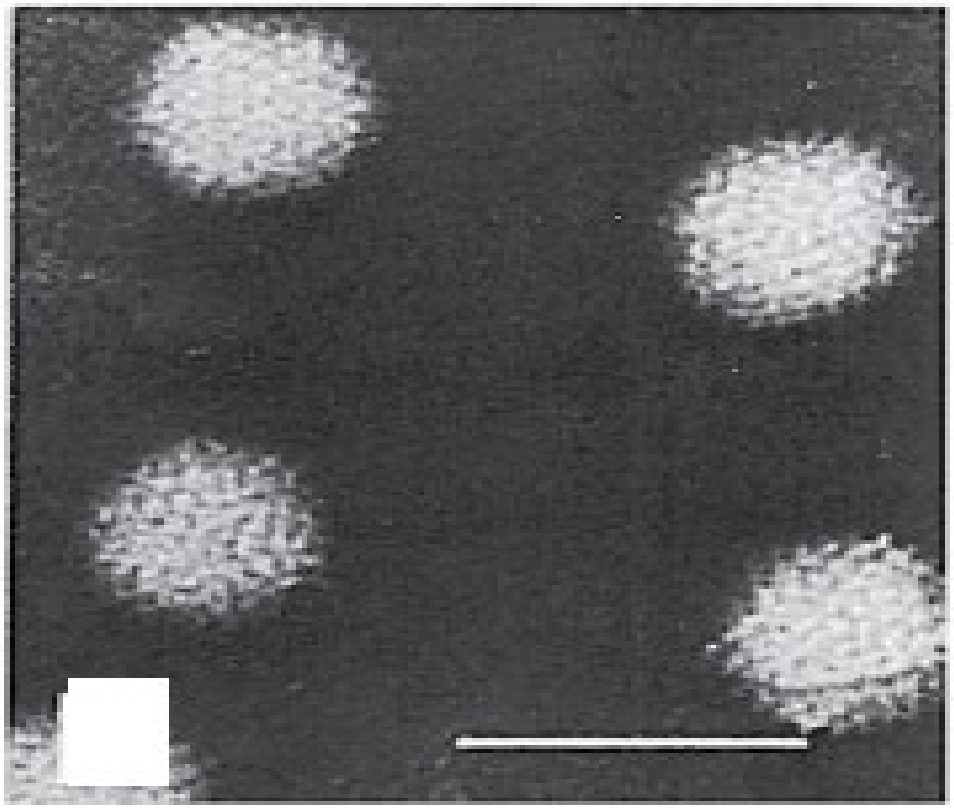
1.Viral isolation

Cell culture using human embryonic kidney & human fetal cells

- **Observe CPE in cultured cells**

2. Rapid Detection

- **ELISA**
- **Immuno fluorescence : for Ag detection using electron microscope**
- **PCR**



Adenovirus particles found in stool

Prevention and control

- No vaccine/ specific treatment
- Good hand washing
- Adequate chlorinating of swimming pools
- Sterilization & disinfection of ophthalmologic equipment

Properties:

- **5 Antigenic groups are known (A - E)**
- **Most human strains are found in group A**
- **Unable to grow in routine cell cultures (*in vitro*)**
- **Hard & may survive in sewage**

- **Protective immunity is directed to 2 surface proteins: G (VP7) and P (VP4)**
- **Reassortment with different rotaviruses can occur (segmented genome)**
- **Human disease is caused by rotaviruses that specifically infect humans**
- **But, there are rotaviruses that infect other animals as well.**
- **Animal rotaviruses can infect humans, but do not cause disease**
- **Immunity following first infection is incomplete & humans can have repeated infections with rotaviruses of a different G or P subtype**
- **Repeated infections tend to be less severe**

- **Disease severity is linked to the age of first rotavirus encounter**
- **Most children encounter rotavirus for the first time during the first year of life (in the first 3 months) in developing countries**
- **Largest cause of diarrhea in children (50 - 80%)**
- **Account for more than half million deaths of infants in 3rd world/yr**
- **Maternal antibody may reduce disease severity in the neonatal period, but protection is short lived**
- **First exposure is associated severe disease**
- **Infection is followed by partial immunity (immunity to severe disease) and is cross protective. i.e. protects from severe disease due to rotaviruses of another serotype**

Incubation: 1-3 day

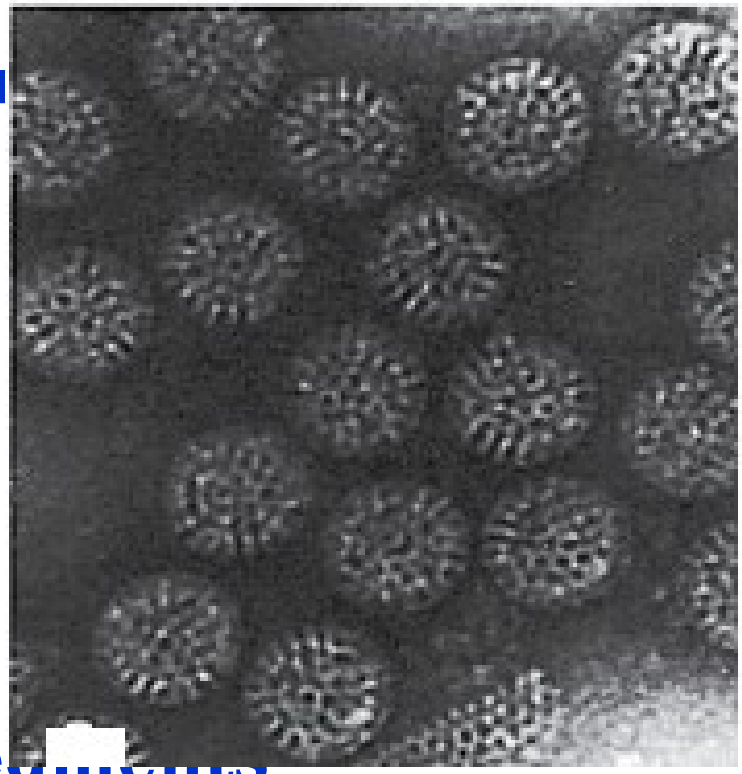
Complications

- **Sudden onset watery diarrhea**
- **vomiting**
- **dehydration**

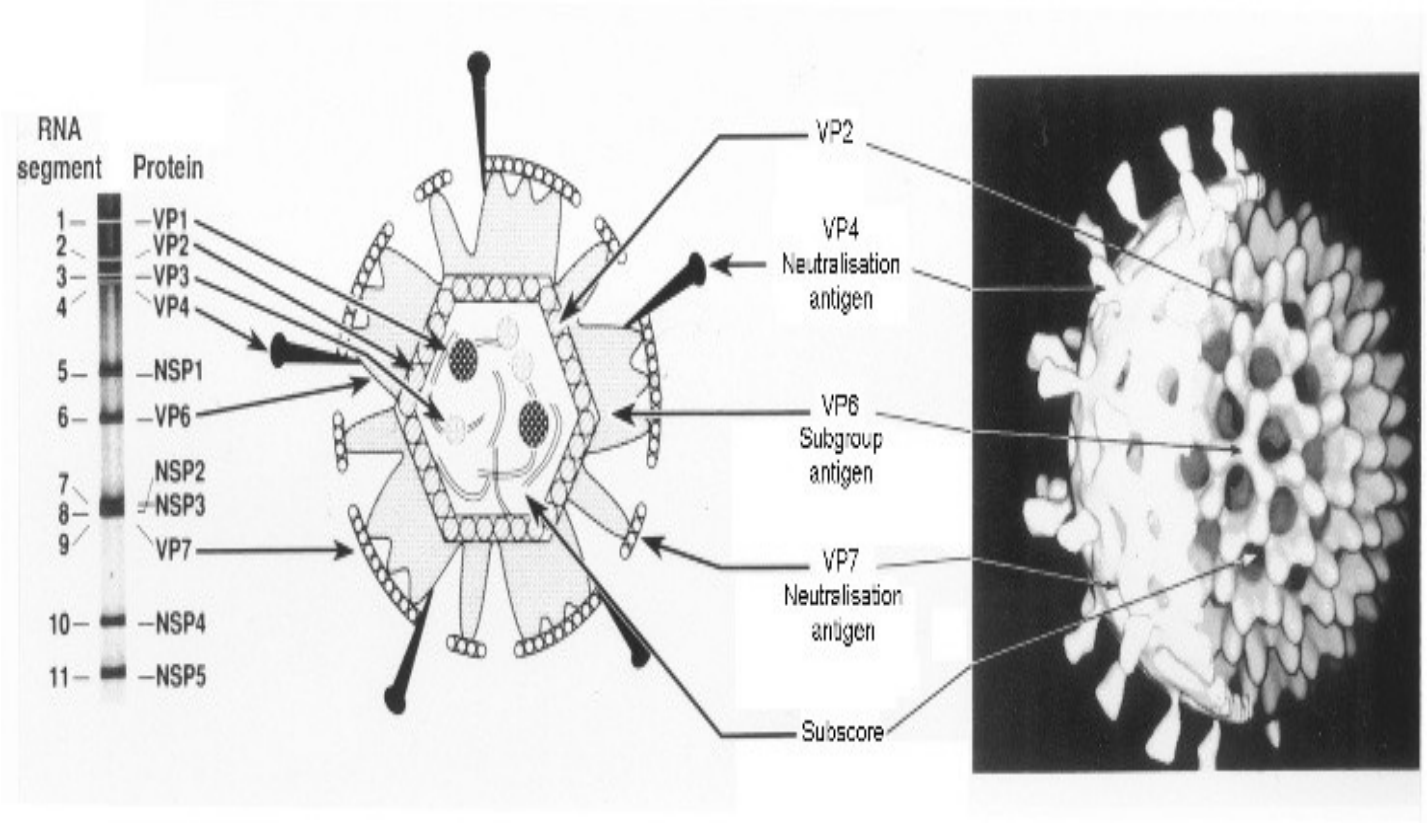
Laboratory Dx

Detection of virus in stool u

- Latex agglutination
- ELISA
- electron microscope
- electrophoresis of RNA segments

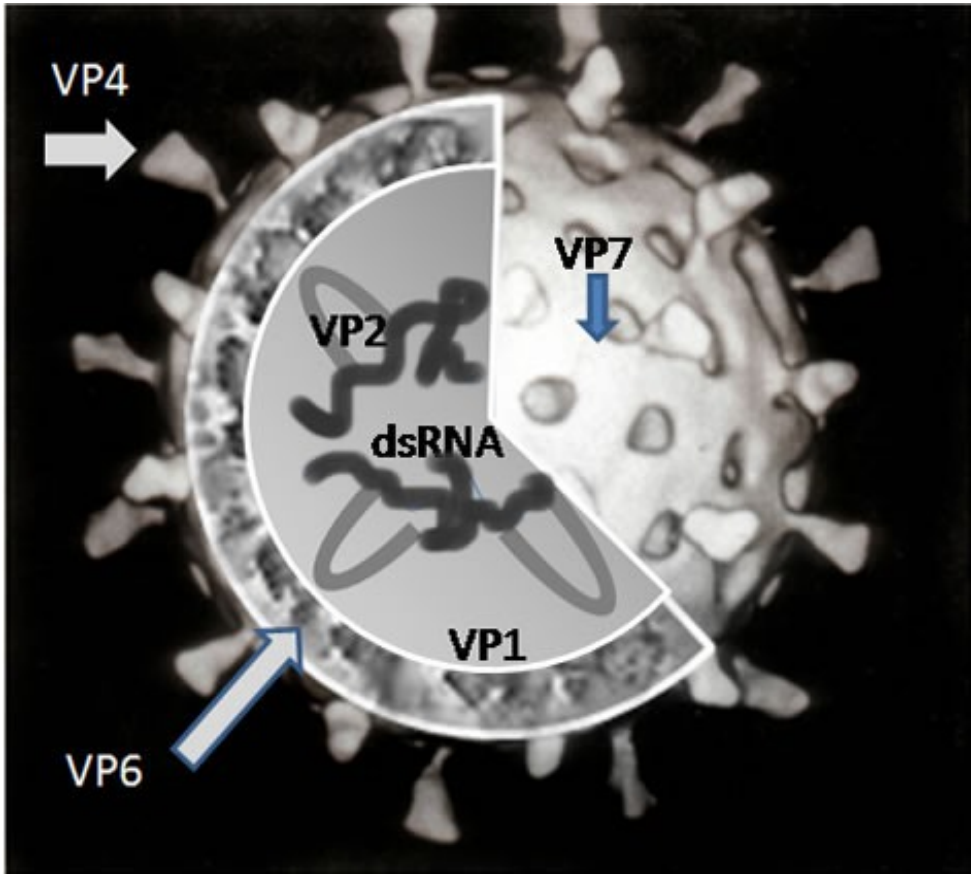


Electron micrographs of virus particles found in stool



Protein products and particle of Rotavirus

(Mattion et al. 1994)



A simplified diagram of the location of rotavirus structural proteins

RX

- **No specific treatment but management of dehydration**

Rotavirus vaccines

- **Protection from re-infection/disease is antibody mediated (especially secretory IgA).**
- **2 oral live attenuated rotavirus vaccines that protect against severe rotavirus disease are in current use:**

1. Rotarix **(**infant EPI programme in South Africa in 2009)

- **Is a live attenuated monovalent vaccine derived from a human rotavirus.**
- **It induces broadly cross reacting immunity to many rotavirus serotypes.**
- **Immune response to this is enough to reduce disease severity on second encounter with any other common rotavirus type.**
- **Infants receive 2 doses of vaccine at 10 and 14 weeks**.

2. Rotateq

- **A pentavalent human & bovine rotavirus reassortant vaccine**

- **Is a bovine rotavirus that has been reassorted to contain the genes that code for the commonest P subtype seen in human rotaviruses**

N.B: protective immunity is directed to the G and P structural proteins on the virus surface

Calici viruses (genus Noro viruses)

- **Within the calicivirus family, noroviruses are main cause of gastro-enteritis**
- **Noroviruses:**
  - **account for approximately 10% acute diarrhea in < 5 children in developing countries**
  - **commonest cause of food borne non bacterial gastro-enteritis outbreaks in people of all ages**

Clinical features of infection

- **similar to other viral causes of gastro-enteritis**
- **Stool and vomitus are highly infectious**

Transmission of infection

- **Fecal oral route**
- **Aerosolized droplets.**
- **High attack rates are seen during outbreaks**

(in both children and adults)

- **Immunity following infection is short lived & re-infections are common**
- **There are no vaccines available**

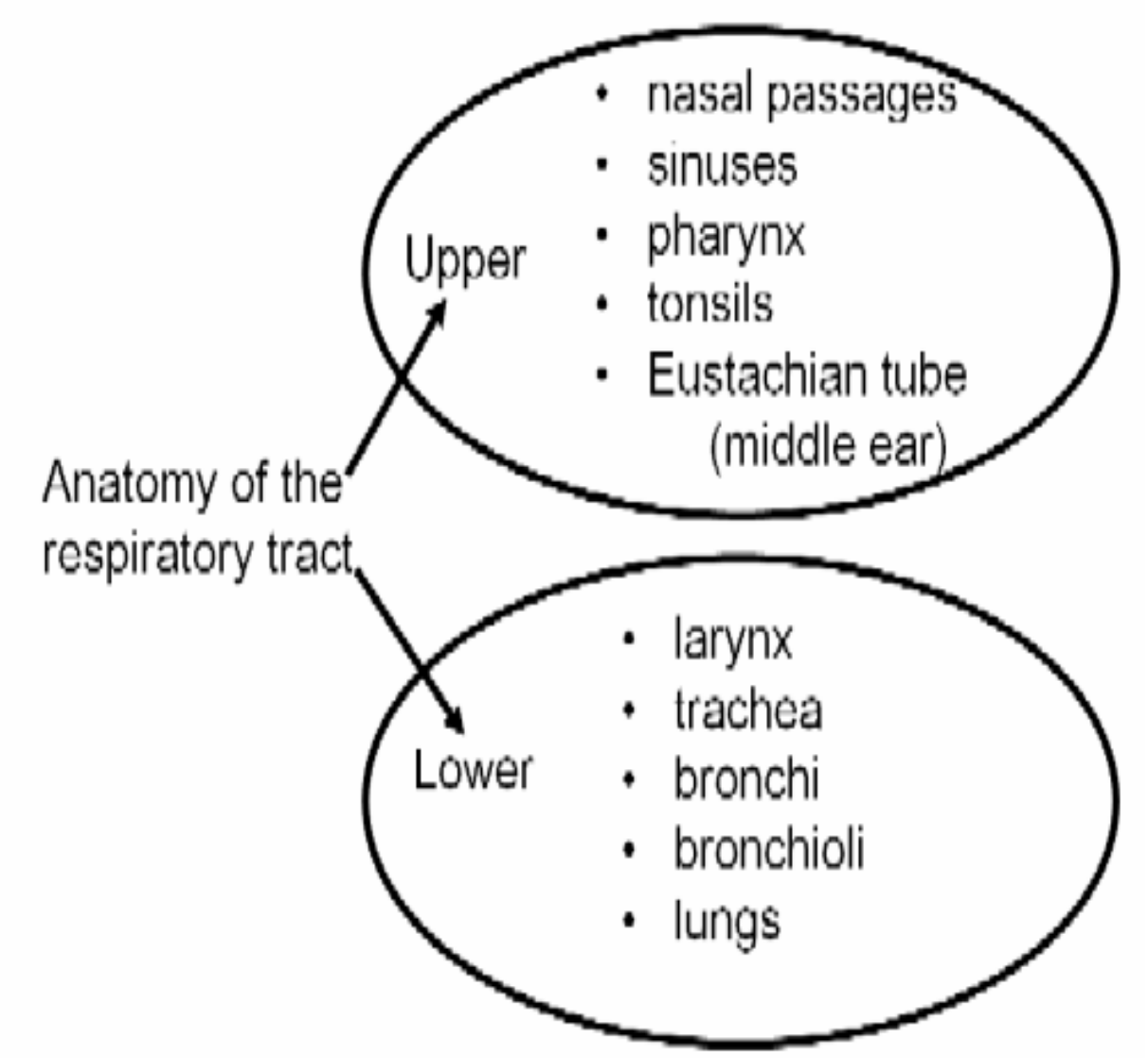
RESPIRATORY INFECTIONS

- **Infections of the respiratory tract are very common in both adults & children**

- **Most are mild & confined to the upper respiratory tract (URT)**

- **However, in infants & young children:**

URT infections may spread downwards and cause more severe & life threatening infections


Anatomy of the respiratory tract
Upper
nasal passages
sinuses
pharynx
tonsils
Eustachian tube (middle ear)
Lower
larynx
trachea
bronchi
bronchioli
lungs

- **1. Upper Respiratory Tract**

A. Colds

- **Characterized by a watery to mucoid, sometimes purulent nasal discharge ("coryza")**
- **Often preceded by a sore throat & may sometimes be accompanied by fever**

  e.g: Rhinoviruses, Coronaviruses and others

- **May be followed by transient opportunist bacterial infection**

B. Pharyngitis ("sore throat")

- **Generalized erythema of pharynx not localized to the tonsils**
- **Some fever may be present e.g: Adenovirus**

C. Tonsillitis

Local infection of tonsils: red, swollen with exudate on the surface e. g: Adenovirus

D. Sinusitis & Otitis Media

- **Inflammation of mucous membranes lining sinuses & middle ear**
- **Impaired drainage of these spaces may lead to bacterial infection.**
- **Bacterial infections are usually secondary to viral infection of the nose & pharynx**

2. Lower Respiratory Tract

A) Laryngo -Tracheo Bronchitis (Croup)

- **An acute inflammation of larynx and trachea.**
- **Often preceded by coryzal symptoms, followed by fever, cough & upper airway obstruction (stridor)**
- **This condition may be life threatening in young children. (para-influenza viruses, RSV, herpes simplex)**

B) Acute Bronchitis

Inflammation of bronchi, accompanied by fever, cough, wheezing & "noisy chest" e.g: RSV, Para-influenza viruses, Adenovirus

C) Acute bronchiolitis

- **Inflammation & narrowing of terminal bronchioles**
- **Bronchiole diameter is larger during inspiration than during expiration**
- **This leads to hyperinflation of air sacs distal to bronchiole**
- **Illness starts with fever & coryzal symptoms, followed by respiratory distress**

& wheezing sound

- **The condition can be life-threatening in babies & small children**

  e.g: Respiratory Syncitial virus, Para-influenza virus

D) Pneumonia & bronchopneumonia

- **Infection of the lung tissue itself**
- **Symptoms include: fever, respiratory distress & cyanosis**
- **can be life-threatening e.g: RSV, Adenovirus, Influenza**

INFLUENZA VIRUSES

Influenza or "flu"

- **is a respiratory tract infection caused by influenza viruses**
- **Flu is a primarily respiratory tract infection but systemic symptoms are common**
- **Influenza infection results in 250,000-500,000 deaths annually in high income countries (WHO,2015)**
- **Influenza virus is able to mutate and cause pandemic with significant morbidity and mortality across the world.**
- **The most notorious 1918 "Spanish flu“ (H1N1) pandemic was estimated to cause 100 million deaths worldwide**

Difference between flu & common cold

Illness	Influenza/flu	Common cold
Clinical spectrum	Often systemic	Mostly local
Speed of onset	Abrupt	Gradual
Fever	Usually high	Usually low-grade
Presentation	Chills, myalgia, malaise, sore throat	Sneezing, sore throat, nasal congestion
Fatigue	Marked	Mild
Course	Unwell for 1-2 weeks, chest problems common, severe malaise	Rapid recovery
Complications	More common and often severe - pneumonia	Uncommon
Occurrence	Seasonal	All year round

Properties

- **Family: Orthomyxoviridae**
- **3 Genera: influenzavirus A,B, & C**
- **ss RNA virus ,with 7-9 RNA segmented genome**
- **Enveloped with a helical symmetry**
- **Possess two surface glycoproteins**

  (Haemagglutinin &Neuraminidase)
- **High frequency of mutability & genetic reassortement**

1. Influenza type A

- **Responsible for pandemics**
- **Further subtyped according to its H and N group**
- **There are 16 H types and 9 N types of influenza A that exist in nature, mainly found in the natural reservoir wild aquatic birds**
- **Only H1, H2 and H3 and N1 & N2 subtypes circulate widely in human**
- **Possess highly variable antigens**
- **Can infect pigs/swine, horse, avian (birds) & human**
- **Swine are known to be susceptible to both avian & human viruses (Mixing vessel)**

2. Influenza type B

- **Demonstrate minor antigenic changes**
- **causes epidemics infrequently**

3. Influenza type C

- **Antigenically stable & causes mild disease**

4 antigenic proteins

1.Haemagglutinin (HA)

2. Neuramindase (NA)

3. Nucelo - capsid proteins (NCP)/Ribon-uceloprotein

4. Matrix proteins (MP)

1. HA

- **Responsible for viral attachment**
- **Account for about 25% of viral protein**

2. Neuraminidase (NA)

Responsible for viral exit from infected cell

Note:

Both HA and NA are important Ags that confer subtype specific immunity & are therefore, used in the vaccine designs

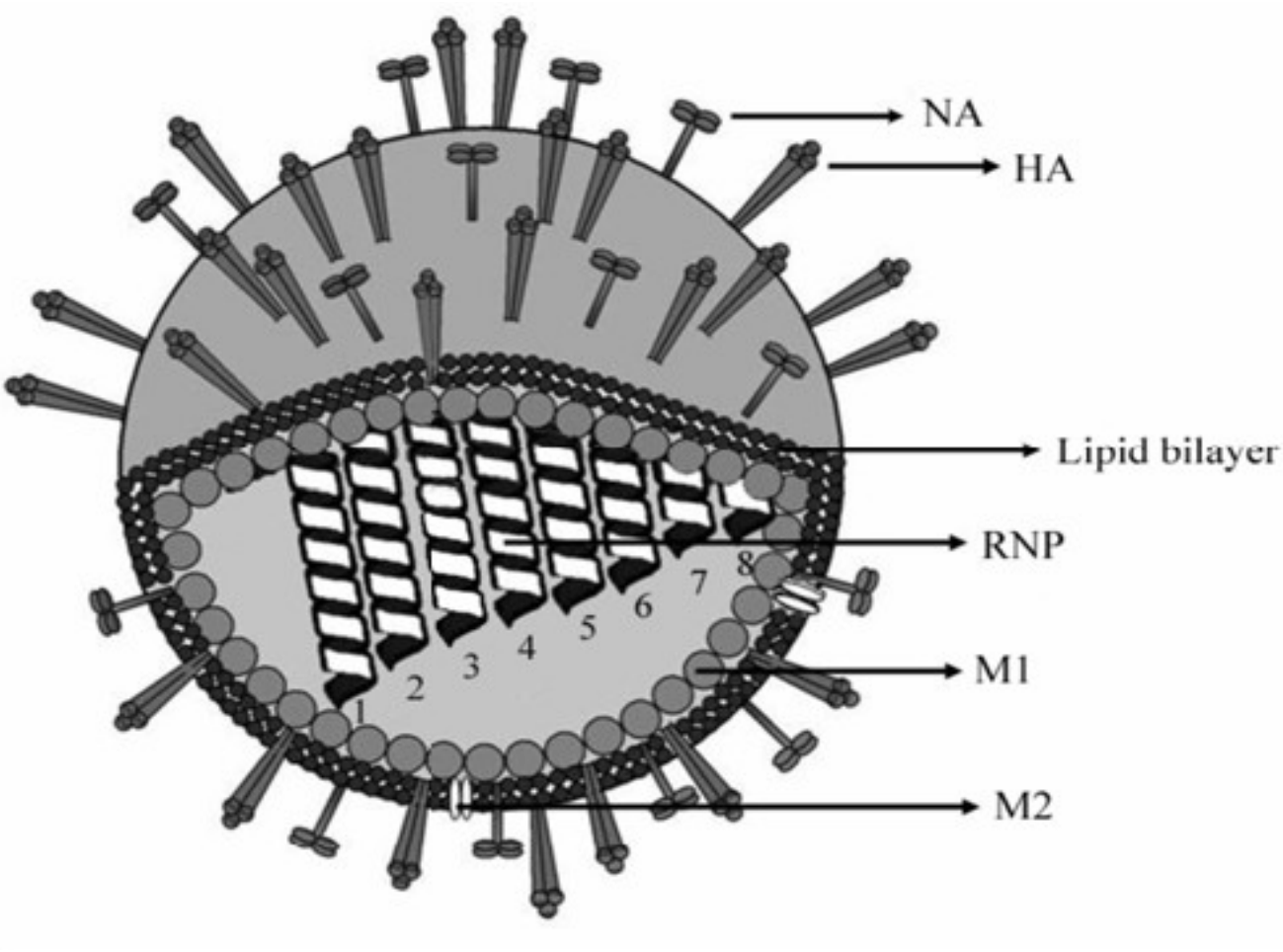
3. NCP

- **Occurs in 3 forms; A, B & C antigens of the viruses**
- **Provide basis for classification of human IVs (A-C)**
- **Important to form helical symmetry of the virus**

4. MP

- **Surrounds the nucelocapsid**
- **Account for about 45% of total viral protein**
- **Important in viral morphogenesis**

Figure 1. Structure of Influenza A virus



Standard nomenclature for IV isolates:

- **type**
- **host of origin**
- **geographical origin**
- **strain number**
- **year of isolation**

e. g: A/Hong Kong /1/ 68 (H_3 N_2) signifies:

Influ. A virus isolated from a patient in 1968 & of sub type H_3N_2

Antigenic shift

- **Is major antigenic changes in HA & NA**
- **Responsible for unique epidemiological features**

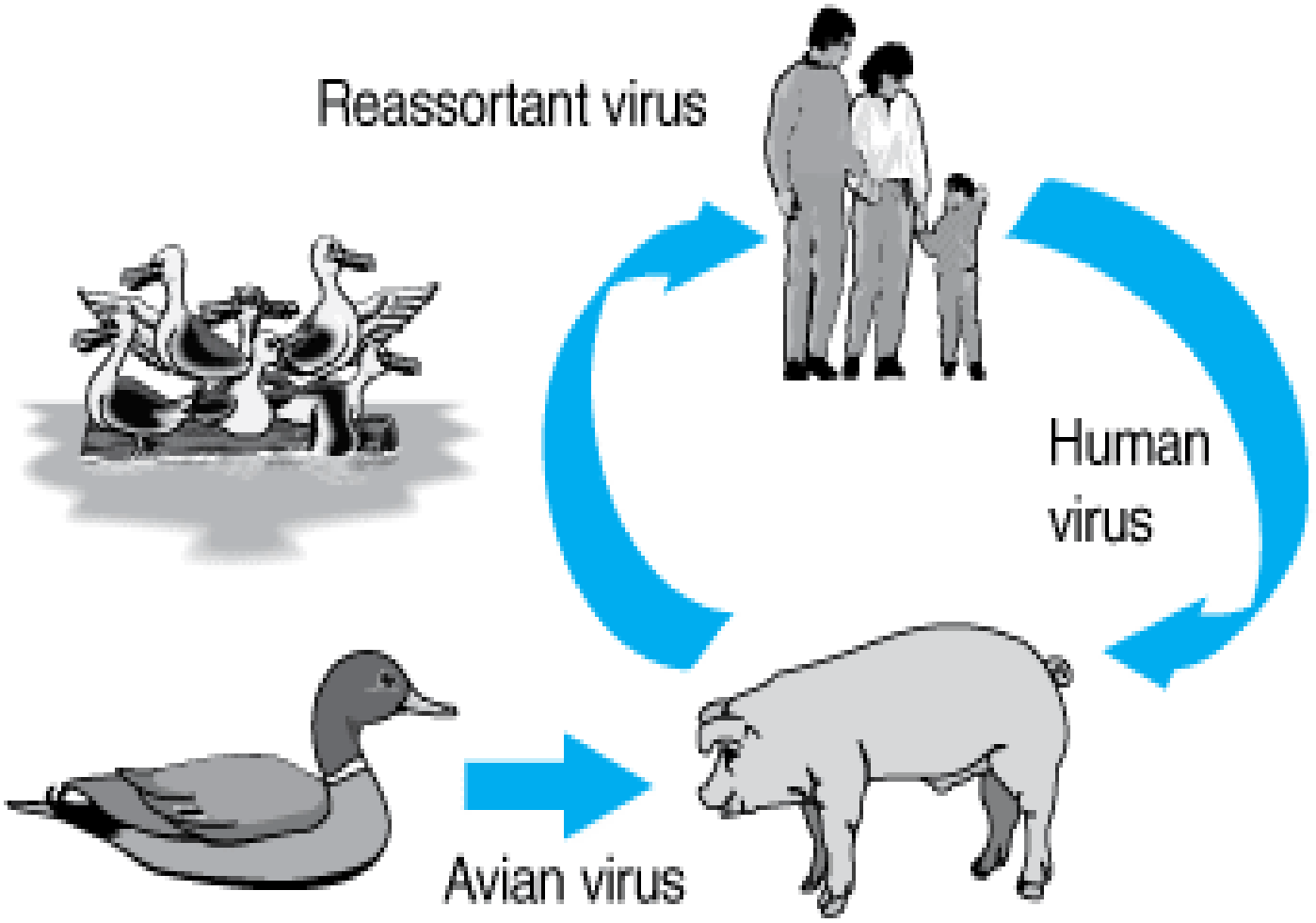
  ►**Appearance of new influenza sub types / strains against which the population has no immunity ;**

  ►**Cross spp. barrier; e.g. Infu.type A (H5N1 or Bird flu)**

  N.B: Pig is the most mixing vessel for formation of new strain

Re: Pig can be infected by both human & avian IVs (Resentment)

Reassortment: Responsible for emergence of flu pandemics

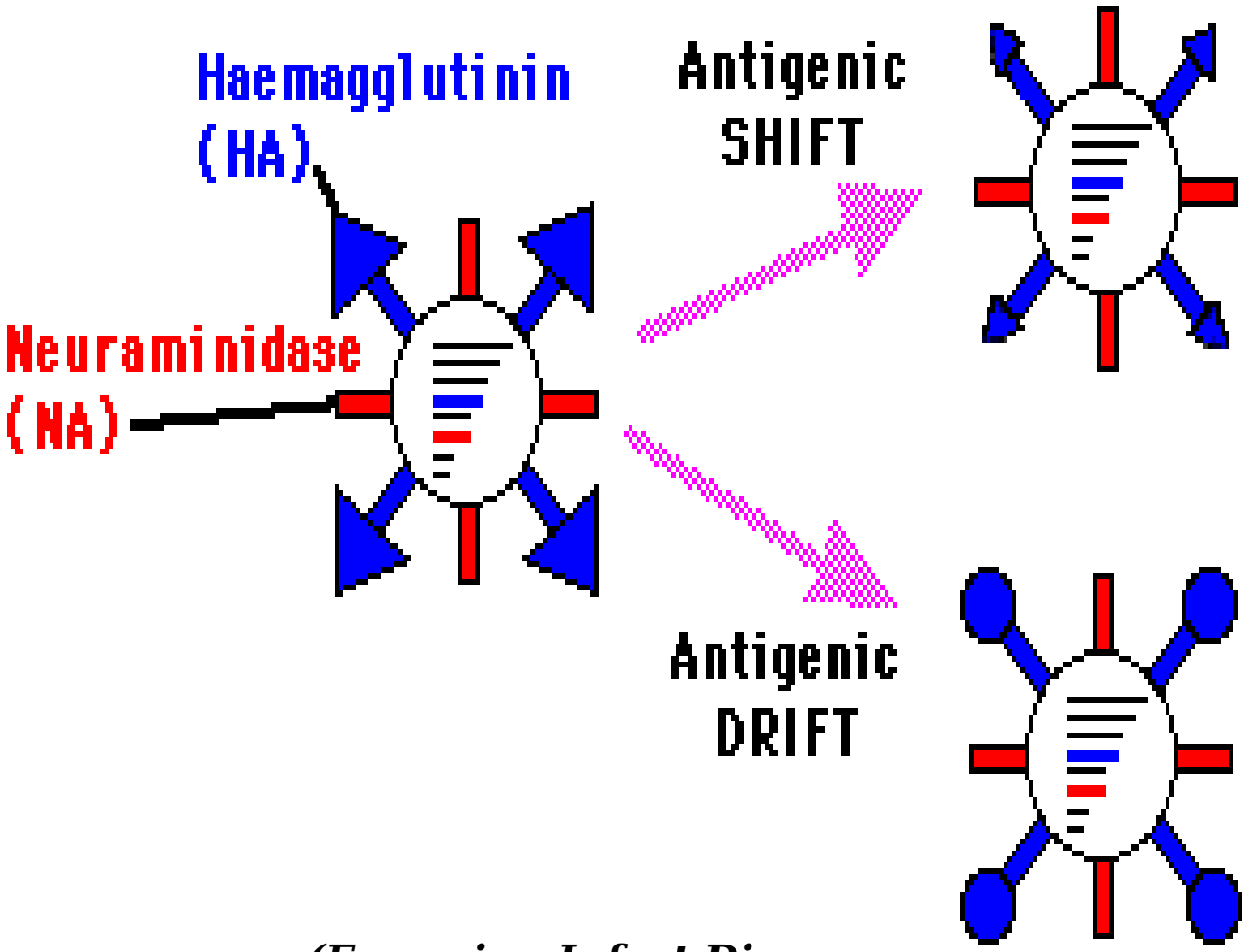


(Source: Science, 2005;310:77)

Antigenic drift

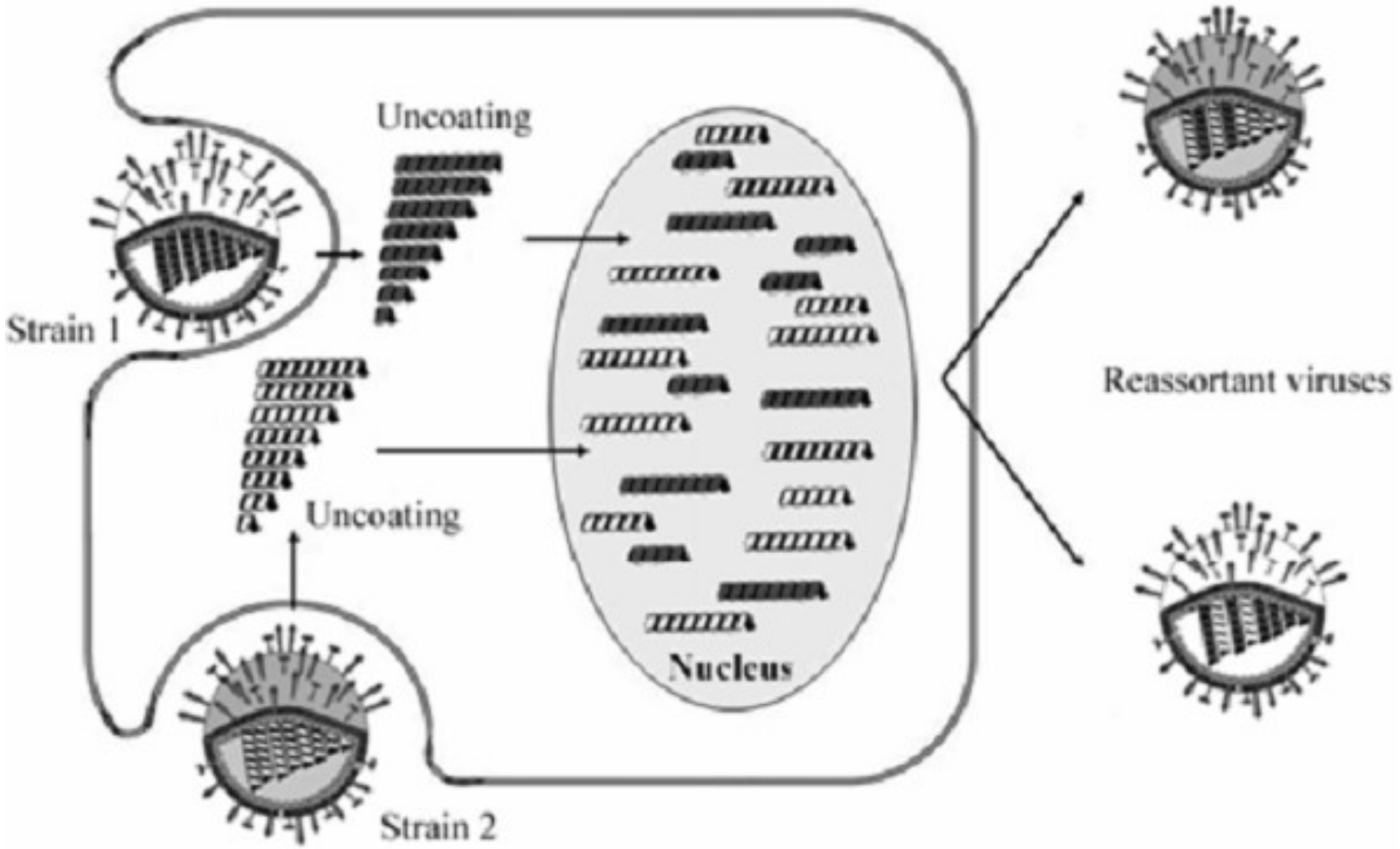
- **Is minor antigenic change in HA or NA**
- **Arise via point mutation: amino acid change**
- **Epidemics due to new virus strains is very less**

Fig.2 Two mechanisms allow IV to Alter its Antigenic Structures



(Emerging Infect Dis, 2006:12:1)

Fig. 3. Influenza A antigenic shift: genetic reassortment b/n two viruses strains infecting



Risk factors for severe influenza disease

- pregnancy
- chronic pulmonary, cardiac or renal disease
- diabetes mellitus,
- individuals over 65 years
- children less than 5 years

Modes of transmission of Human influenza virus

- **Inhalation (aerosol) of small particles**
- **Fomites (contamination & subsequent self-inoculation)**
- **Human became infected with H5N1:**
  - **Exposed to an infected bird**
  - **Plucking and preparing diseased birds**
  - **Handling fighting cocks**
  - **Consumption of duck's blood or undercooked poultry**

- **Recent evidence showed limited human-to-human transmission of H5N1**

E.g. Indonesia epidemiologic and genetic sequencing data

suggest that a 10-year-old boy contracted the virus from his

aunt and then passed it on to his father !!

(Journal of Brazilain Infectious Disease, 2007)

Pathogenesis

- Virus inhaled & reach respiratory epithelium
- Replicate & produce new progeny virion
- NA decrease viscosity of mucous
- Spread of the virus
- Viral invasion & damage of host cells

Clinical features

- **High grade fever (> 38°C)**
- **Bleeding from the nose & and gums (early stages of illness)**
- **Lung symptoms (respiratory distress, tachypnea)**
- **Sputum production (occasionally, hemoptysis)**

Complications

- **Pneumonia: 1º viral or 2º bacterial pneumonia**
- **Tracheo-bronchitis: Severe respiratory symptoms**
- **Viral encephalitis**

Lab. diagnosis

Potential specimens

- **Throat swabs , nasal washings , nasopharyngeal aspirations & blood**

Lab diagnostic techniques

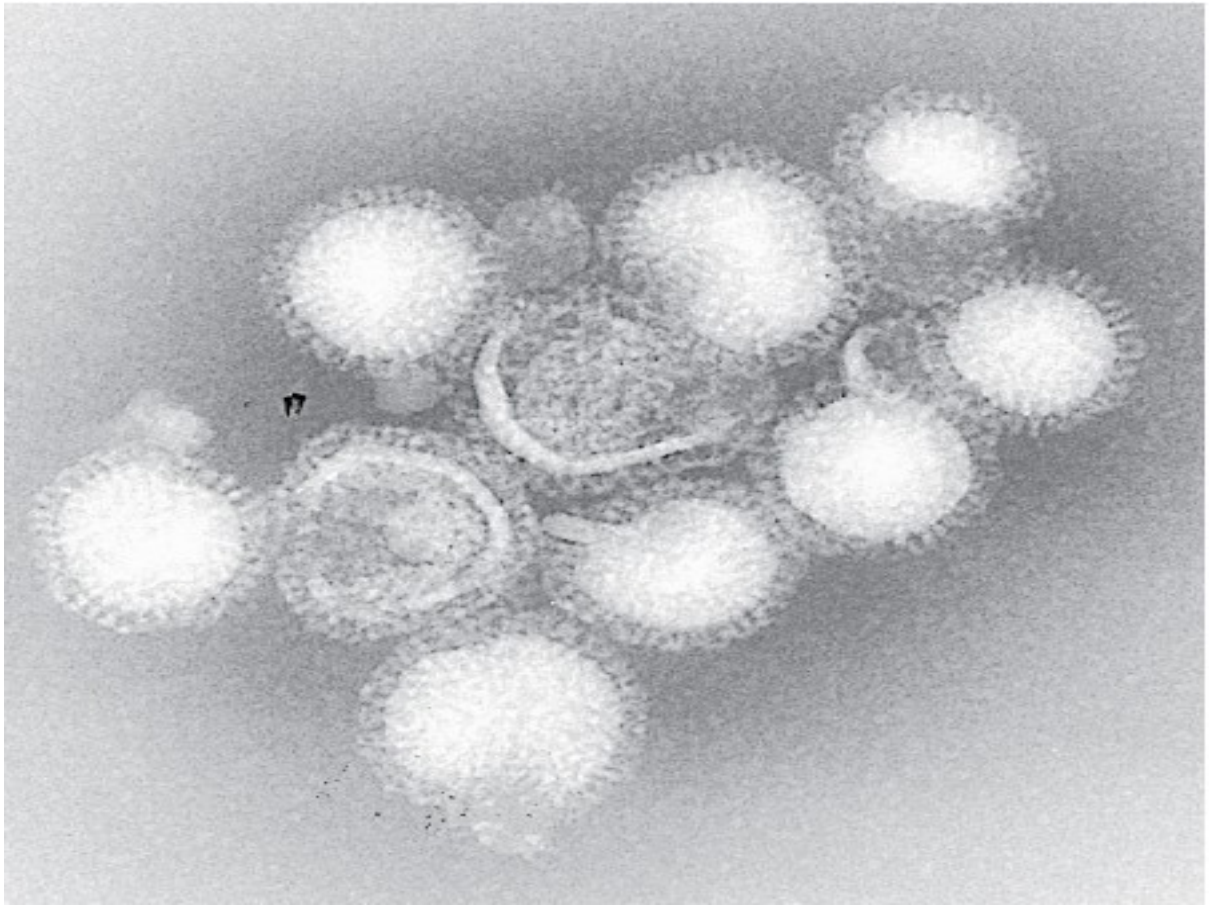
1. Virus isolation in cell cultures such as Madin-Darby canine Kidney cells

- **Laborious & limited to specialized lab. due to bio safety**

2. Detection of virus-infected cells by immunofluorescence

- **Cells can be identified using specific monoclonal Abs**
- **Infected cells synthesize & express viral proteins (Ags)**
- **Presence of these can be detected using specific mono-clonal Abs labelled with fluorescene**
- **The Ab. binds to the cells if they express the corresponding Ag**
- **The cells can then be visualized by examination under a fluorescent microscope**
- **Positive cells fluoresce a bright green color**

Influenza virus particle



3. Detection of Non Structural Proteins 1 (NS1)

- **Found only in infected cells by avian influenza**
- **Rapid & distinguish avian influenza from non-avian human Ivs**

4. Serologic tests

5. Molecular techniques

Detection of viral RNA by RT- PCR

Treatment (Rx)

Two classes of antivirals against influenza viruses

1.M2 channel inhibitors : **Amatidine & Rimantidine**

- **prevent the entry of the virus to the cells**
- **But, check for resistance to the drug (AST)**

2. The neuraminidase inhibitors : **Zanamivir & Oseltamivir (Tamiflu)**

- **prevent budding off newly formed virions**
- **Only effective if given early (first 48 hours of infection)**
- **Resistance to neuraminidase inhibitors is common among some subtypes of influenza virus**

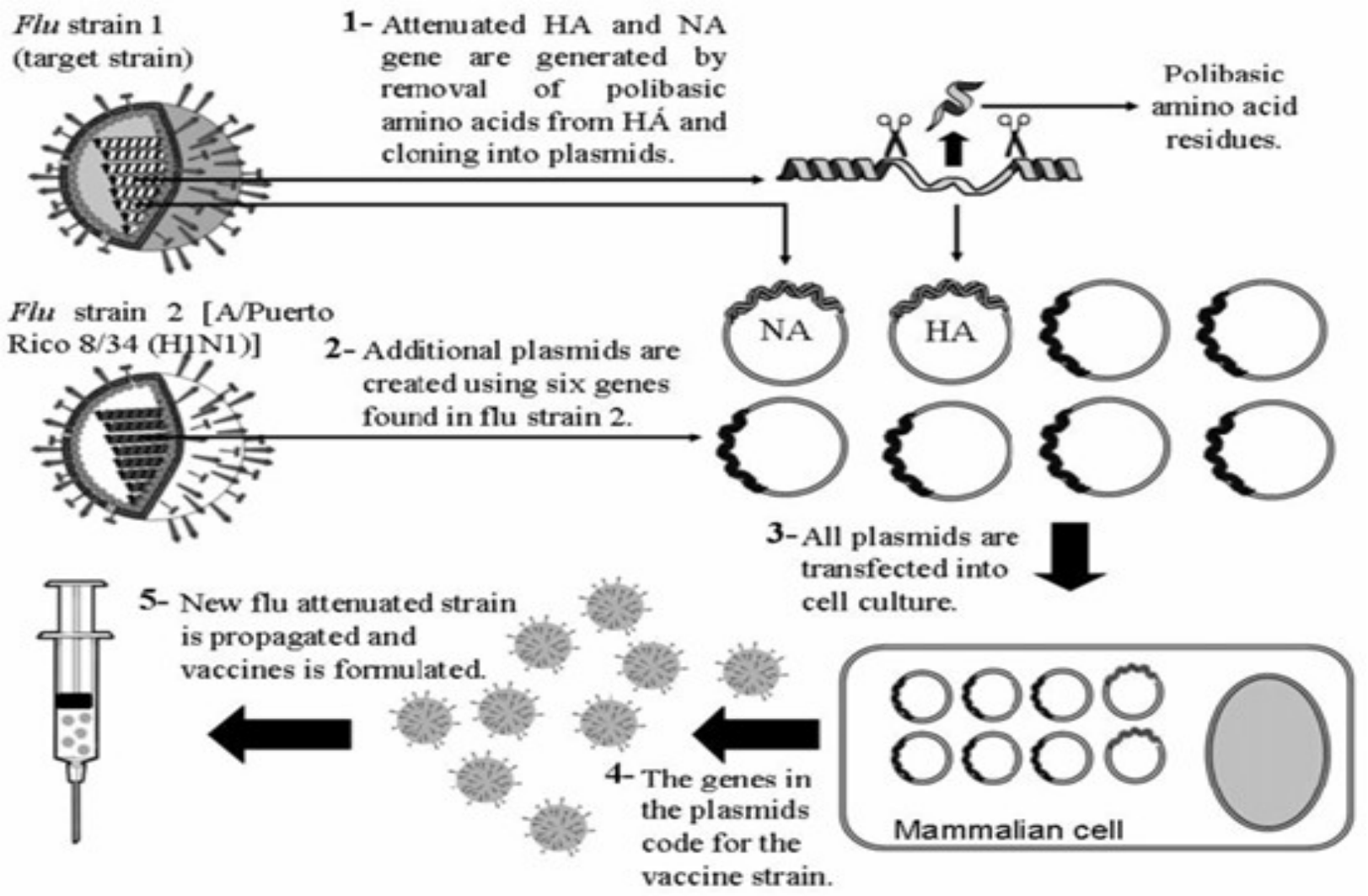
Prevention and Control

- **viral surveillance : monitoring ongoing influenza evolution, & transmission**
- **culling of all infected poultry ….. economic impact?**
- **Use of vaccine in poultry… affordability?**
- **Temporary quarantine**
- **Recombinant adenovirus-based vaccine**

Use of influenza vaccines

- **Are usually trivalent; consisting of envelope (HA and NA) antigen from all three circulating influenza strains**
- **Vaccine viruses are cultured in embryonated hen's eggs and the envelope proteins are purified**
- **The subtype composition of the vaccine is updated every year based on influenza isolates of the preceding year.**
- **The only contraindication to the vaccine is egg allergy and concurrent febrile illness**
- **All patients with significant risk factors for influenza morbidity are advised to receive the vaccine annually.**
- **Health care worker & other high risk individuals should also be immunized annually**

Reverse genetic strategy to produce vaccines against pandemic influenza virus



(Ref: Science, 2006;312:384)

PARA INFLUENZA VIRUSES

Para influenza viruses (PIVs)

Properties

- 5 antigenically distinct para-influenza viruses that infect humans (1, 2, 3, 4a & 4b)
- Family: paramyxoviridae
- enveloped ssRNA viruses
- About 150-200nm in diameter
- Pleomorphic with helical symmetry
- Cross - reactivity with different sero types & mumps virus

- **Antigenically stable --- but, highly infectious?**
- **Morphologically resembles influenza virus**
- **Possess structural proteins analogous to influenza virus**
- **Inner helical core that protects ssRNA genome**

Types of glycoproteins in the envelope

A) HN (haemagglutinin)

- **Binds, agglutinates red blood cells**

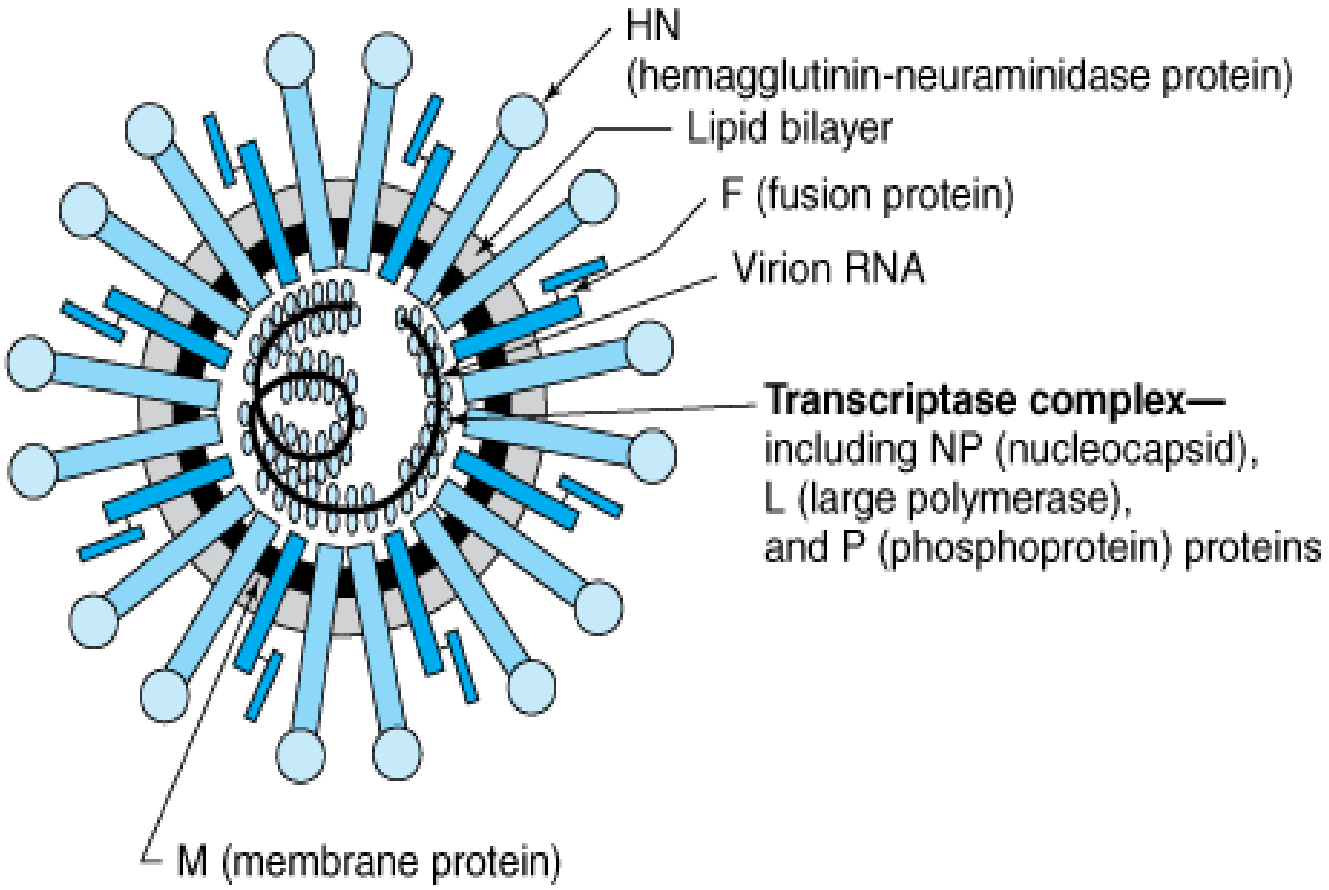
B) NA (neuraminidase)

- **Enzyme that degrades sialic acid (detaches the virion from the cell surface)**

C) F (Fusion)

- **Causes membrane fusion, syncitium formation**

Para-myxovirus particle showing major viral components



(Clin Microbiol Rev 2003;16:242)

Epidemiology

- **Causes respiratory tract infections in all age groups**
- **First infections occur in early childhood (< 3yrs of age)**
- **Immunity following infection is poor and re-infections are common**
- **Common in temperate zones**
- **Transition: inhalation of droplet nuclei**
  - ► **High prevalence rate during wintertime**
- **virus is delicate & does not survive long in the environment**
- **Type 1& 2 are associated with epidemics (RTI/crop)**

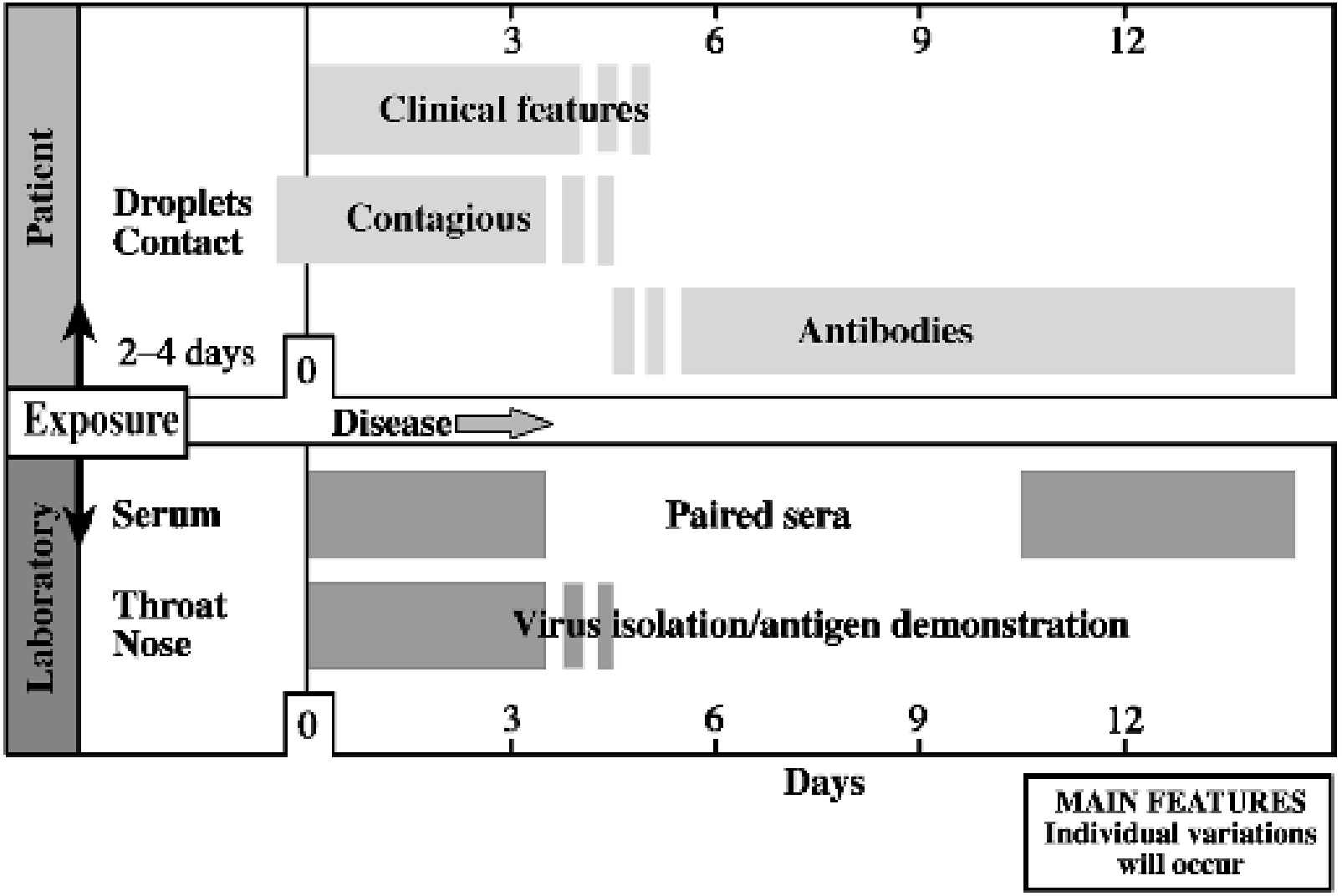
Viral disease & responsible viral agent

Disease	Virus type
Minor upper respiratory disease	1,3, 4
Bronchitis	1, 3
Bronchopneumonia	1,3
Croup (laryngo - tracheo-bronchitis)	1,2,3

Clinical features

- **Cough with fever**
- **Respiratory difficulty**
- **Swelling of epiglottis**
- **Acute laryngo-tracheo bronchitis (Croup)**
  - ➡ **airway obstruction (needs tracheotomy)**
- **Bronchiolitis**
- **Pneumonia**
- **Re-infections cause "common cold" symptoms**

Para influenzavirus (respiratory infection)



Lab. DX

Specimens: Naso-pharygeal secretions , mouth gargle, etc

A. Direct method

- **Using electron microscopy**

B. Virus isolation

- **Cell culture using monkey kidney & human fibroblast cells**

N.B: **- PIV require proteases for their replication**

- Majority of them don't produce CPE

PIV with surface projections along the edge of the particle



C. Serology

- **HAI test , CFT , ELISA/RIA**

Prevention & Control

- **Noso-comial spread is well documented**
- **Elective admissions (epidemic period)**
- **Vaccine production?**

Summary : Comparison of Viruses that Infect the Human Respiratory Tract.

Virus	Disease	Number of Serotypes	Lifelong Immunity to Disease	Vaccine Available	Viral Latency
RNA viruses					
Influenza A virus	Influenza	Many	No	+	-
Parainfluenza virus	Croup	Many	No	-	-
Respiratory syncytial virus	Bronchiolitis	One	No	-	-
Rubella virus	Rubella	One	Yes	+	-
Measles virus	Measles	One	Yes	+	-
Mumps virus	Parotitis, meningitis	One	Yes	+	-
Rhinovirus	Common cold	Many	No	-	-
Coronavirus	Common cold	Many	No	-	-
Coxsackievirus	Herpangina, pleurodynia	Many	No	-	-
DNA viruses					
Herpes simplex virus type 1	Gingivostomatitis	One	No	-	+
Epstein-Barr virus	Infectious mononucleosis	One	Yes	-	+
Varicella-zoster virus	Chickenpox, shingles	One	Yes[1]	+	+
Adenovirus	Pharyngitis, pneumonia	Many	No	-	+