Microbiology in Bioengineering

Course 8

Viral Diagnosis, Treatment and Some Important Viruses

Assoc.Prof. Dr. Emrah Şefik Abamor

Laboratory Diagnosis

- Five pathways are used to diagnose viral diseases using clinical specimens;
- Identification of the virus in cell culture
- Direct recognition by microscope
- Increase in antibody titer or serological procedures for detection of IgM antibody
- Detection of viral antigens in blood or body fluids
- Detection of viral nucleic acids in blood or patient cells

Diagnostic Methods in Virology

1. Direct Examination
2. Indirect Examination (Virus Isolation)
3. Serology

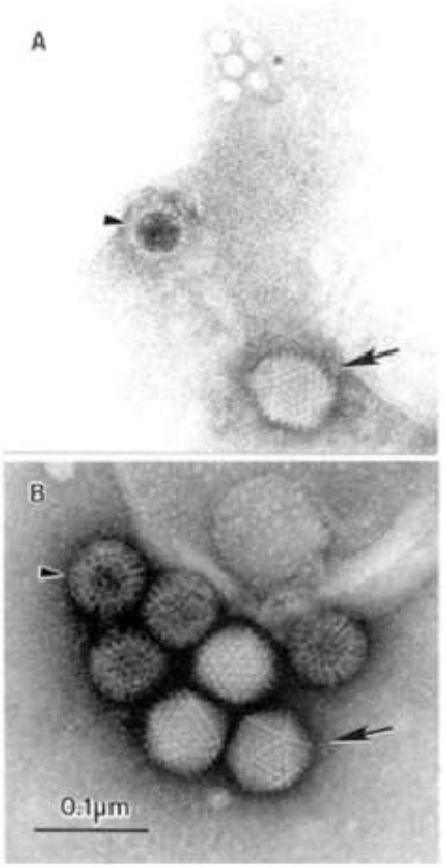
Identification in Cell Culture

- Although most bacteria grow on cell-free media, because of the replication of viruses only in living cells, cell culture is essential for the reproduction of viruses.

- Virus growth in cell culture often causes cytopathic effect (CPE) in infected cells.
- CPE are changes in the appearance of an infected cell
- These changes can be in the form of fusion of cells, shape and multi-nucleated giant cells (sincytium).
- The type of cell that will form the CPE of the virus provide important clues in the preliminary diagnosis.

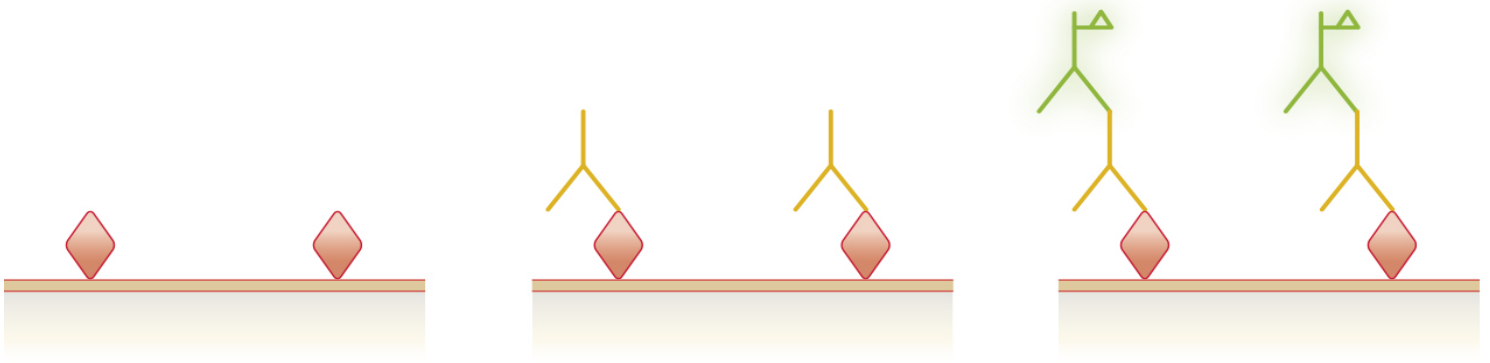
Direct Detection

- Electron Microscopy
  - Examine specimen for viruses
- Immuno-electron microscopy
  - Labeled antibody
- Immunofluorescence
  - Fluorescent tag bound to Fc region of Ab



Fluorescence Antibody Test

- If the virus-infected cells and the fluorescently labeled antibody are homologous, fluorescent microscopy shows typical green fluorescence in cells.

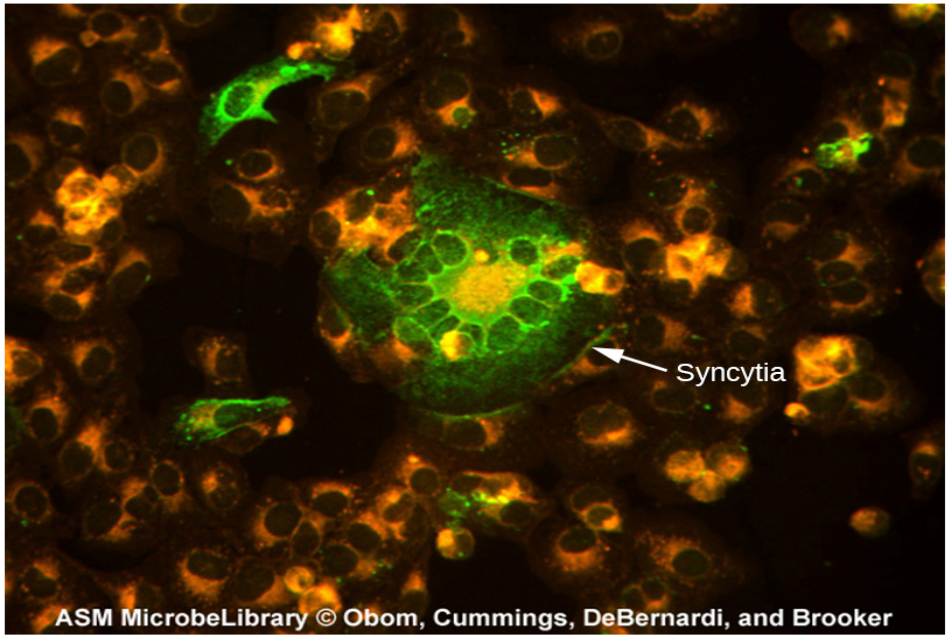


1 Antigen is fixed to a surface.

2 Patient serum is added; if antibodies are present, they bind to the antigen.

3 Secondary antibody (with florescent label) is added; if patient antibodies are present, the secondary antibody binds to the patient antibodies.

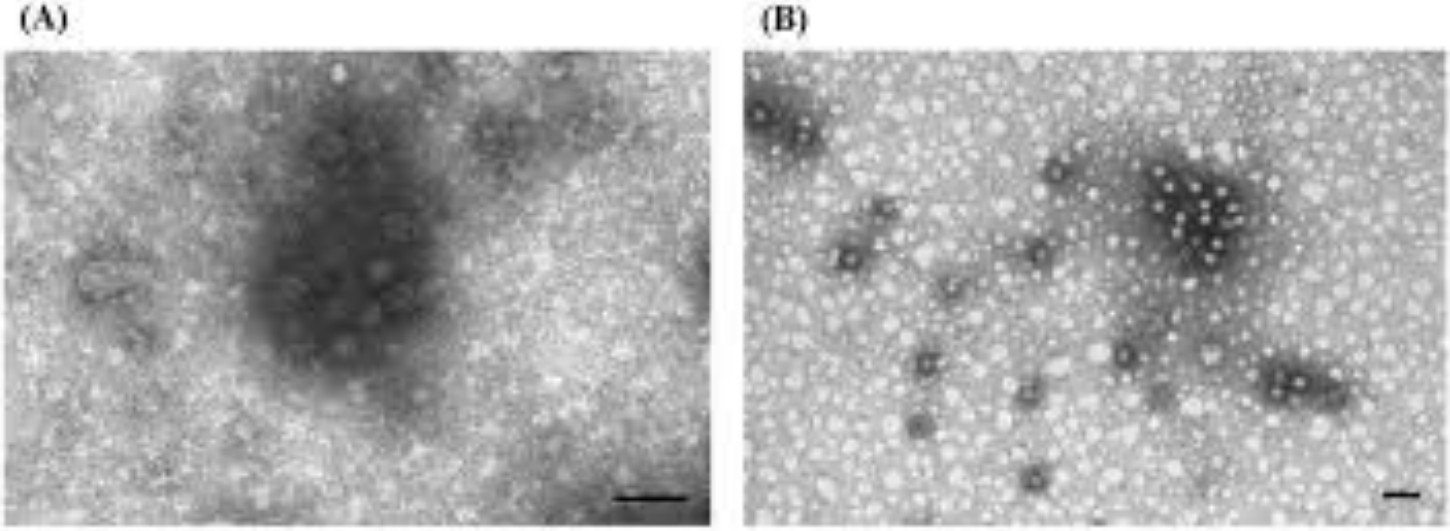
(a)



(b)

İmmune Electron Microscopy

- If the antibody is homologous to the virus, aggregates produced by the virus-antibody mixture are seen in the electron microscope.



Serology

- Since the isolation and identification of viruses is not commonly done in the clinical laboratory, the clinical picture and serology plays a greater role in the diagnosis of viral disease. The major types of antibodies that are assayed for are neutralizing, haemagglutination inhibiting and complement fixing antibodies. Complement fixing antibodies follow the kinetics of IgM and are most useful in indicating a current or recent infection

Viral Serology

- Indirect
  - Primary and secondary responses to viral infections
    - IgM (1st exposure)
    - IgG (2nd exposure)

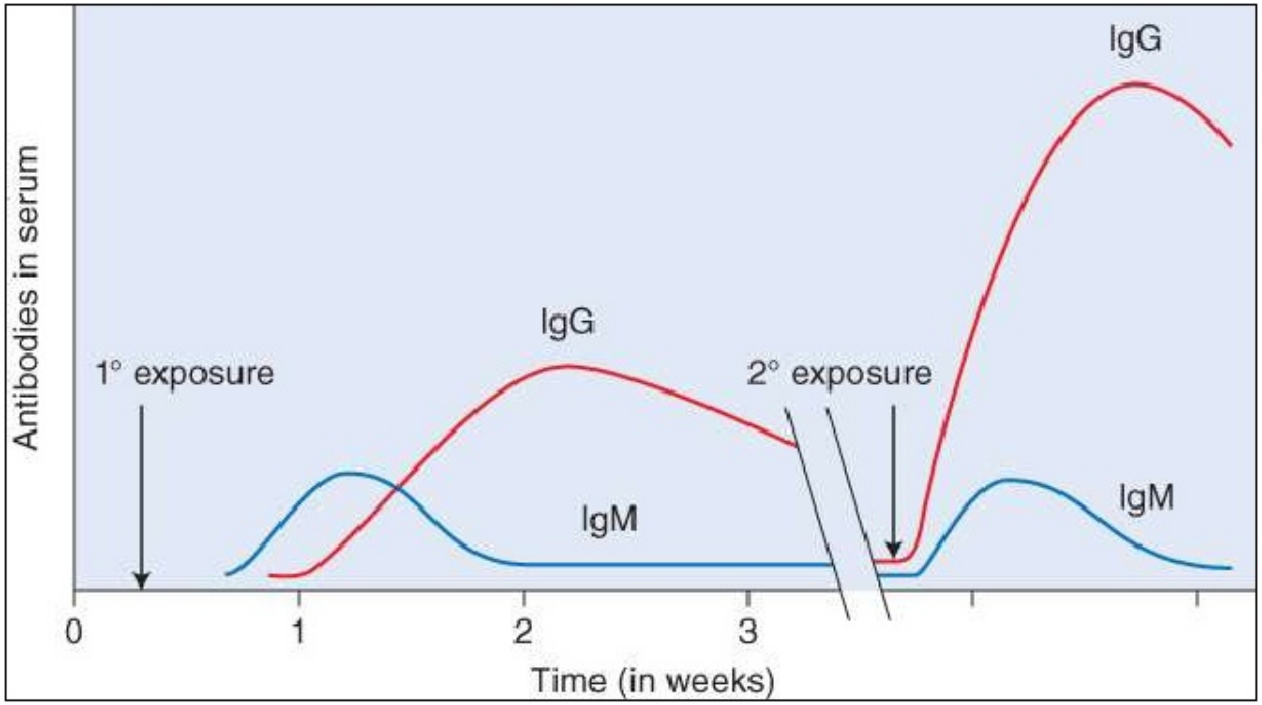


Figure 5.18: Primary (1 degree) and secondary (2 degree) antibody responses toward a viral pathogen.

Serological Diagnosis

- Detection of Immunologlublins Ig G. Ig M Ig A
- Raise of titers Ist sample later sample **(convalescent sample)** tested after 10 – 14 days Raise of titer is diagnostic

Viral Serology

- Enzyme-Linked Immuno-Sorbant Assays (ELISAs)
  - Enzyme reacts with substrate to produce colored product
  - Very sensitive
    - HIV test
      - If positive twice, Western Blotting is performed next

ELISA

- In the enzyme-linked immunosorbent assay, a known antibody is bound to the surface to type a virus.
- If there is a virus in the sample taken from the patient, it will be bound to this antibody.
- An antibody sample bound to an enzyme is added, which will bind to the virus.
- The substrate of the enzyme is added and the amount of bound enzyme is determined.

ELISA for HIV antibody

Micro plate ELISA for HIV antibody: colored wells indicate reactivity

ELISA Procedures

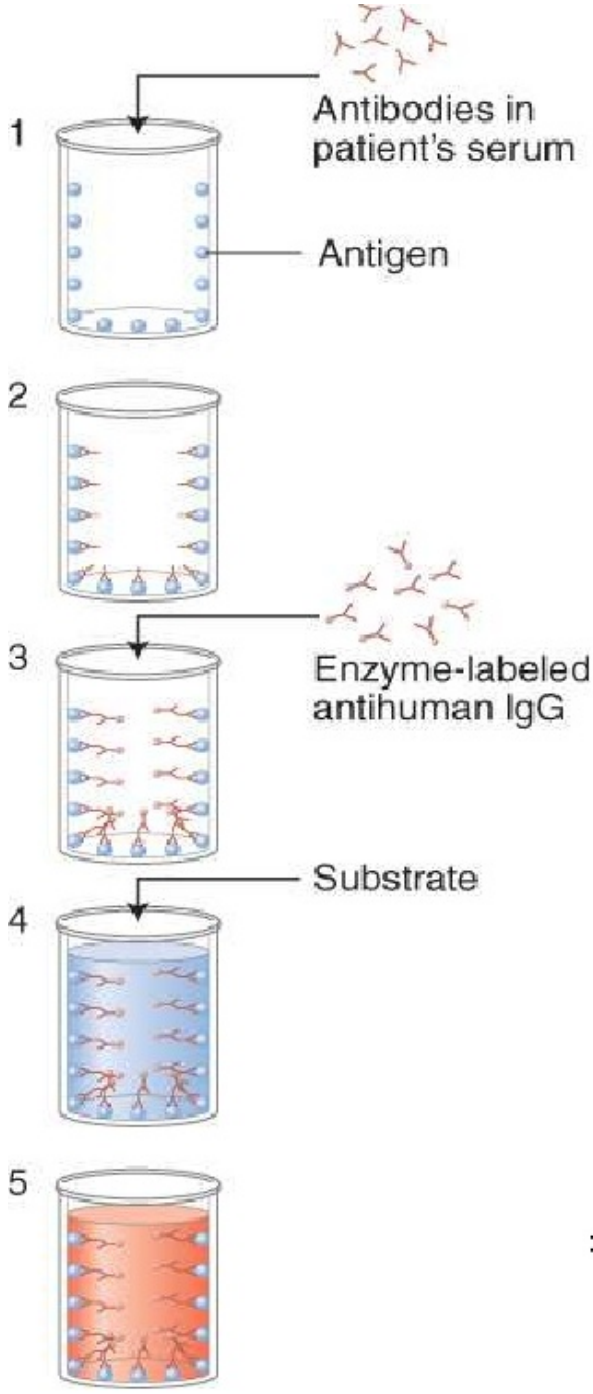


Figure 5-19a

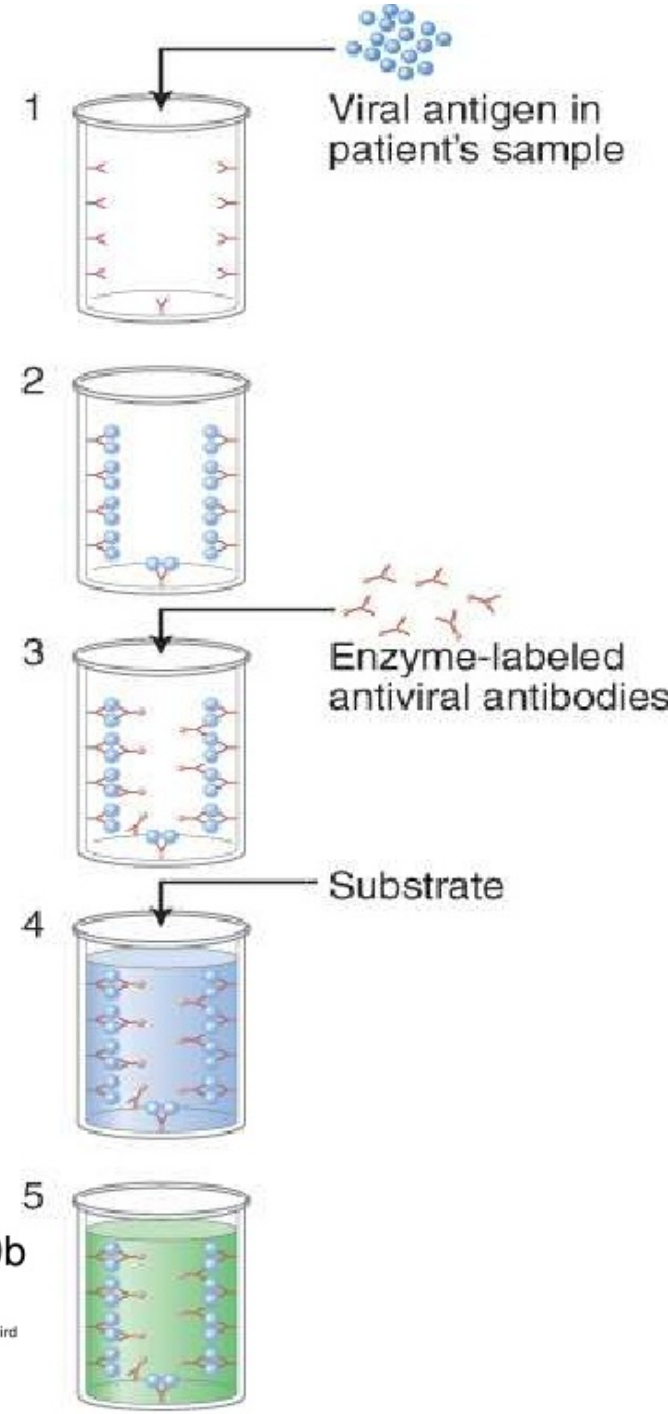


Figure 5-19b

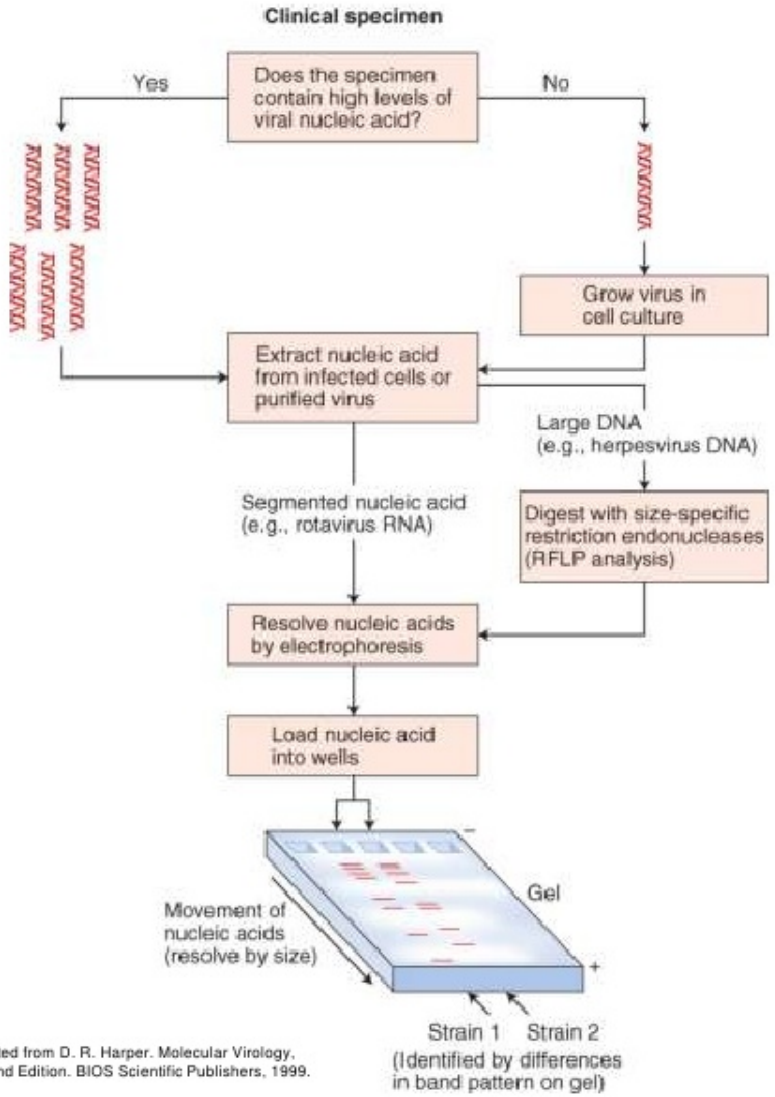
Modified from Specter, S. C., R. L. Hodinka and S. A. Young. Clinical Virology Manual, Third Edition . ASM Press, 2000.

Detection of Viral Nucleic Acids

- The presence of viral DNA or RNA in the diagnosis of viruses has increasingly become the golden standard.
- The labeled probes are highly specific and the results are rapidly taken.
- Small amounts of viral nucleic acids can be increased to the amounts that can be determined by the probe using reverse transcryptase.
- An important example of this is the determination of the 'virus burden' of HIV RNA.

Virus Isolation

– Nucleic acid methods
  - PCR (DNA), RT-PCR (RNA)
  - Can be used to detect viruses that are noncultivatable
  - Rapid identification (e.g. RT-PCR—4 Corners outbreak of hantavirus or FRET in the field)
  - Can be used to manage patients (e.g. HIV viral load)



Adapted from D. R. Harper. Molecular Virology, Second Edition. BIOS Scientific Publishers, 1999.

Polymerase Chain Reaction

- Advantages of PCR:
  - Extremely high sensitivity, may detect down to one viral genome per sample volume
  - Easy to set up
  - Fast turnaround time
- Disadvantages of PCR
  - Extremely liable to contamination
  - High degree of operator skill required
  - Not easy to set up a quantitative assay.
  - A positive result may be difficult to interpret, especially with latent viruses such as CMV, where any seropositive person will have virus present in their blood irrespective whether they have disease or not.
- .

HOW DOES COVID-19 REAL-TIME RT–PCR TESTING WORK?

1 Sample

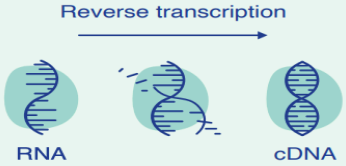
A person's blood, saliva or mucus is sampled.

Chemical solutions are added to remove substances such as proteins and fats.

The sample contains a mix of a person's genetic material and the virus's RNA (if present).

2 From RNA to DNA

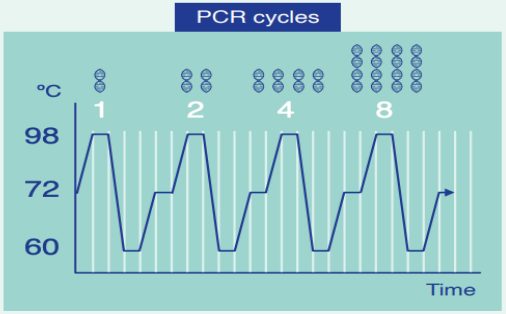
In order for PCR to work, the RNA needs to be converted into cDNA.



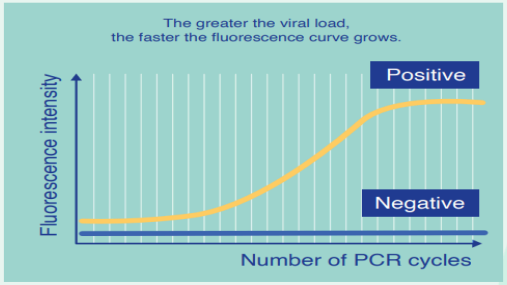
3 Copying and dyeing the DNA

Chemical reagents, including probes with fluorescent dyes that will mark any viral cDNA found, are added in order to build copies of the genetic material.

The samples are then placed in a PCR thermocycler machine.

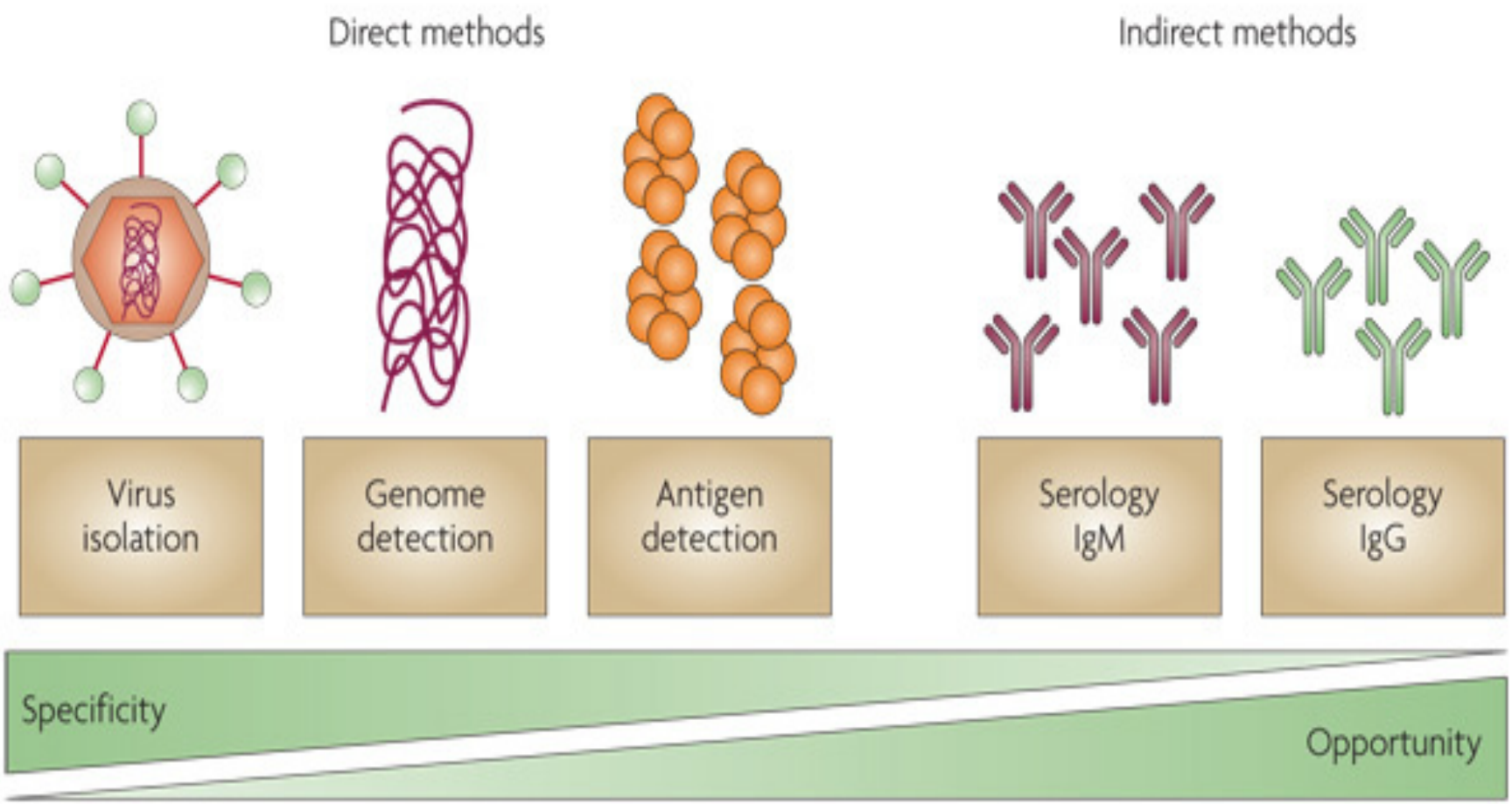


Different temperature cycles trigger chemical reactions that replicate the original genetic material.



If the virus is present, the copies will generate fluorescence. The more fluorescence, the more viral material.

In about one hour, billions of copies of the original genetic material are made, revealing if the virus is present or not.


Direct methods
Indirect methods
Virus isolation
Genome detection
Antigen detection
Serology IgM
Serology IgG
Specificity
Opportunity

Antiviral Drugs

- Compared to the number of drugs that can be used for the treatment of bacterial diseases, the number of antiviral drugs is quite low.

- The main reason for this difference is **the difficulty of providing selective toxicity against viruses**; the replication of these cells is accompanied by normal synthesis events of the cell.

- Another limiting factor that renders antiviral drugs ineffective is the fact that most of the viral replication cycle takes place during the incubation period in which the patient feels good.

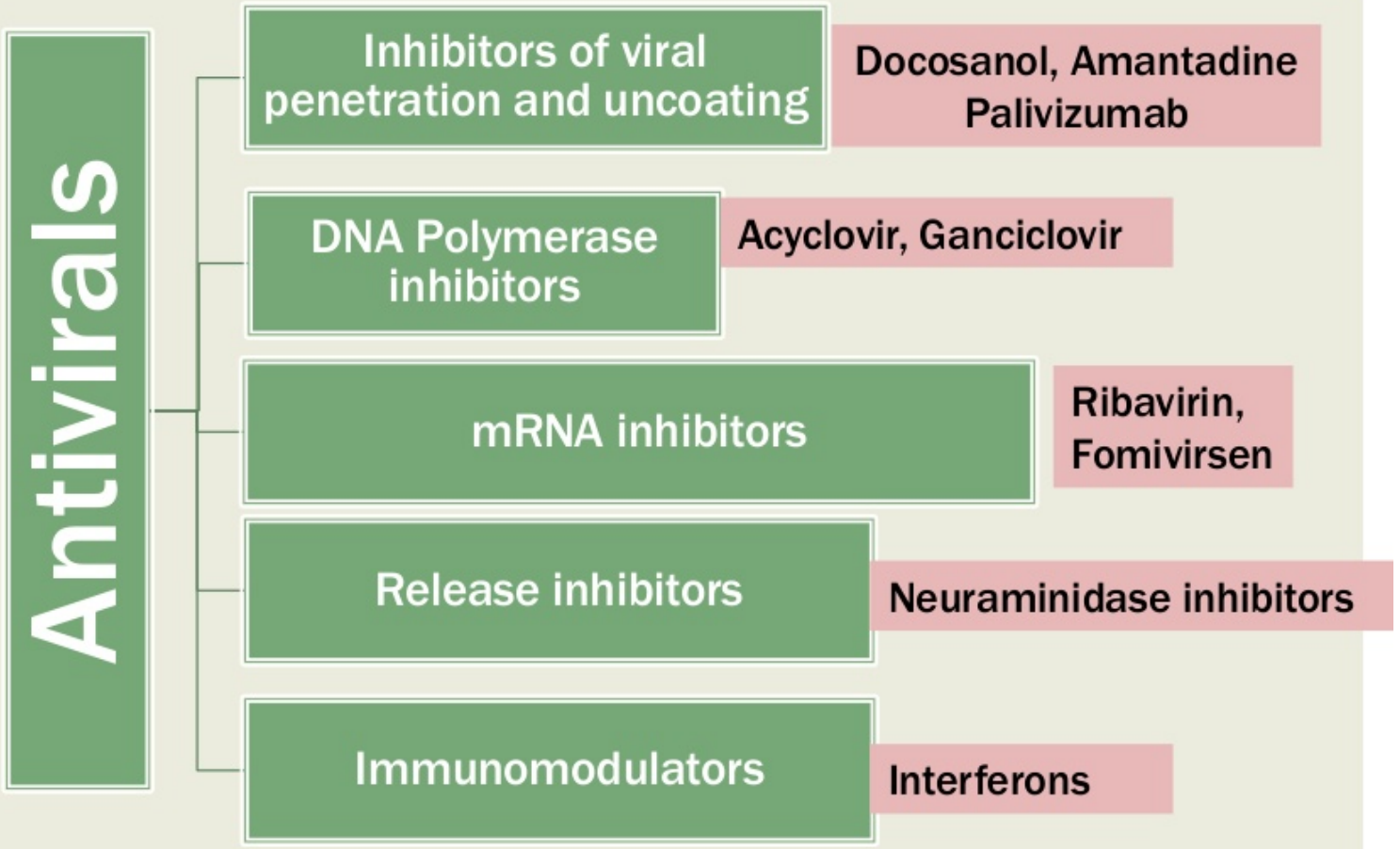
- **When the patient was diagnosed with a systemic viral disease, the virus spread throughout the body and it was too late to prevent it.**

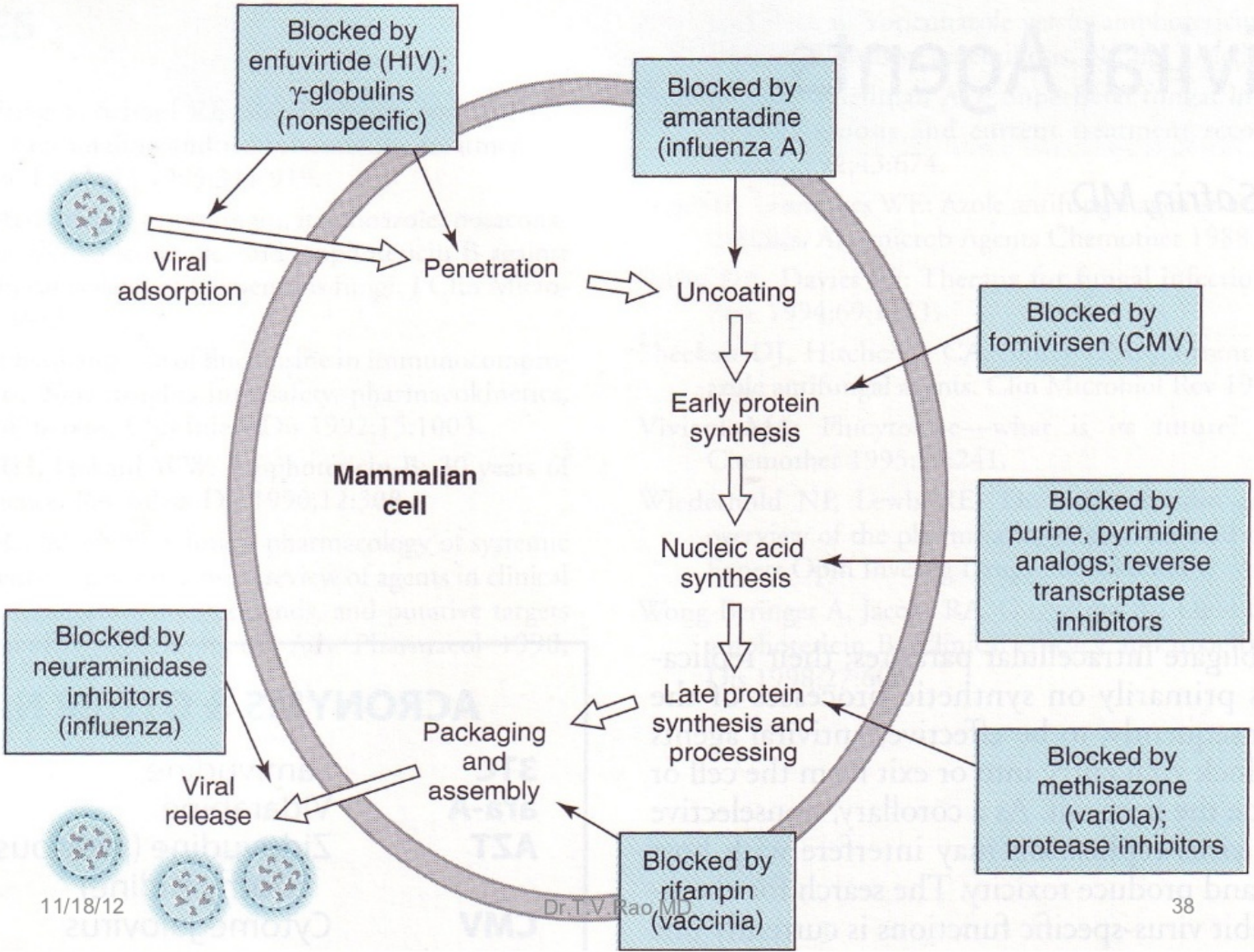
- Another potential limiting factor is the emergence of drug-resistant viral mutants. This phenomenon is of great clinical importance today.

- Due to the fact that the virus can only reproduce in cells and use a large number of cellular functions during reproduction, it is very difficult to obtain highly selective toxicity in antiviral drugs.

- Selective toxicity **is the ability of a drug to inhibit viral replication without significant damage to the host cell.**

CLASSIFICATION




Blocked by enfuvirtide (HIV); γ-globulins (nonspecific)
Blocked by amantadine (influenza A)
Viral adsorption
Penetration
Uncoating
Blocked by fomivirsen (CMV)
Early protein synthesis
Mammalian cell
Nucleic acid synthesis
Blocked by purine, pyrimidine analogs; reverse transcriptase inhibitors
Blocked by neuraminidase inhibitors (influenza)
Late protein synthesis and processing
Packaging and assembly
Blocked by methisazone (variola); protease inhibitors
Viral release
Blocked by rifampin (vaccinia)

Inhibitors of Viral Penetration and Uncoating

- Amantadine inhibits the removal of the influenza A virus capside by blocking the ion channel activity of the viral matrix protein.

- The drug has no effect on influenza B or C viruses.

Anti-viral drugs

Respiratory viral infections

Influenza –

- Amantadine / Rimantadine
- Oseltamivir / Zanamavir

(Neuraminidase inhibitors)

RSV bronchiolitis –

- Ribavirin

Anti-viral drugs

Amantadine and Rimantadine : Influenza

- Prevention & Treatment of influenza A
- **Inhibition of viral uncoating** by inhibiting the viral membrane protein M2
- Influenza A virus
- Amantadine has anti-parkinsonian effects.

Anti-viral drugs

Neuraminidase inhibitors : Influenza
Oseltamivir / Zanamavir

- **Influenza** contains an enzyme *neuraminidase* which is essential for the replication of the virus.
- ***Neuraminidase inhibitors*** prevent the release of new virions and their spread from cell to cell.

Anti-viral drugs

Neuraminidase inhibitors : Influenza
Oseltamivir / Zanamavir

- These are effective against both types of influenza A and B.
- Do not interfere with immune response to influenza A vaccine.
- Can be used for both prophylaxis and acute treatment.

Inhibitors of Herpesviruses

- **Acyclovir**
- **Gancyclovir**
- **Foscarnet**
- inhibits DNA polymerase of herpesvirus.

Anti-viral drugs

Mechanism of action of Acyclovir and congeners :

- All drugs are phosphorylated by a viral thymidine-kinase, then metabolized by host cell kinases to nucleotide analogs.
- The analog inhibits viral DNA-polymerase
- Only actively replicating viruses are inhibited

Anti-viral drugs

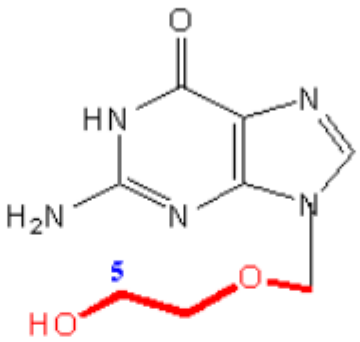
- Acyclovir is thus selectively activated in cells infected with herpes virus.
- Uninfected cells do not phosphorylate acyclovir.

1- Antiviral drugs used against DNA viruses

Inhibitors of DNA polymerase

A) CHANGES IN SUGAR

Acyclovir
zovirax®

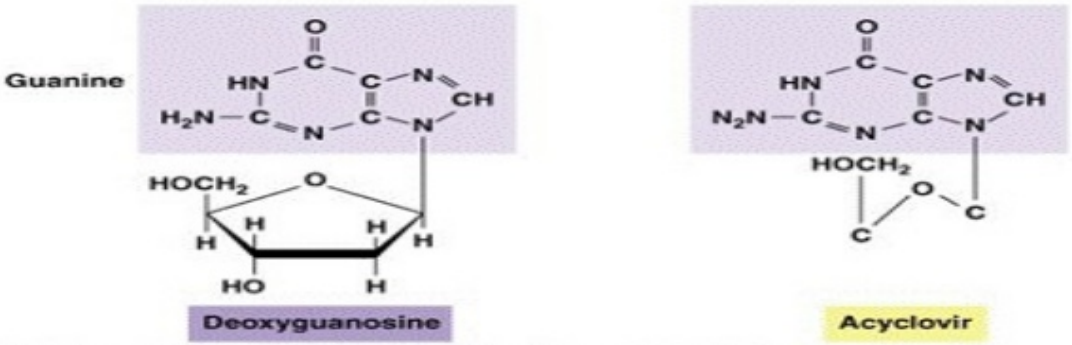


MOA

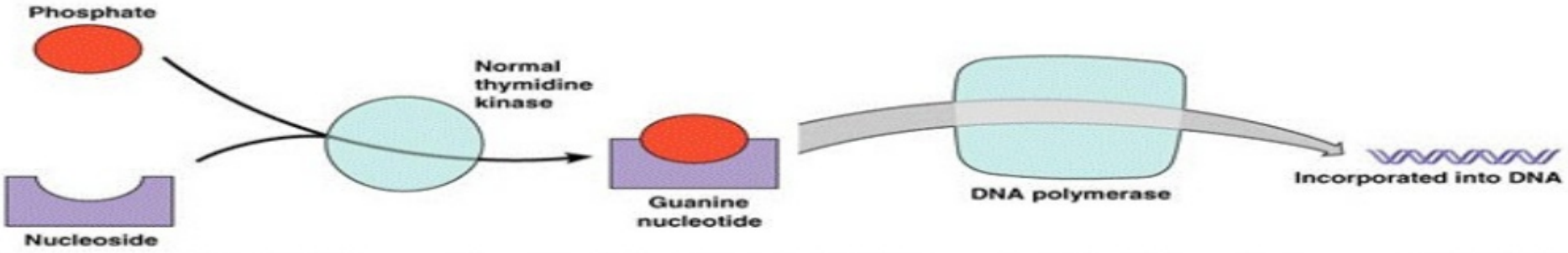
- Acyclovir is phosphorylated by viral thymidine kinase → monophosphate → then by cellular thymidine kinase → triphosphate (active form)
- The active forms:
  - Incorporated in viral DNA chain terminate chain elongation (no 3'OH)
  - competitively inhibits viral DNA polymerase leading to arrest of viral DNA replication
- Selectivity of acyclovir to viral thymidine kinase is 200 times that of mamalian enzyme therefore in normal uninfected cells it remains as prodrug

USES: Herpes Simplex

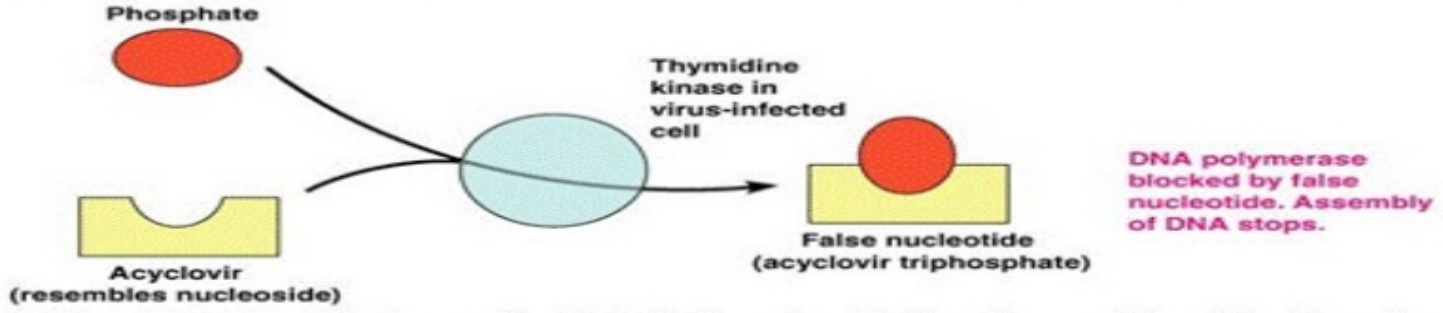
Mechanism of Action of Acyclovir



(a) Acyclovir structurally resembles the nucleoside deoxyguanosine.



(b) The enzyme thymidine kinase combines phosphates with nucleosides to form nucleotides, which are then incorporated into DNA.



(c) Acyclovir has no effect on a cell not infected by a virus, that is, with normal thymidine kinase. In a virally infected cell, the thymidine kinase is altered and converts the acyclovir (which resembles the nucleoside deoxyguanosine) into a false nucleotide—which blocks DNA synthesis by DNA polymerase.

Inhibitors of Retroviruses

- Azdotimidin
- Nevirapin, delavirdin ,
- didanozin, zalsitabin, stavüdin, lamivudin, abakavir,vetenofovir inhibits DNA polymerases of retroviruses

Viral Protein Synthesis Inhibitors

- **Interferons** primarily inhibit viral replication by blocking viral mRNA production and blocking viral protein production.

- These allow the synthesis of a ribonuclease, which specifically cleaves the viral mRNA instead of cell mRNA

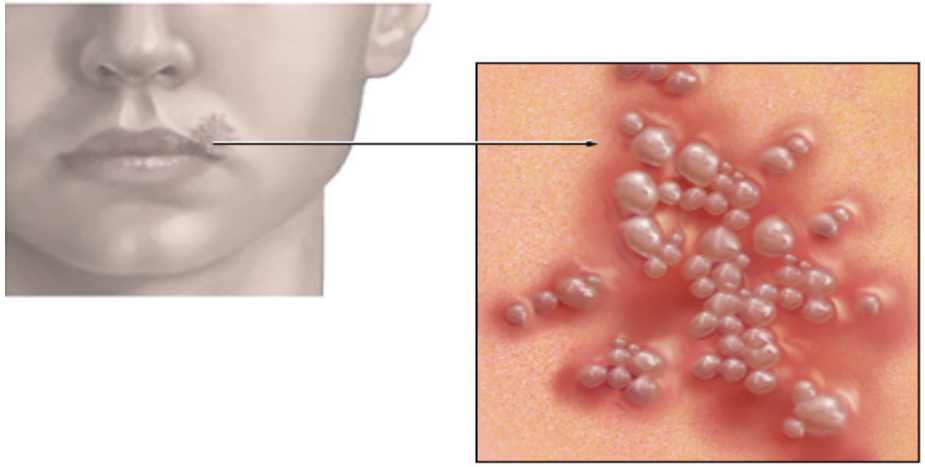
ENVELOPED DNA VIRUSES

Herpes Simplex Virus Type 1

Photo courtesy of CDC - Dr. Herrmann

Diseases

- Herpes labialis
- keratit (cornea inflammation),
- Ensephalite (brain inflammation)



Pathogenesis

- The first lesions appear on the mouth or face.
- The virus then travels along the axon and becomes latent in nerve cells in the face.
- Cellular immunosuppressed individuals may experience life-threatening internal organ proliferation.

Treatment

- Acyclovir is used for treatment in encephalitis and in cases spread to internal organs
- Trifluorotimidine is used for keratitis
- No vaccine

Herpes Simplex Virus Type 2

- Diseases

  - Herpes genitalis (lesions in genital region),

  - Aseptic meningitis,

  - Neonatal infections

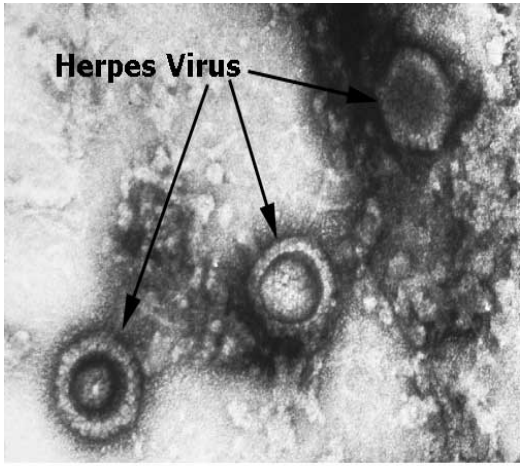


Photo Courtesy of CDC - Dr. John Hierholzer

Varicella-Zoster Virusu

Diseases

- Varicella Zoster virus causes chickenpox in children and shingles in adults
- Shingles disease is a disease that occurs by spontaneous repetition of chickenpox virüs localized in nerves after years. Causes painful spills on skin

Pathogenesis

- The initial infection of the virus is in oropharynx.
- The virus spreads through the blood to the internal organs such as the liver and then to the skin. After an acute seizure of chickenpox, the virus remains latent in sensory ganglia
- After years, it can be reactivated in the form of zona, especially in elderly and immunocompromised individuals.

Smallpox Virus

- Disease

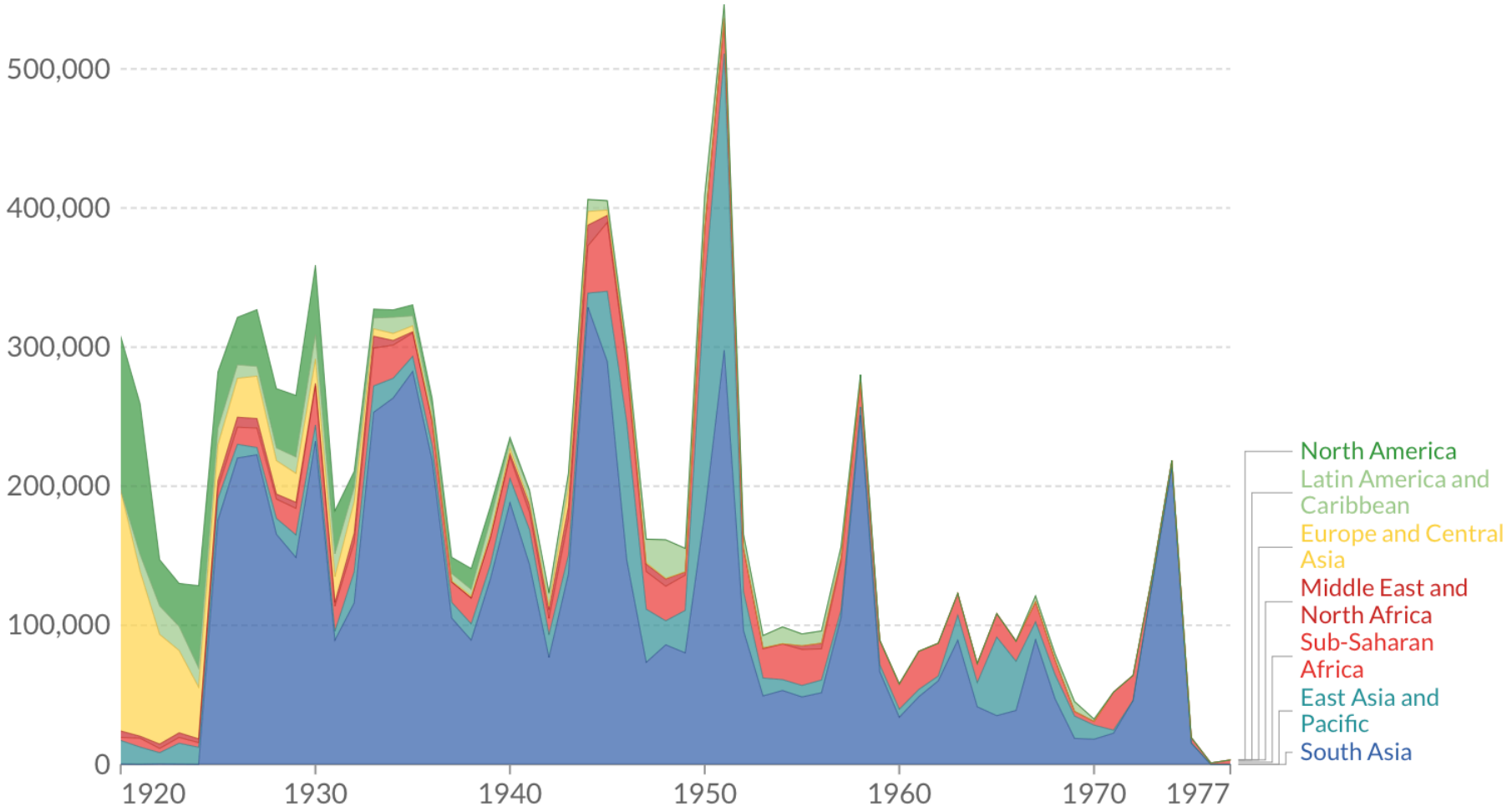
  Smallpox (Variola) disease.

  The disease has lost its effect by the use of an effective vaccine. The last known case was seen in Somalia in 1977.

Reported number of smallpox infections by world region, 1920 to 1977

Our World in Data

Relative



Source: World Health Organization (1969-1988)

NON-ENVELOPED DNA VİRUSES

Human Papilloma Virus

Diseases :

- Papillomas
- Cervix cancer
- Penis cancer

Human Papilloma Virus

Transmission:

- **Human papillomavirus** (**HPV**) is a viral infection that is passed between people through skin-to-skin contact.

Pathogenesis:

- This virus forms proteins that inhibit the activity of proteins encoded by tumor-inhibiting genes.

Treatment:

- No treatment and vaccine

ENVELOPED RNA VIRUSES

Influenza Virus

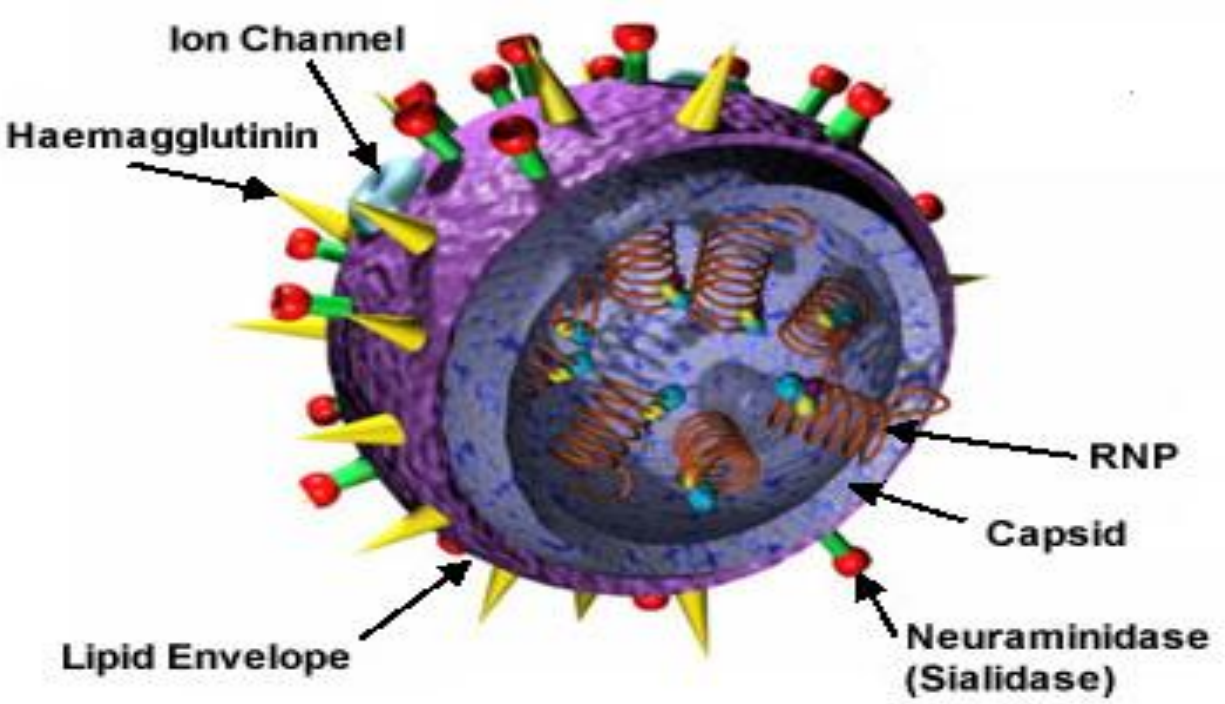
Disease:

- Cold Flu
- Influenza A virus is the main cause of epidemics and pandemics worldwide.

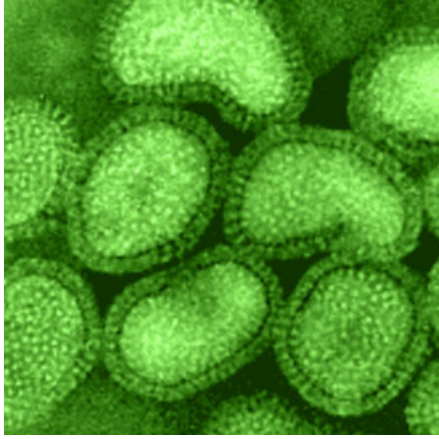
Features:

- **The two main antigens of influenza virus are hemagglutinin and neuraminidase.**

Influenza Virus



Influenza Virus



Features:

- Mutations-induced antigen deviation may cause changes in the influenza virus.
- There are many serotypes of the virus due to these antigenic shifts and deviations.
- The A, B or C influenza virus is called the antigenic property of the inner capsid protein.

Influenza Virus

Treatment :

- Amantadin
- Rimandatin
- In addition, zanamivir and oseltamivir, a neuraminidase inhibitor can be also used.

İnfluenza Virus

Protection:

- Two vaccines:
  - A dead vaccine containing hemagglutinin and neuraminidase
  - A live vaccine containing a temperature sensitive mutant of the Infulenza virus
  - Live vaccine is proliferated in cold airways and induces secretory IgA; cannot reproduce in the hot environment of the lower respiratory tract.

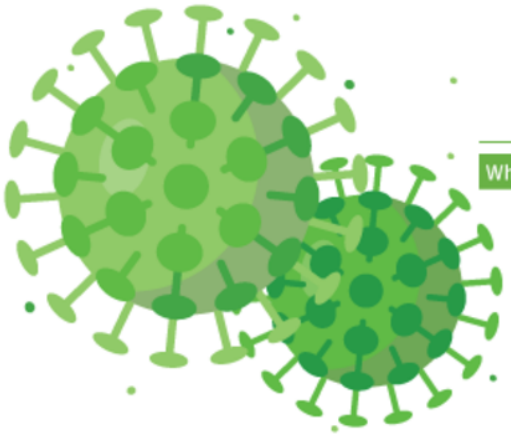
COVID-19

Disease caused by the SARS-CoV-2 virus

Novel coronavirus

Coronaviruses are viruses that **circulate among animals** but some of them are also known to affect humans.

The 2019 novel coronavirus was identified in China at the end of 2019 and is a new strain that has not previously been **seen in humans**.



Prevention

When visiting affected areas

- Avoid contact with sick people
- Wash your hands with soap and water
- If you develop cough, use a medical face mask

Wherever you travel apply general hygiene rules

Symptoms

- FEVER
- COUGH
- DIFFICULTY BREATHING
- MUSCLE PAIN
- TIREDNESS

Transmission

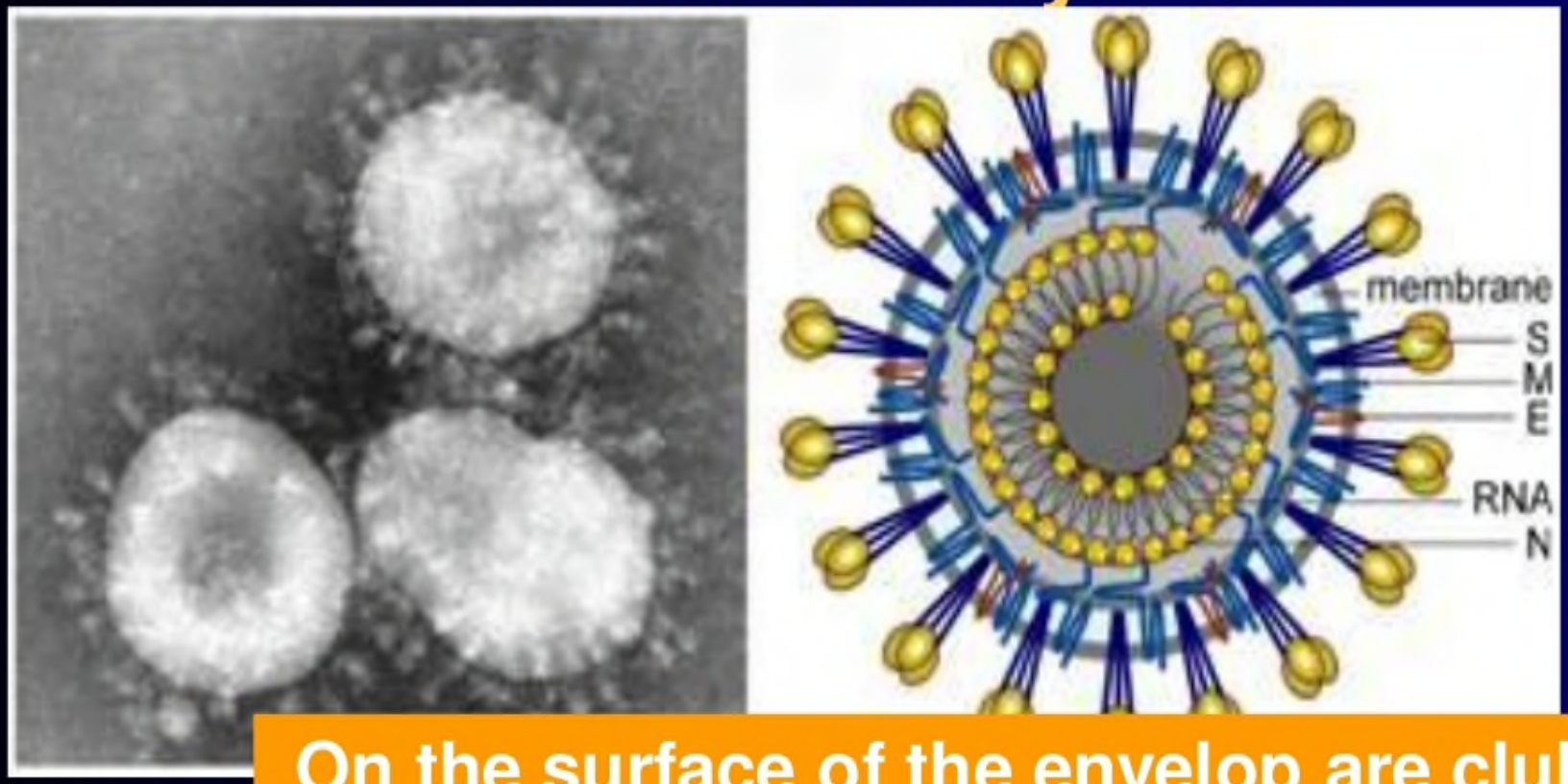
VIA RESPIRATORY DROPLETS

2–14 days

estimated incubation period

ecdc.europa.eu/en/novel-coronavirus-china

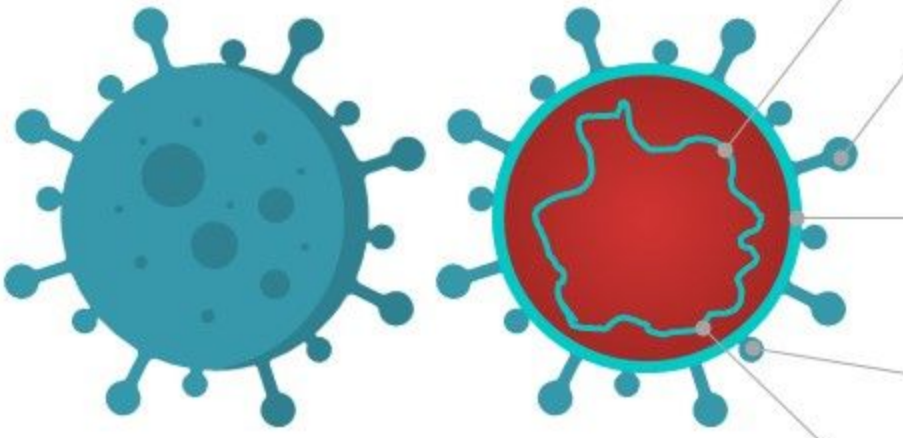
A Crown-like Appearance when viewed by EM



On the surface of the envelop are club • shaped projections that resemble a solar corona

Detailed Structure of Coronavirus

This slide provides information about the structure of coronavirus comprising of various proteins and the functioning of each protein.



Structural Protein	Function of Protein
Nucleocapsid Protein (N)	• Bound to RNA genome to make up nucleocapsid
Spike Protein (S)	• Critical for building of host call receptors to facilities entry of host cell
Envelope Protein (E)	• Interacts with M to from viral envelope
Membrane Protein (M)	• Central organizer of CoV assembly • Determines shape of viral envelope
RNA	

It has been noted that some CoVs do not need to have the full ensemble of structural proteins to make virions, highlighting that certain proteins may be dispensable or compensated by the function of non-structural proteins.

What are Corona viruses?

University Health Center

- Coronaviruses are believed to cause a significant percentage of all common colds in human adults. Coronaviruses cause colds in humans primarily in the winter and early spring season.

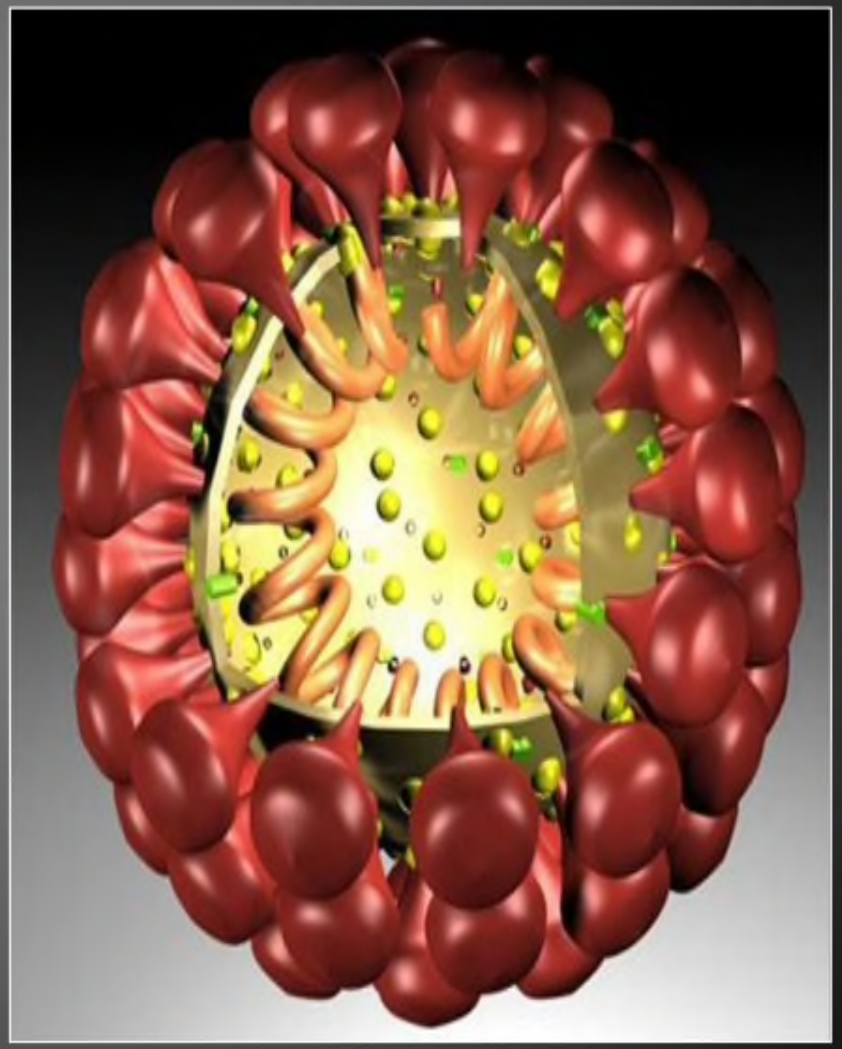
- Coronaviruses primarily infect the upper respiratory and gastrointestinal tract of mammals and birds.

What are Corona viruses?

University Health Center

- Four to five different currently known strains of Coronaviruses infect humans. The most publicized human Coronavirus, is SARS.

- A sixth was discovered last year, known as Novel Coronavirus 2012.



SARS Corona Virus

University Health Center

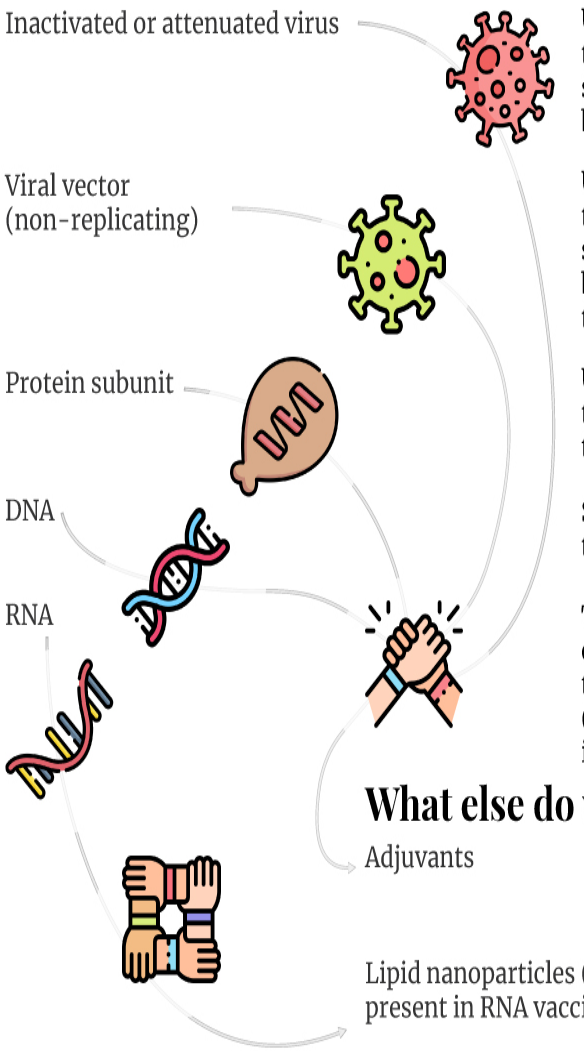
- This has a unique pathogenesis because it causes both upper and lower respiratory tract infections and can also cause Gastroenteristis.

April 2021

The different types of COVID-19 vaccines

Vaccines will play a major role in ending the COVID-19 pandemic.
COVID-19 vaccines have already been proven highly effective at preventing severe illness, hospitalisation and death.

iap SCIENCE HEALTH POLICY
the interacademy partnership



Approach	How does it work?	SARS-CoV-19 vaccines *	Similar vaccines
Inactivated or attenuated virus	Uses a form of the virus that has been inactivated or weakened so it doesn't cause disease, but still generates an immune response	Sinopharm, Sinovac	Cholera, Polio, MMR, Yellow fever, TBC
Viral vector (non-replicating)	Uses a virus that has been genetically engineered so that it can't cause disease but produces coronavirus proteins to safely generate an immune response	Gamaleya Research Inst. (Sputnik V), AstraZeneca and Univ. Oxford, CanSino Biological Inc., Johnson & Johnson	Ebola
Protein subunit	Uses harmless fragments of proteins that mimic the COVID-19 virus to safely generate an immune response	EpiVacCorona, Novavax	Seasonal influenza, Hepatitis B, Tetanus
DNA	Synthetic DNA fragment (plasmid) that encodes a COVID-19 antigen	Inovio	None (new tech)
RNA	Typically the RNA segment of the viral genome that codes for the virus spike protein (or other antigenic region) is prepared in a suspension of lipid nanoparticles	Pfizer/BioNTech, Moderna, Curevac	None (new tech)

What else do vaccines contain?

Adjuvants

Lipid nanoparticles (NLPs), present in RNA vaccines only

Why are they there?

To enhance the immune response and reduce the dose of antigen needed, e.g. by stimulating the body to produce more antibodies or a longer-lasting immune response

Encapsulate and protect the RNA and help it enter the body's cells where the RNA can start producing the desired protein that will produce the antigenic response

*Mention here is for illustrative purposes only and does not signify endorsement by the InterAcademy Partnership (IAP).
Other vaccines produced using similar processes may be equallyor more effective or still under trial. Icons designed by Freepik from Flaticon.com.

Meaning of AIDS.....?

Acquired
(not born with)

Transmitted from person to person

Immune
(body's defense system)

It affects the body's immune system, the part of the body which usually works to fight off germs such as bacteria and viruses

Deficiency
(not working properly)

Malfunctioning of the body's immune system

Syndrome
(a group of signs and symptoms)

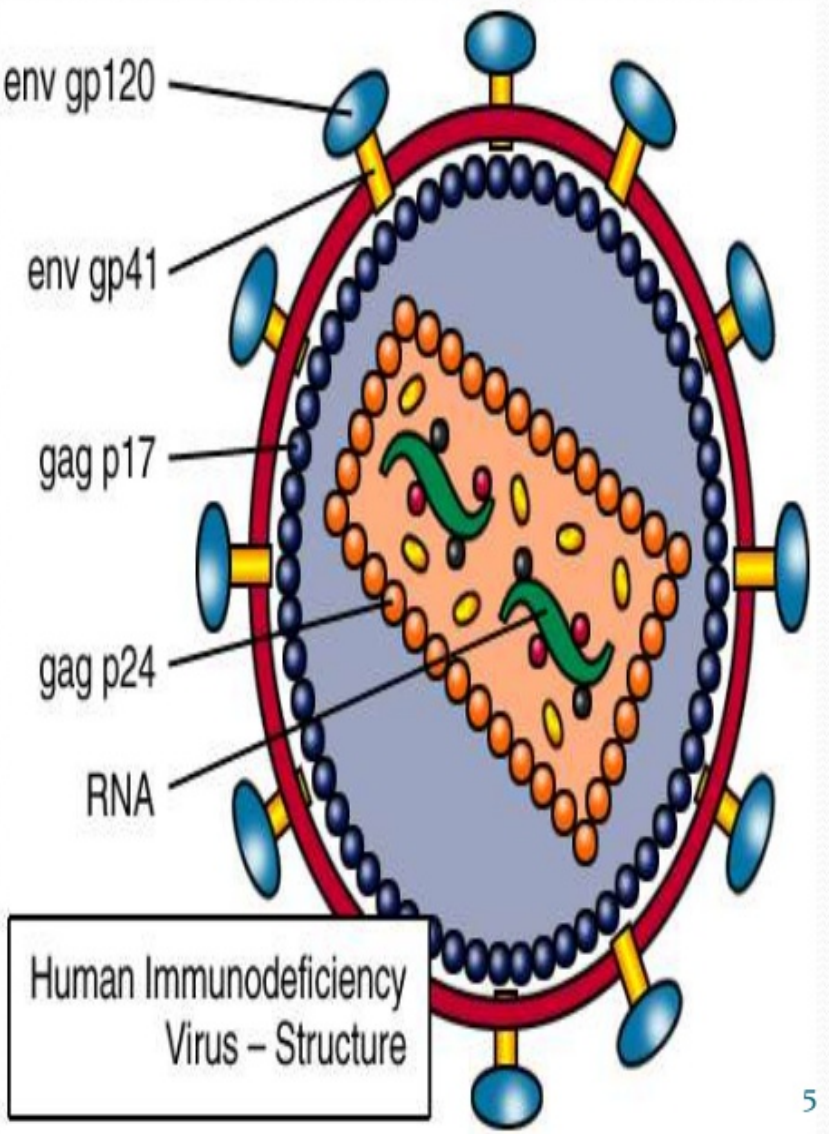
Someone with AIDS may experience a wide range of different diseases and OIs

INTRODUCTION

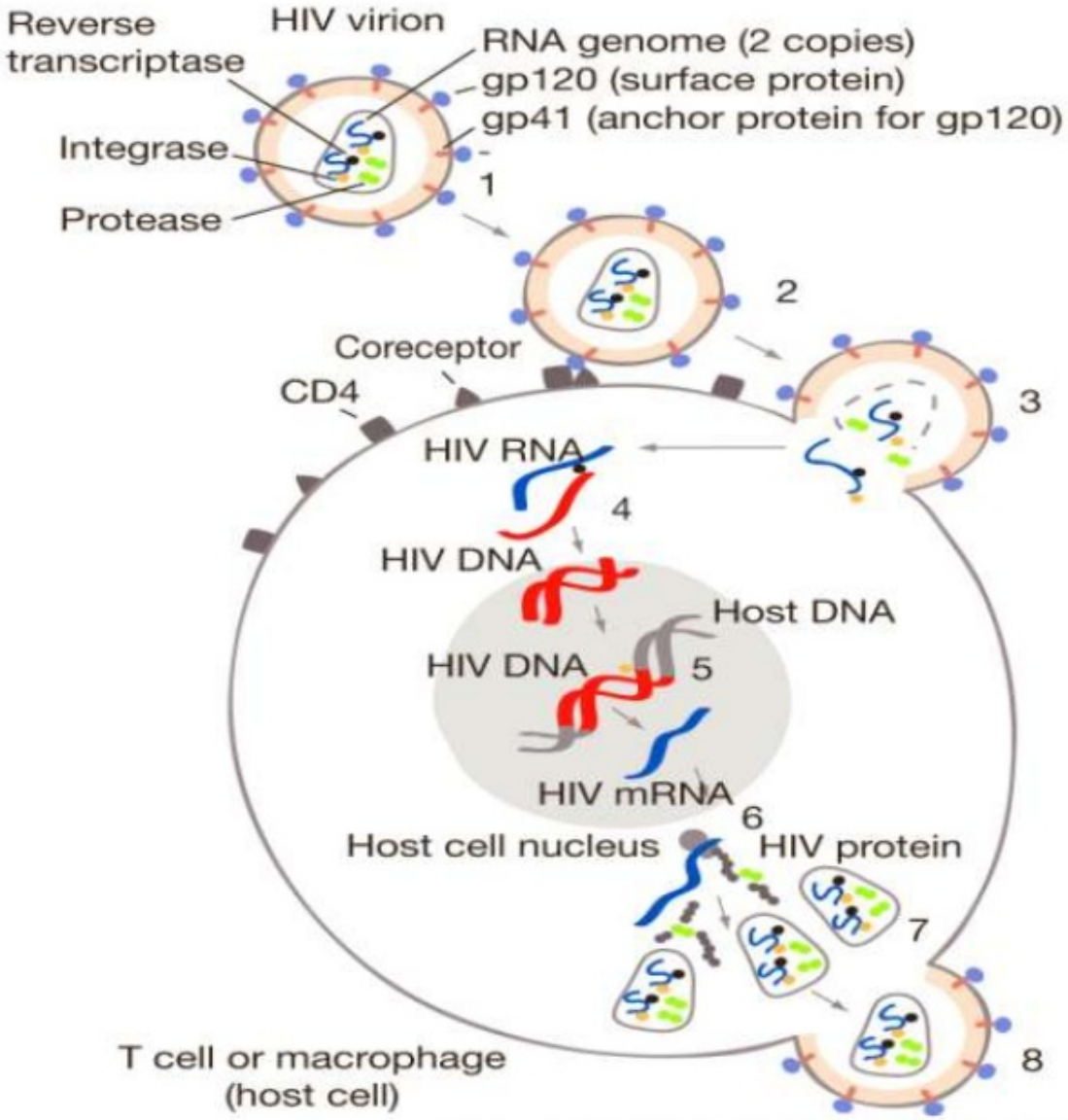
Family : Retroviridae

Subfamily : Lentivirus

- RNA virus, 120nm in diameter
- Envelope gp160; gp120 & gp41
- Icosahedral symmetry
- Nucelocapsid
  - Outer matrix protein (p17)
  - Major capsid protein (p24)
  - Nuclear protein (p7)
- Diploid RNA with several copies of reverse transcriptase



HIV life cycle



1) HIV's extracellular, or virion stage
2) HIV's gp120 protein binds to CD4 and coreceptor on host cell
3) HIV's RNA genome, reverse transcriptase, integrase, and protease enter host cell
4) Reverse transcriptase synthesizes HIV DNA from HIV's RNA template
5) Integrase splices HIV DNA into host genome. HIV DNA is transcribed to HIV mRNA by the host cell's RNA polymerase
6) HIV mRNA is translated to HIV precursor proteins by host cell's ribosomes. Protease cleaves precursors into mature viral proteins
7) New generation of virions assembles inside host cell
8) New virions bud from host cell's membrane

Component of antiviral response

- **Both humoral and cell-mediated immune responses partially control the viral production but in this process they destroy the infected CD4+T cells, leading to a gradual decline of CD4+ T cells**

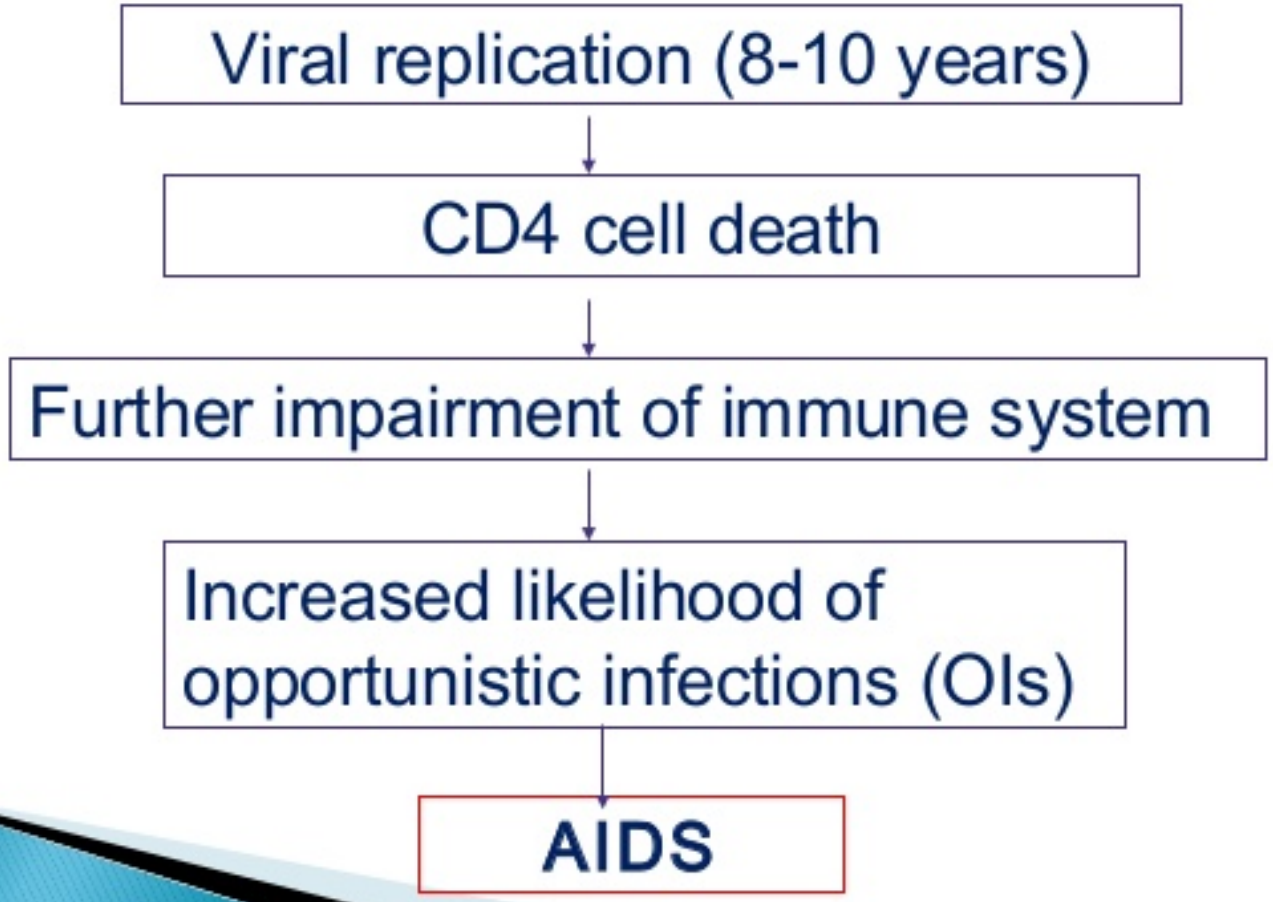
Innate Immunity

- Antigen presenting cells: Macrophage and Dendritic cells
- Other cell types: NK, gamma- delta , NKT cells
- Soluble factors: Chemokines

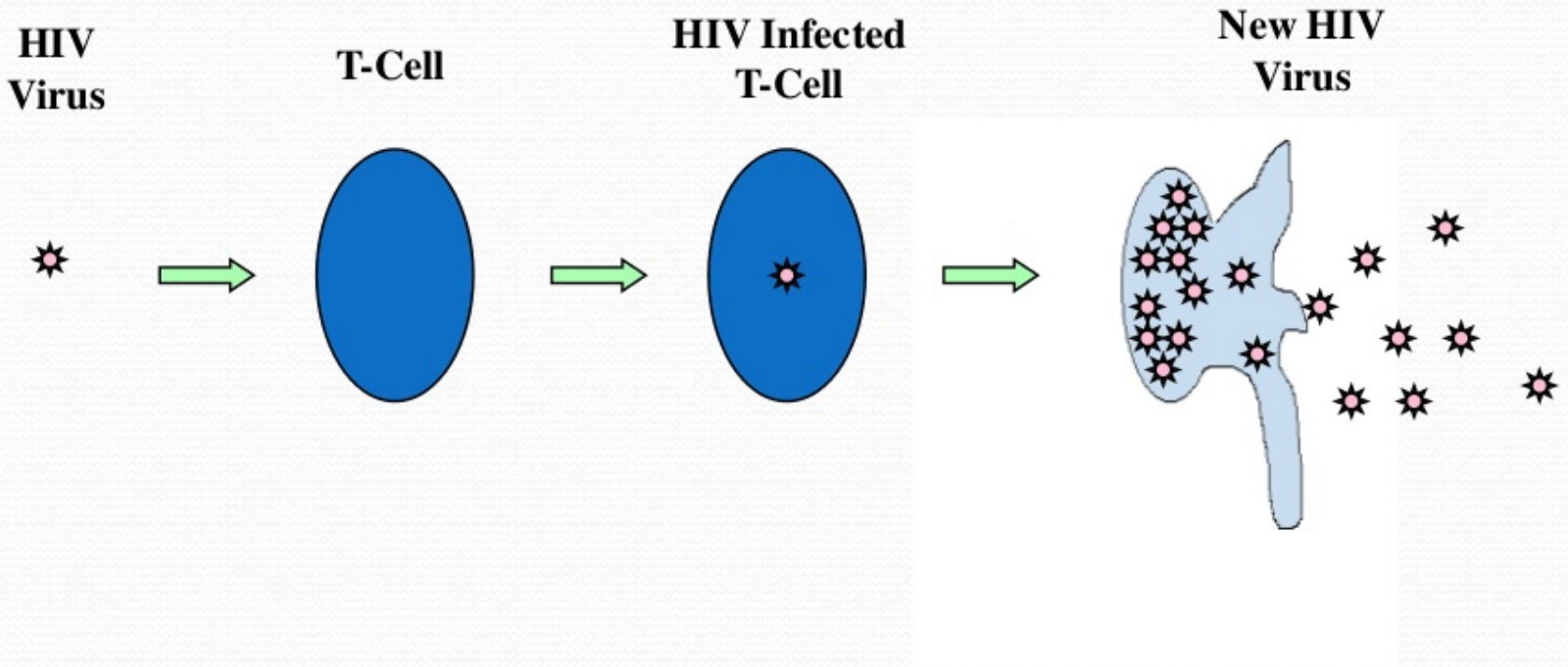
Adaptive Immunity

- CD4 T helper cells
- CD8 T cells (CTL)
- B cells: Neutralizing antibodies

How Does HIV Cause AIDS ?



Pathogenesis of HIV / AIDS
Infected T-Cell



NON-ENVELOPED RNA VIRUSES

Hepatitis Viruses

- Hepatitis viruses keep cells that form liver tissue, these cells become unable to function over time, and individual cell death may begin, then liver tissue loss may develop.
- hepatitis A virus (HAV)
- hepatitis B virus(HBV)
- hepatitis C virüs (HCV)
- hepatitis D virüs (HDV)
- hepatitis E virus (HEV)

- The five hepatitis viruses that infect humans and cause hepatitis are now known and called hepatitis viruses A, B, C, D, and E, respectively.
- Among these, hepatitis D is an incomplete virus that can only infect and replicate in people who carry the hepatitis B virus.

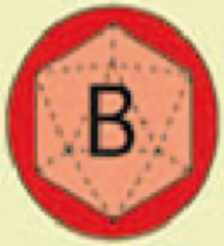
Virus	Nucleic Acids (Number of Bases)	Envelop	Infection Route	Persistent Infection	Type of Hepatitis
	RNA (approx. 7,500 bases)	No	Drinking Water Foods	No	Acute Hepatitis Fulminant Hepatitis
	RNA (approx. 7,600 bases)	No	Drinking Water Foods	No	Acute Hepatitis Fulminant Hepatitis
	DNA (approx. 3,200 bases)	Yes	Bloods	Yes	Acute Hepatitis Fulminant Hepatitis Chronic Hepatitis Liver Cirrhosis Hepatocellular carcinoma
	RNA (approx. 9,500 bases)	Yes	Bloods	Yes	Acute Hepatitis Chronic Hepatitis Liver Cirrhosis Hepatocellular carcinoma

Fig3 : Properties of four Hepatitis Viruses

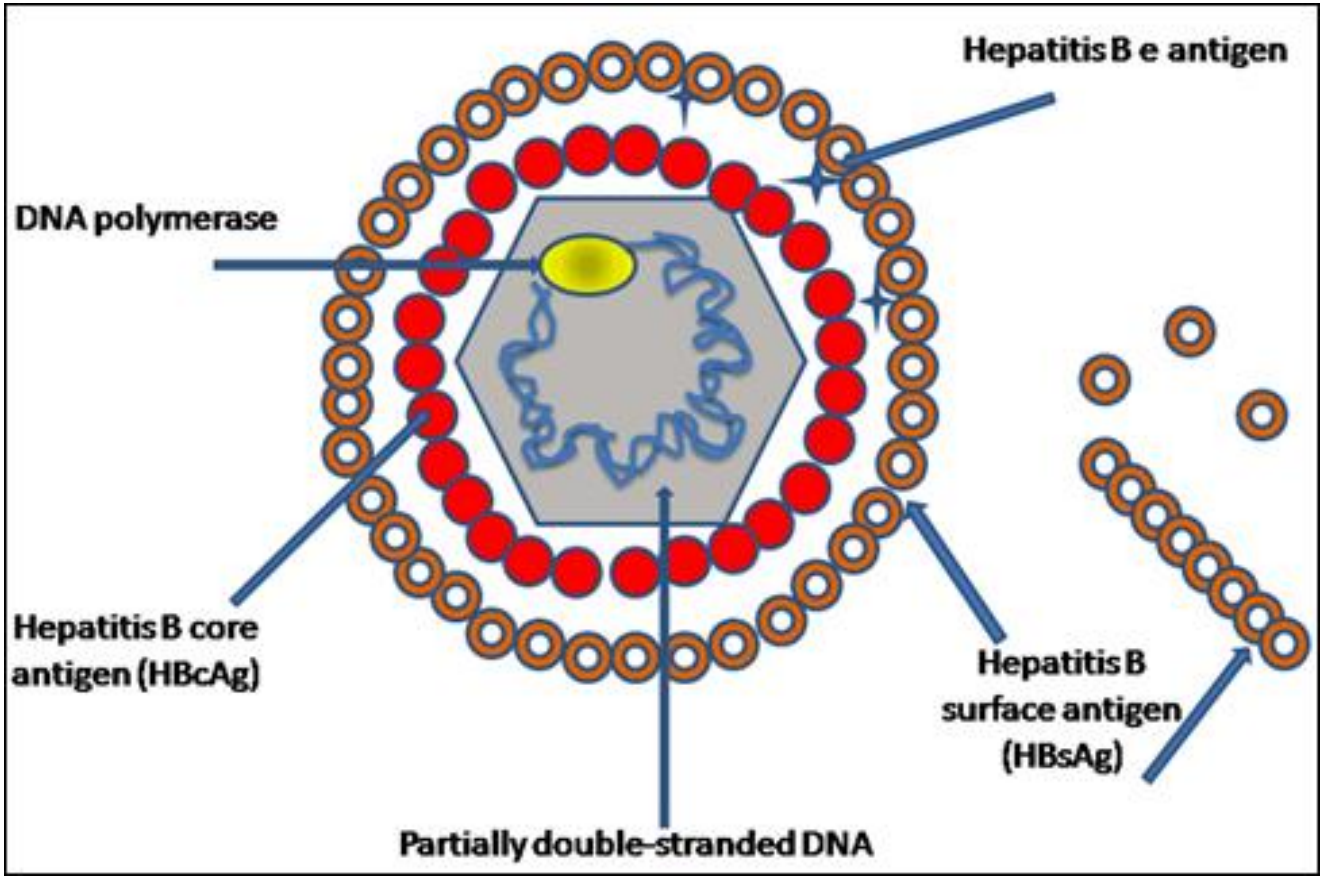
Table 25.7 : Characteristics of hepatitis viruses.

Disease/Virus	*Classification*	*Genome*	*Transmission*	*Prevention*
Hepatitis A	Picornaviridae, Hepatovirus	RNA	Food,Water	Killed HAV
Hepatitis B	Hepadnaviridae, Orthohepadnavirus	DNA	Blood,needle, body secretion, placenta	Recombinant HBV vaccine
Hepatitis C	Flaviviridae, Pestivirus or Flavivirus (?)	RNA	Blood	Routine blood screening
Hepatitis D	Unclassified	RNA	Blood	HBVvaccine
Hepatitis E	Caliciviridae (?)	RNA	Faecal/oral	Maintain Hygeine

- Hepatitis A and E viruses are transmitted orally through drinking water and food.
- Because the infection is short-lived and does not last period than 6 months, persistent infection does not occur.
- Therefore, these viruses cause acute hepatitis only and not chronic hepatitis. If the child is infected, symptoms may be mild but tend to be severe in adult.

- On the other hand, hepatitis B and C viruses are transmitted through the bloodstream.
- Hepatitis B virus can cause acute hepatitis, which can lead to frightening, fulminant hepatitis and Infection with hepatitis C virus rarely causes acute hepatitis with symptoms.

- Both viruses can cause persistent infection. Many people have no symptoms, but some develop hepatocellular carcinoma after chronic hepatitis and subsequent cirrhosis.
- Thus, these virus are much more frightening than the type A and E viruses.


Hepatitis B e antigen
DNA polymerase
Hepatitis B core antigen (HBcAg)
Hepatitis B surface antigen (HBsAg)
Partially double-stranded DNA

HBV Replication