Microbiology Team
Med441

Introduction to Medical Virology

MED441
KING SAUD UNIVERSITY

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Editing File

Color Index:
- Main text
- Boys slides only
- Girls slides only
- Doctor's notes
- Extra information
- ***Important***

Objectives

- Distinguish viruses from other microorganisms.
- General characteristics of viruses.
- Structure and symmetry.
- Classification of viruses.
- Steps of virus replication.
- Laboratory diagnosis of viral infections.

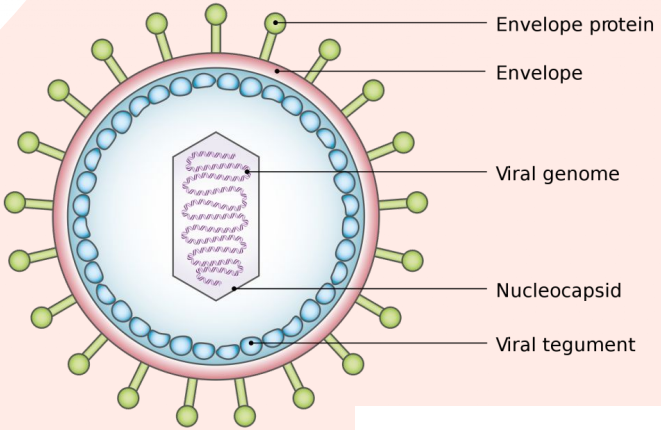
Properties of microorganisms:

Characteristic	Parasite	Fungi	Bacteria	**Virus**
Cell	yes	yes	yes	**No**
Type of nucleus	eukaryotic	eukaryotic	prokaryotic	**-----**
Nucleic acid	Both DNA & RNA	Both DNA & RNA	Both DNA & RNA	**DNA or RNA**
Ribosomes	Present	Present	Present	**Absent**
Mitochondria	Present	Present	Absent	**Absent**
Replication	Mitosis	Budding or mitosis	Binary fission	***Special***

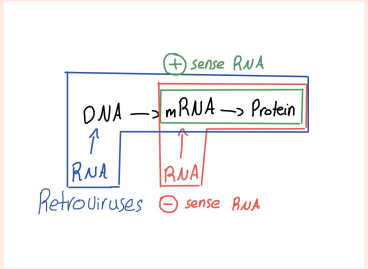
characteristics of viruses:

- Acellular (non-cellular).
- Has tiny particles:
  - 1. Internal core.
  - 2. Protein coat.
  - 3. Some viruses have lipoprotein membrane **(envelope)**.
- Obligate intracellular organisms.
- Replicate in a manner different than other cells. (1 virus → many viruses).
- Size: 20-300 nm.

Viral structure:



1. **Viral genome**
2. **Capsid**
3. **envelope**



1. Viral genome:

DNA

- (deoxyribonucleic acid).
- All DNA vs have ds except Parvoviruses.
- Single molecule.

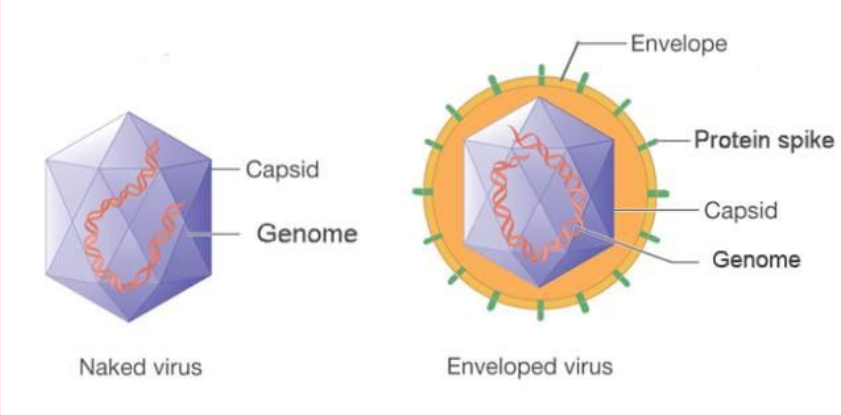
RNA

- (ribonucleic acid).
- All RNA vs have ss except Reoviruses.
- Single/ double molecule.
- (+) polarity. (-) polarity.

All viruses are haploid except for retroviruses are diploid.

2. Capsid:

- A protein coat.
- Subunits (capsomeres)
- Genome (nucleic acid) + capsid = nucleocapsid
- Function:
  - Protects nucleic acid.
  - Facilitates its entry into the cell.

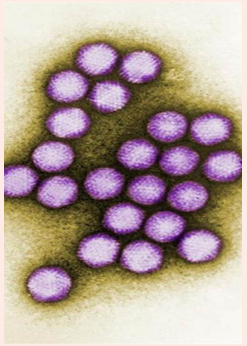
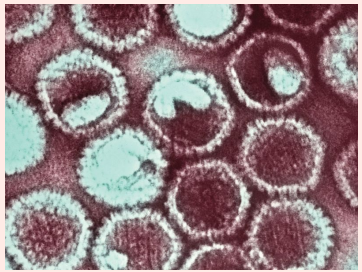
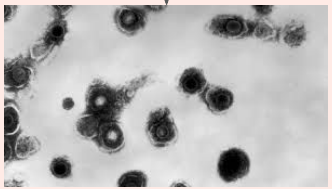
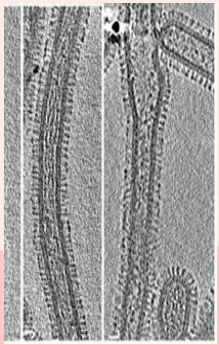


3. Envelope:

- Lipoprotein membrane (host lipid, virus specific protein).
- During Budding:
  - Envelope is derived from cell membrane (except for herpes virus it is from nuclear mb).
  - Enveloped viruses are more sensitive to heat, dry, **ether** **and other factors** than non-enveloped viruses.
  - Glycoprotein attaches to host cell receptor.

Symmetry:

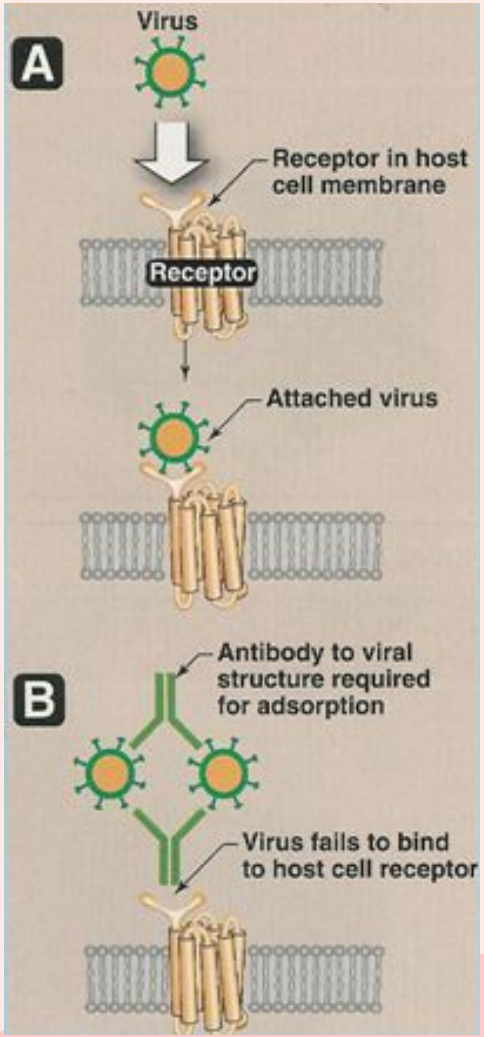
(arrangement of capsomeres)

- Cubic symmetry (icosahedral)
  - Adenovirus
    - 
  - Herpes virus
    - 
- Complex symmetry
  - Poxviruses
    - 
- Helical symmetry
  - Elongated (filovirus)
    - 
  - Pleomorphic (influenza virus)
    - 

Viral proteins:

- **The outer viral proteins:**
  - Mediate attachment to specific receptors.
  - Induce neutralizing antibodies.
  - Target of antibodies.

- **The internal virus proteins:**
  - Structural proteins (capsid proteins of enveloped viruses).
  - Nonstructural proteins (enzymes)
    - All ssRNA viruses (-) polarity have transcriptase (RNA dependent RNA polymerase) inside virions.
    - Retroviruses & HBV contain reverse transcriptase.



Classification of viruses

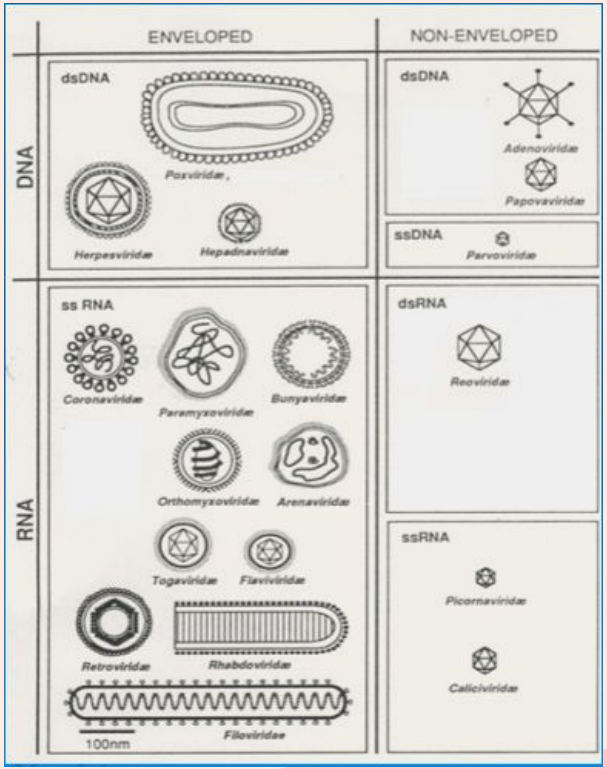
01 Type of nucleic acid.

02 Number of strands.

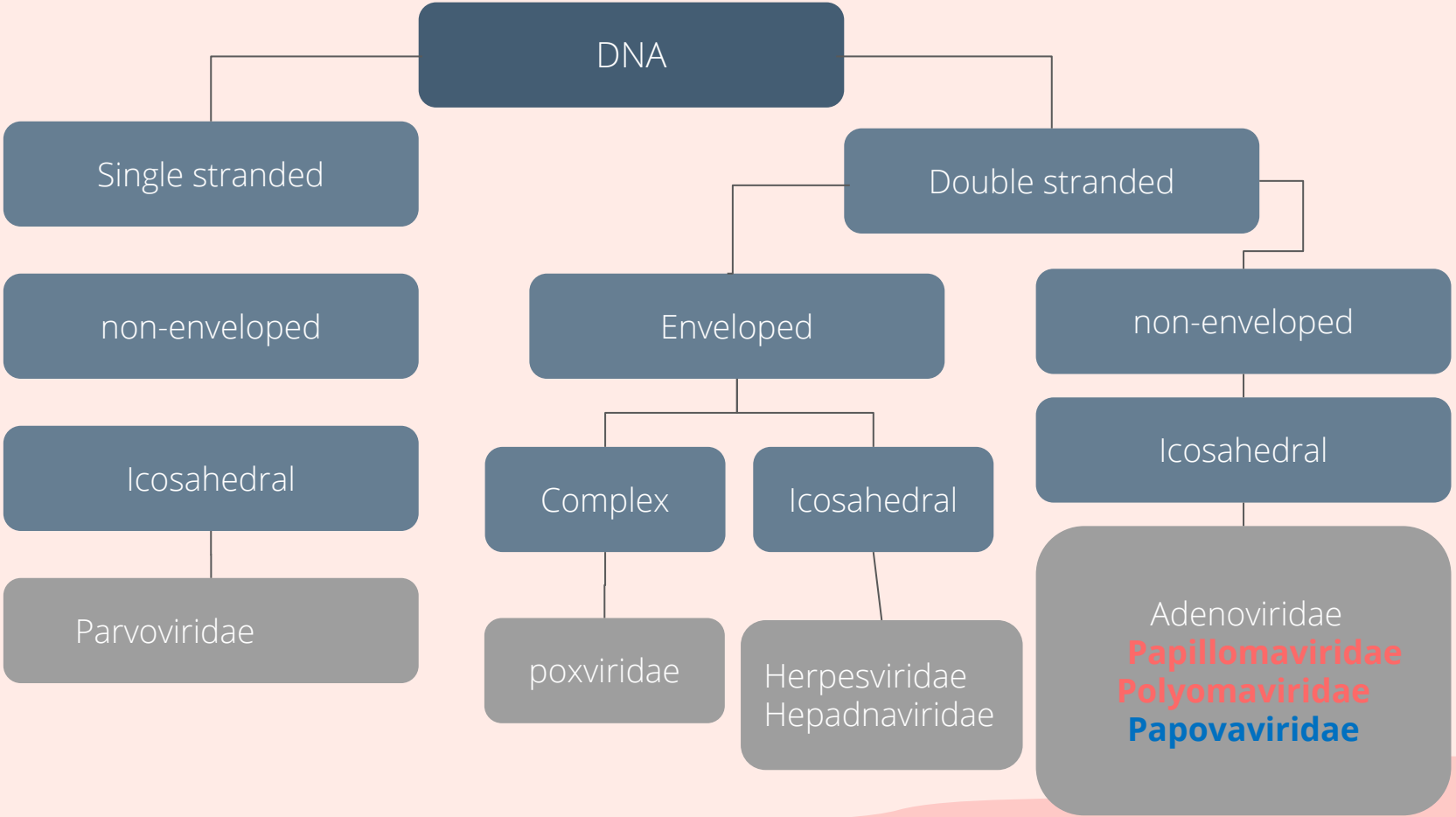
03 Polarity of viral genome.

04 The presence or absence of envelope.

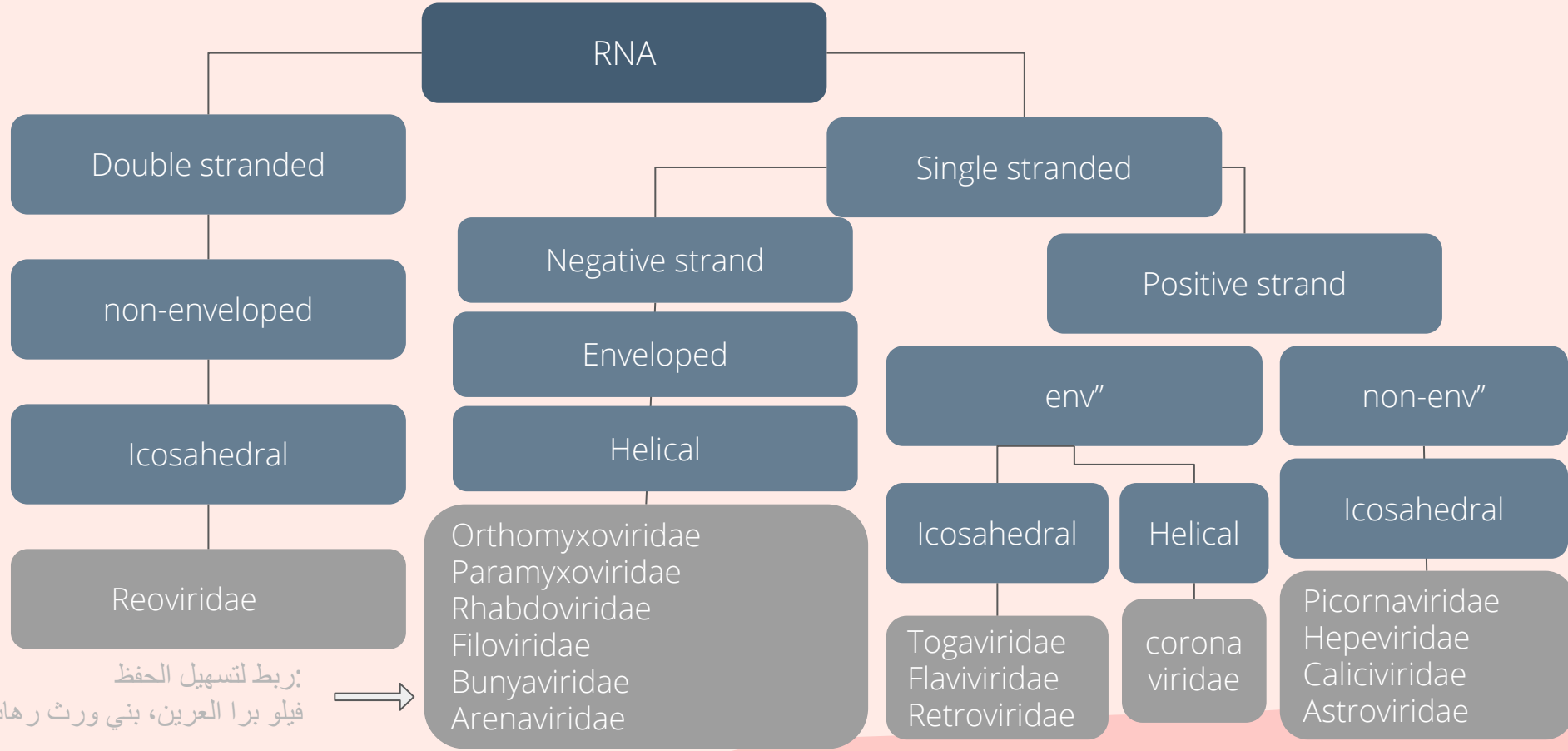
05 Type of symmetry.



Medically important viruses



Medically important viruses



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فيلو برا العرين، بني ورث رهاب

VIRUS REPLICATION STEPS:

1- Adsorption **(Attachment)**	- **glycoprotein fiber** (envelope) (specific structures that attach to specific receptors) -folding in the capsid proteins.(Non envelope)
2- Penetration	(the attachment of the virus from the outer surface of the host across the membrane to the cytoplasm) 1. **Fusion** (envelope) viral envelope fuse with the cell membrane and enter the host cell and the envelope remains attach to the cell membrane 2. **Endocytosis** : (*Enveloped* virus fuses with endosome membrane) (*Non-enveloped* virus pore [virus will create a pore in the endosome] or lysis endosome membrane)
3- Uncoating	(remove the capsid protein) Release of viral genome in **(cytoplasm for RNA virus)** or **(nucleus for DNA virus)** to continue the replication

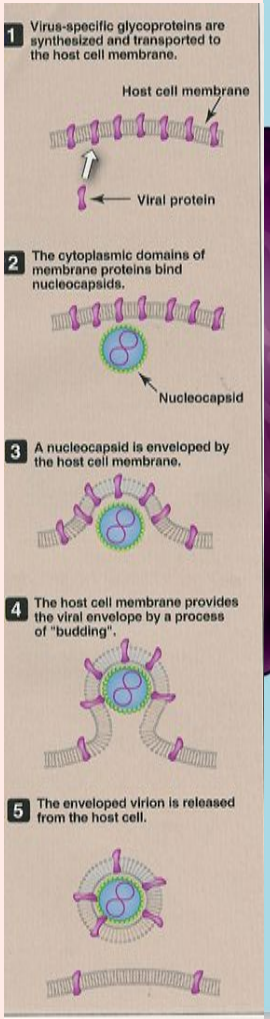
VIRUS REPLICATION STEPS: CONT..

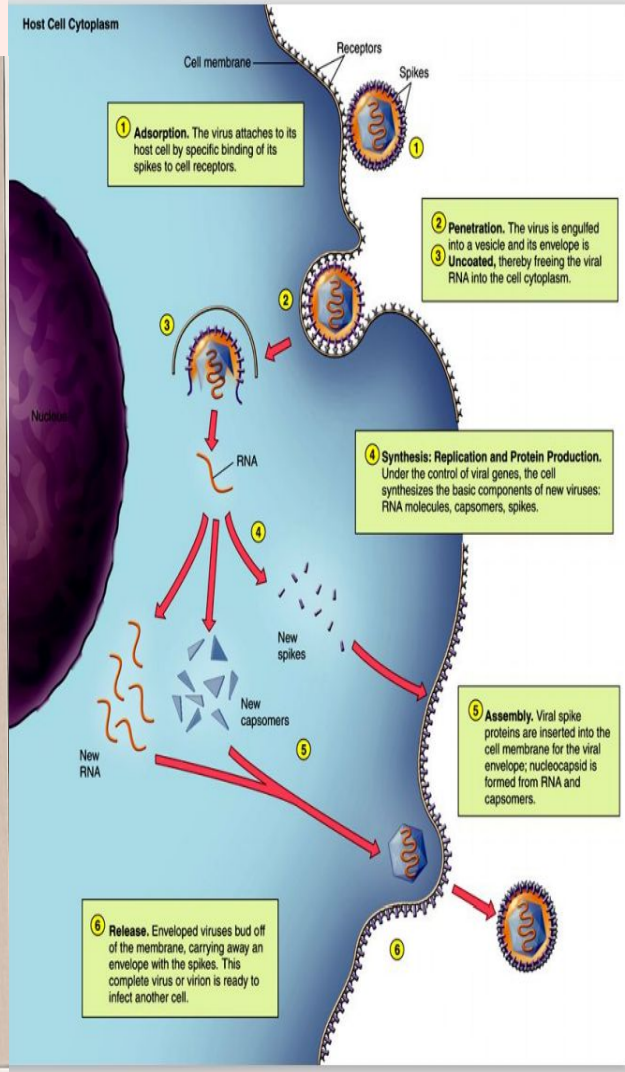
4- Synthesis of viral components	- **Positive single strand RNA (+ssRNA) viruses** their single strand of RNA acts directly as mRNA so no need for transcription **(direct)** which will be **translated** to structural proteins and enzymes (with help of the cell's ribosomes) - **Negative RNA viruses** their RNA must be **transcribed** into mRNA first **by (RNA dependent RNA polymerase)** then **translated** to make structural proteins (by help of cell's ribosomes which make enzyme [non-structural protein requires for the synthesis of viral genome] and structural protein [capsid]) - viral genome is replicated in both cases
5- Assembly	- (**Nucleic acid (viral genome) + viral protein = virions** (an entire viral particle)
6- Release	(from the virus infected cell) - **Envelope viruses** : undergo budding and get their membrane either from the **cell membrane** (in RNA viruses) or **nuclear membrane** (in DNA viruses e.g herpes viruses). **"Budding"**: a process by which the host cell membrane provides the viral envelope. - **Non-enveloped virus** : virus **lysis** or ruptures from the cell membrane **(cell is damaged)**.

laboratory diagnosis of viral infections

- Cell culture
- Viral antigen/antibody infection
- Molecular method
- Microscopic examination
- Serological tests

Budding :



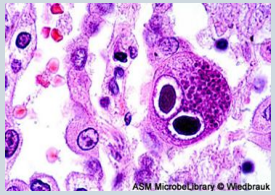


Microscopic examination:

Light Microscopy

- Shows **histological appearance** and **cytopathic effect**
(used to see the effect of virus in the host cell not the virus itself)

Ex. Inclusion bodies (collection of the virus particle inside the cell)

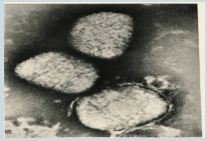


Owl's eye (CMV)

Electron microscopy

- Shows **morphology** and **size** of the virus **(to see virus itself).**

E.g.
-Diagnosis of skin lesions caused by herpes or poxvirus.
-**Diagnosis of viral GE (gastroenteritis) such as rota & adenovirus.**
- It is replaced by antigen detection & molecular tests (because it has a lot of disadvantages; it's expensive and the sensitivity is variable, it is used in research and discovering new viruses)



poxvirus

Herpesvirus

Virus cultivation

Methods of virus cultivation

- Laboratory animals **(effectiveness of vaccine)**
- Embryonated eggs **(to develop vaccine)**
- Cell culture **(isolation of virus)**

- Cell culture has 3 types of sub passage

Primary cell culture	Diploid cell culture (semi-continuous)	Continuous cell line
1 or 2 sub-passage (lasts a very short period which is culture why it is **difficult to maintain**)	**20 to 50 sub-passages (lasts for a few weeks)**	**Indefinite (cancerous cell line)**

Cell culture

Dr's note: cell culture is not definite diagnosis

- Cell culture refers to removal of cells to see their subsequent growth ender suitable environment After isolating the cells form the tissue.

Purpose: **isolation of virus (DNA or RNA)**, new virus diagnosis.
Used to see: **cytopathic effect (not virus or type of virus)**

Problems with cell culture:

-Long incubation (5 days or more),
it can be solved with rapid culture technique.

- Sensitivity is variable.

- Susceptible to bacterial contamination.

- Some viruses don't grow in cell culture (HCV -Hepatitis C virus)

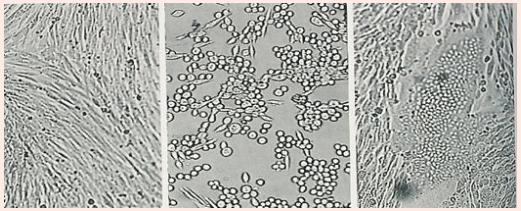
Dr's note: there is no single cell line capable in growing all medically important viruses so, there must be combination between them

Rapid cell culture technique

- A modification of cell culture called Shell Vial Assay (for rapid detection of viruses)
- Based on immunofluorescent detection of viral antigens
- 1-3 days

Detection of Viral Growth :

1. **Cytopathic effects:** the affected cell will have rounding, shrinking and aggregation (تشبه عنقود العنب) (in a group). Syncytium (giant multinucleated cell) and loses adherence. Basis of cell culture.



Uninfected cc | Cell rounding | syncytium

2. **Immunofluorescence** (IF) basis of Shell vial assay

3. Other methods

439 team (for your understanding)

1st step: **cell culture**
Tells whether or not there is infection through cytopathic effect. It is not a definitive diagnosis .

2nd step: **further investigation** to identify pathogen through other tests like :
1-Immunofluorescence (IF) .
2-ELISA.
3- molecular testing .

Serological test:

Antibody and Antigen detection:

techniques

Only for antibody detection:

Complement fixation test(CFT)

Immunofluorescence (IF)

Enzyme-linked immunosorbent assay (ELISA)

Dr's note: : (rapid result also used to diagnoses viral disease)
Used specifically for viruses to detect:
- **Antigen (Direct indication of viral infection)**
- **Antibody (indirect indication of viral infection)**

Antigen detection: it is test that looks for (antibodies) from a sample to determine the availability of antigen

Test	Sample	virus
IF	Nasopharyngeal aspirate	Influenza V
IF	Skin scrapings	HSV
ELISA	Feces	Rotavirus
ELISA	Blood	HBV(HBsAg)

Immunofluorescence (IF)

Uses fluorescent microscope and antibodies labeled with fluorescent dye to detect infection

- **Green** fluorescent means **positive result**
- No fluorescent means **negative result .**

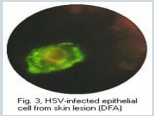
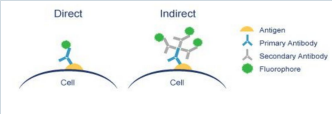


Fig. 3. HSV-infected epithelial cell from skin lesion (DFA)

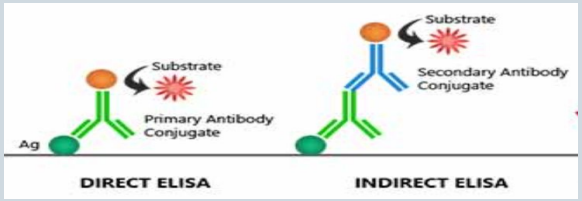
Direct (antigen detection)	Indirect (antibody detection)
Add antibody labeled with fluorescent dye and look through a fluorescent microscope; if green fluorescent the result is (+)	First add patient serum then add secondary-antibodies labeled with fluorescent dye



ELISA
Enzyme-linked immunosorbent assay

Same principle as IF but Uses **antibodies labeled with enzymes** instead of fluorescent dye and is read through a **spectrometer** . (it uses plate instead of slides)

- Yellow: positive result
- Colorless: negative result
- **Direct: antigen detection.**
- **Indirect: antibody detection**



Molecular test

- **Detects the viral genome.**

Uses Polymerase Chain Reaction (PCR)

- Amplification of viral genome and nucleic acid

Uses:

- Used for diagnosis as a confirmatory test .
- **It is the only way to monitor a patient's response to treatment by measuring viral load/concentration.**

MCQs

1) All viruses are haploid except for:			
A) Icosahedral viruses	B) herpes	C) retroviruses	D) Parvoviruses
2) The “budding”process happens at which viral replication step?			
A) Penetration	B) uncoating	C) synthesis of components	D) release
3) which one of these tests detects the viral genome?			
A) Molecular test	B) IF	C) ELISA	D) complement fixation test
4) poxviruses’ symmetry is:			
A) Helical	B) complex	C) Icosahedral	D) elongated

4- B
3- A
2- D
1- C

Questions and Answers (SAQ)

1-what are the viral structure component ?	1. Viral genome 2. Capsid 3. envelope
2-what are the types of symmetry ?	1-Cubic symmetry (icosahedral). 2-Complex symmetry 3-Helical symmetry
3-what is the Serological test technique for antibody detection ?	Complement fixation test(CFT)
4-what are the problems of cell culture?	Slide 16

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