Version 2

Viral Structure & Classification

-Important -Extra -Notes

-In boy's slides -In girl's slides

In this link, you will find any correction or notes unmentioned in the team's work. Please check the link below Frequently.
https://docs.google.com/document/d/1WvdeC1atp7J-ZKWOUSukSLsEcosjZ0AqV4z2VcH2TA0/edit?usp=sharing

MICROBIOLOGY TEAM 438

Objectives:

- General characteristics of viruses.
- Structure & Symmetry of viruses.
- Classification of viruses.
- Steps of virus replication.
- Laboratory diagnosis of viral infection.

Abbreviations:

- V(s): Virus(es).
- NA: Nucleic Acid.
- ds: double stranded.
- ss: single stranded.
- mb: membrane.
- Ps: proteins.
- Rs: Receptors.
- AB(s): Anti-body.
- Ag: Antigen.
- RetroVs: Retroviruses.
- IF: Immunofluorescence.
- ELISA: Enzyme-linked immunosorbent assay
- HBV: Hepatitis B virus
- HCV: Hepatitis C virus
- HSV: Herpes simplex virus

Properties of Microorganisms

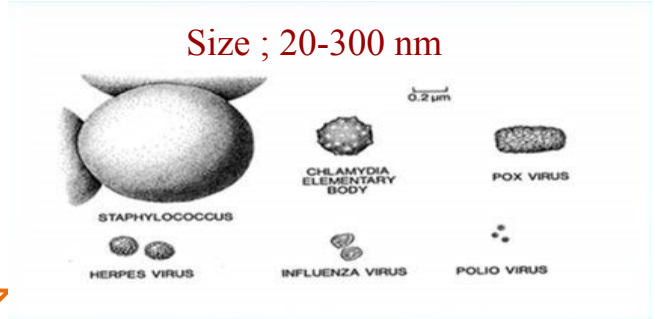
Characteristic	Parasite	Fungi	Bacteria	Virus
Cell	Yes	Yes	Yes	No
Types of Nucleus	Eukaryotic	Eukaryotic	Prokaryotic	-
Nucleic Acid	Both DNA & RNA	Both DNA & RNA	Both DNA & RNA	DNA or RNA (Can't be in both)
Ribosomes	Present	Present	Present	Absent
Mitochondria	Present	Present	Absent	Absent
Replication	Mitosis	Budding or Mitosis	Binary fission	Special

Characteristics of Viruses

★ Obligate intracellular organisms.

*no ribosomes or mitochondria
so it must replicate in a living host.

★ Acellular organisms.
*Not having cells.



Viruses

★ Replicate in a manner diff from cells .
〈 1V ⟶ Many Vs 〉

★ Tiny particles:
★ Internal core of nucleic acid (containing DNA or RNA),
★ Protein coat that surrounds the nucleic acid (Capsid),
★ some Vs have lipoprotein mb (envelope).

Viral Structure

1- Viral genome

(All Viruses have a viral genome either DNA or RNA, **CAN NOT BE BOTH.**)

DNA **(Deoxyribonucleic acid)**	**RNA** **(Ribonucleic acid)**
All DNA Vs have ds except Parvoviruses (Have ss).	All RNA Vs have ss except Reoviruses (ds).
Single molecule (All of them are **haploid**).	Single / Double (All of them **haploid** **except** Reoviruses are **diploid**).
	(+), (-) Polarity

All Vs are **haploid** (single copy of genetic material), except retroviruses are **diploid** (two copies of the same genetic material).

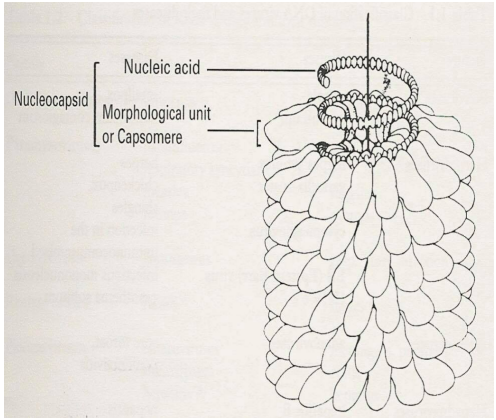
Viral Structure

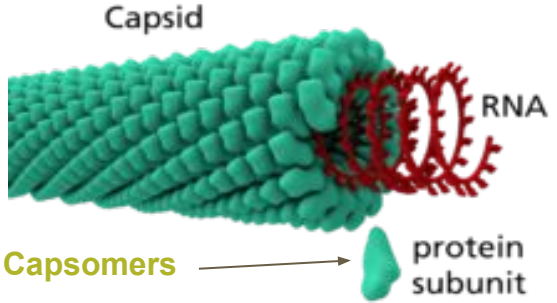
(All viruses have a capsid.)

2- Capsid

- ★ a protein coat.
- ★ Subunits (capsomers) (It's the building unit for Capsid).
- ★ Genome (NA) + capsid = nucleocapsid.
- ★ Function:
- ★ Protects NA,
- ★ Facilitates its entry into cell.

(for non-enveloped viruses)



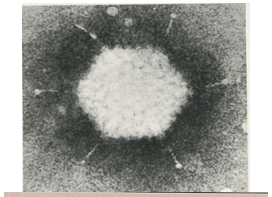


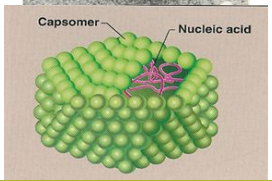
Symmetry

The arrangement of a viruses capsomeres gives it a unique Symmetry.

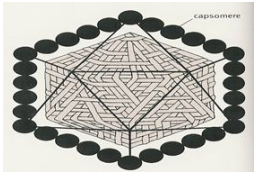
Cubic (icosahedral)

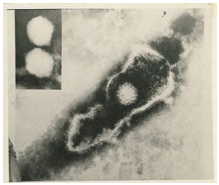
Adenovirus





herpes Virus

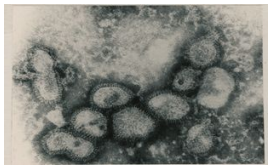




Complex,
(Poxviruses)

Helical

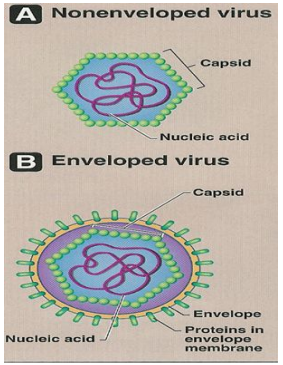
Elongated (filoviruses).



pleomorphic (influenza v.)

3- Envelope

in SOME viruses only.



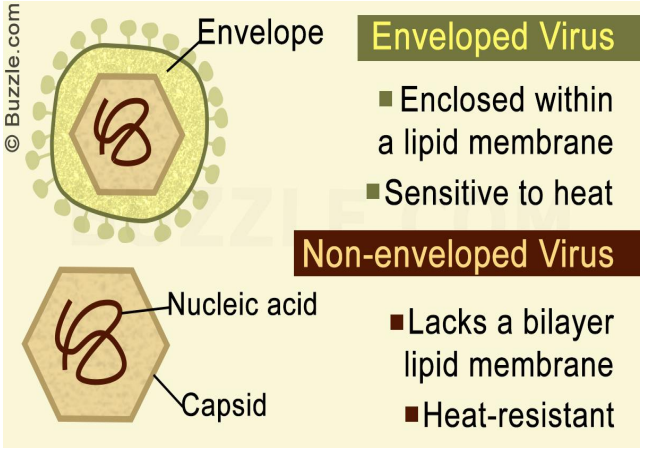
★ it is a lipoprotein mb (membrane), (host lipid + virus specific protein) which contains : Protein and Glycoprotein.

★ **During viral budding :**

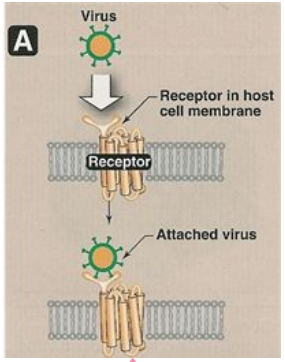
★ Envelope is derived from host cell mb (membrane), **EXCEPT**, Herpesviruses from nuclear mb (membrane).

★ Enveloped Vs (viruses) are more sensitive to heat, dry & other factors than non-enveloped Vs (viruses).

★ Glycoprotein attaches to host cell receptors, usually in the form of spikes / finger like projection.



Viral Proteins

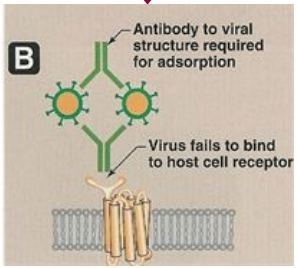


The outer viral Ps (proteins):

★ Mediate attachment to specific Rs (receptors).
★ Induce neutralizing Abs (Anti-Bodys).
★ Target to Abs.

The internal viral Ps (proteins):

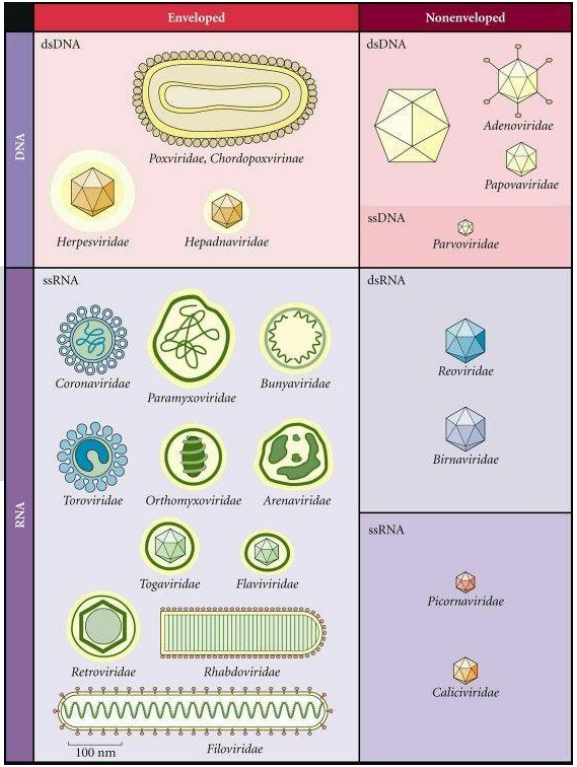
★ Structural Ps (Capsid Ps of enveloped Vs).
★ Non-structural Ps (enzymes).
- All ssRNA Vs (-) Polarity have transcriptase (RNA dependent RNA polymerase) inside virions.
- Retroviruses & HBV (Hepatitis B viruses) contain reverse transcriptase.



Classification of Viruses

Classification of Viruses

- Enveloped or non-enveloped.
- Type of NA, (DNA or RNA)
- Number of strands, (single or double stranded)
- Polarity (-ve or +ve), only in RNA viruses.
- Type of symmetry. (Helical, cubic or icosahedral, complex)



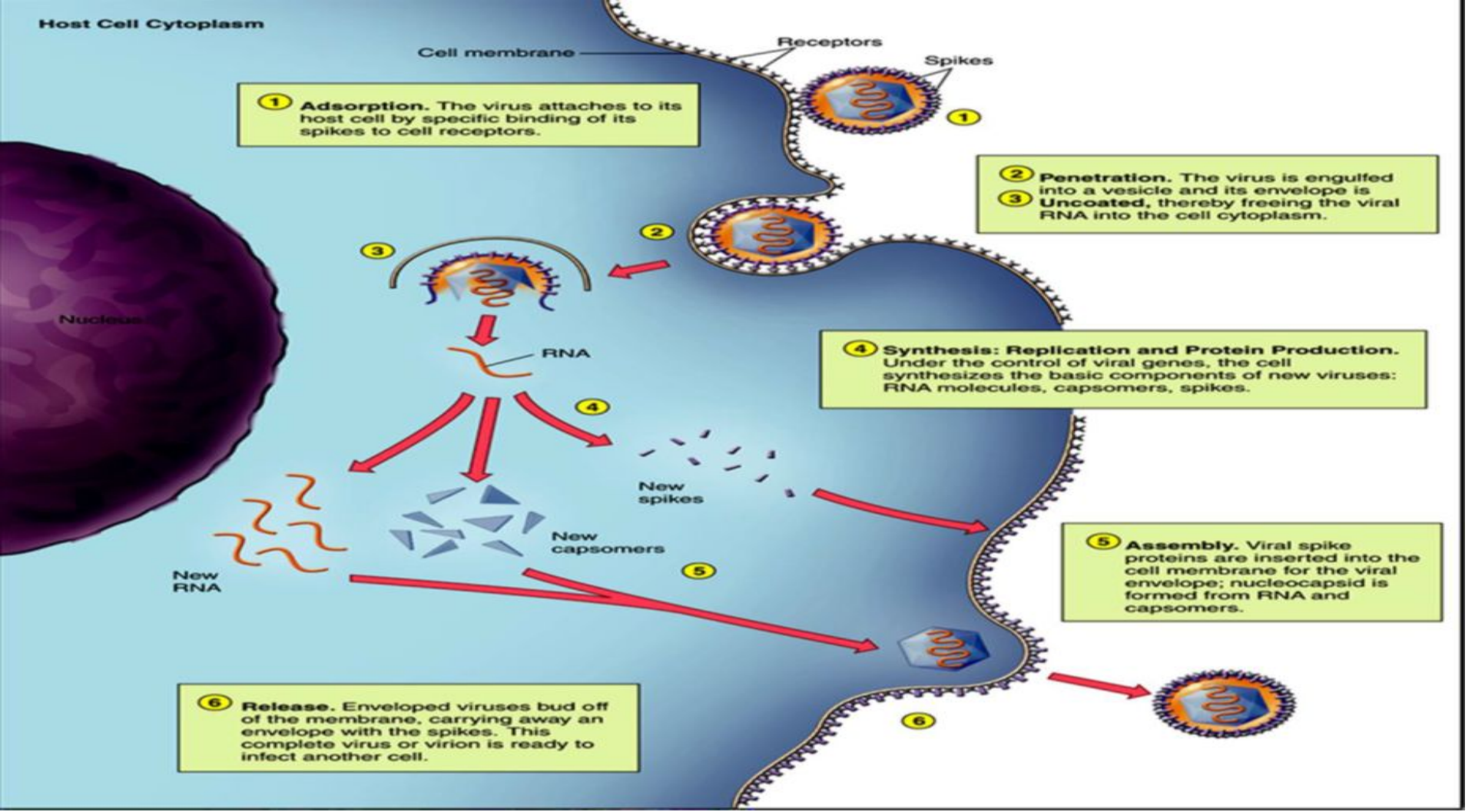
Medically Important Viruses:

1- DNA			
Single Stranded	**Double Stranded**		
Non- Enveloped	**Enveloped**		**Non- Enveloped**
Icosahedral	Complex	Icosahedral	Icosahedral
Parvoviridae	Poxviridae	Herpesviridae	Adenoviridae

2- RNA			
Single Stranded			**Double Stranded**
Neg-Strand	**Pos-Strand**		**Non-Enveloped**
Enveloped	**Enveloped**	**Non-Enveloped**	**Icosahedral**
Helical **Filoviridae**	**Helical** **Coronaviridae** **Icosahedral** **Retroviridae**	**Icosahedral** **Hepeviridae**	**Reoviridae**

VIRUS REPLICATION STEPS

1-Adsorption	○ Attachment site: Glycoprotein (if enveloped) ○ Folding in the capsid proteins (if non-enveloped)
2-Penetration	○ Fusion (if enveloped): viral envelope fuses with host cell membrane and enters cell. ○ Endocytosis (if enveloped: viruses fuse with endosome mb),(if non-enveloped; vesicles form and transfer the virus inside the cell).
3-Uncoating	○ Release of viral genome in cytoplasm (for RNA viruses) or nucleus (for DNA viruses).
4-Synthesis of viral components	○ For –ve RNA viruses, the viral genome is transcribed into mRNA and then translated to make viral proteins (indirect). ○ For +ve RNA viruses, their viral genome (ssRNA) can be translated directly to make viral proteins (direct). ○ The viral genome is replicated in both cases.
5-Assembly	○ Nucleic acid and viral proteins assemble to make virions (an entire viral particle). Millions of virions are produced.
6-Release	○ For enveloped viruses: the virus "buds" & gets their membrane from the cell membrane (in RNA viruses) or nuclear membrane (in DNA viruses). ○ For non-enveloped viruses: the virus lysis or ruptures from the cell membrane.(cell is damaged)


Host Cell Cytoplasm
Cell membrane
Receptors
Spikes
1 Adsorption. The virus attaches to its host cell by specific binding of its spikes to cell receptors.
2 Penetration. The virus is engulfed into a vesicle and its envelope is
3 Uncoated, thereby freeing the viral RNA into the cell cytoplasm.
Nucleus
RNA
4 Synthesis: Replication and Protein Production. Under the control of viral genes, the cell synthesizes the basic components of new viruses: RNA molecules, capsomers, spikes.
New spikes
New capsomers
New RNA
5 Assembly. Viral spike proteins are inserted into the cell membrane for the viral envelope; nucleocapsid is formed from RNA and capsomers.
6 Release. Enveloped viruses bud off of the membrane, carrying away an envelope with the spikes. This complete virus or virion is ready to infect another cell.

laboratory diagnosis of viral infections

- ★ Microscopic examination.
- ★ Cell culture.
- ★ Serological test.
- ★ Detection of viral Ag.
- ★ Molecular method.

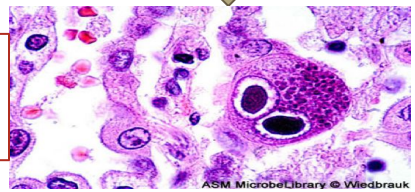
laboratory diagnosis of viral infections

Microscopic examination

light microscopy

Histological appearance "to see what the virus has done to the cell, without seeing the virus itself"
Ex. "Inclusion bodies"

Owl's eye (CMV)



It is replaced by Ag (Antigen detection) detection & molecular tests

- Ex. Diagnosis of viral GE (Gastroenteritis) such as Rota, adenoviruses.
- Diagnosis of skin lesion caused by herpes, or poxviruses.

electron microscopy

see **SIZE** and **Morphology** of virus.

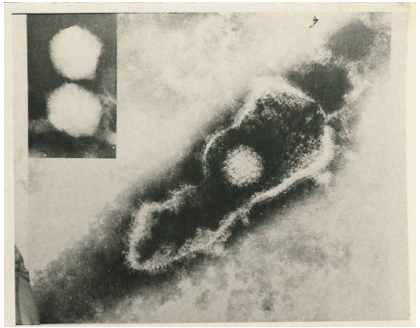
Morphology & size of virions" to see the virus particle itself"

Microscopic examination

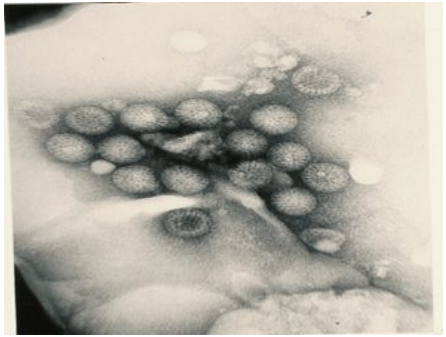
Electron microscopy

Poxvirus
(dsDNA env)

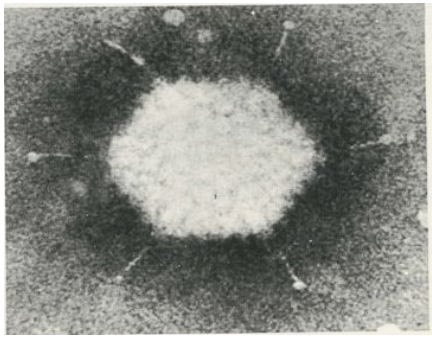
Herpesvirus
(dsDNA env)



Rotavirus
(dsRNA Non-env)



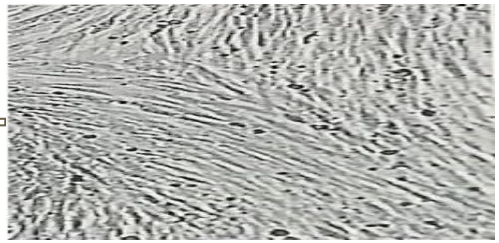
Adenovirus
(dsDNA Non-env)

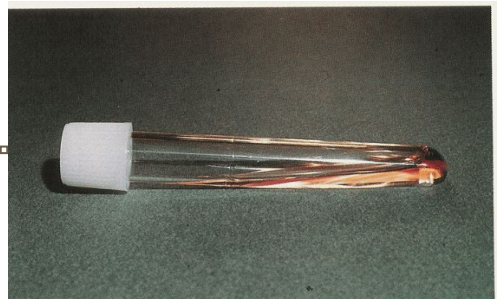


Virus cultivation

Cell culture | **Laboratory animals** | **Embryonated eggs**

★ Cell culture



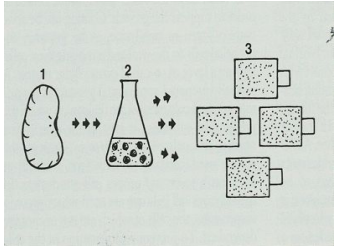


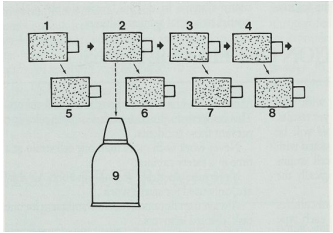
cell culture

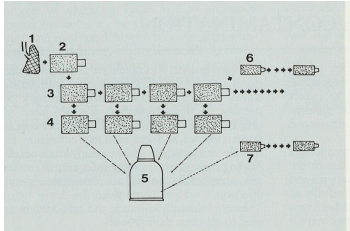
★ Cell culture refers to the removal of cells to see their subsequent growth under suitable environment.

★ After isolating the cells from the tissue, it has 3 types of sub passages

	Cell culture	No of sub passages
01	Primary C/C	1 OR 2 (it lasts for very short period)
02	Diploid C/C [semi continuous]	20 TO 50 (it lasts for few weeks)
03	Continuous cell line	Indefinite







Variation in Sensitivity of cell cultures to infection by viruses commonly isolated in clinical virology laboratories

PMK, primary MK. Degree of sensitivity: +++, highly sensitive;++, moderately sensitive; +, low sensitivity; +/-, variable; -, not sensitive

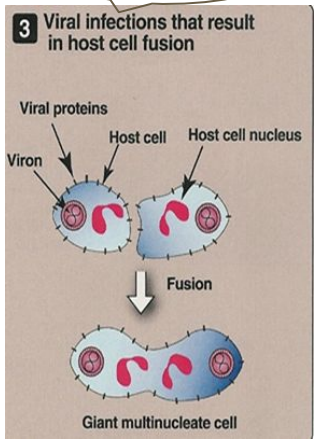
Virus		Cell culture		
		PMK	HDP	HEp-2
RNA virus				
	Enterovirus	+++	++	+/-
	Rhinovirus	+	+++	+
	Influenza virus	+++	+	-
	RSV	++	+	+++
DNA virus				
	Adenovirus	+	++	+++
	HSV	+	++	++
	VZV	+	+++	-
	CMV	-	+++	-

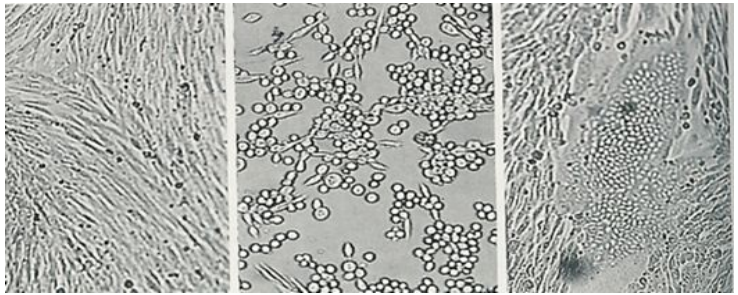
Detection of viral growth

- Cytopathic effects The affected cell will have “Rounding, shrinkage, aggregation(تشبه عنقود العنب). Syncytium(giant multinucleate cell) and loss of adherence
- IF (Immunofluorescence)
- other

صور توضيحية

Syncytium توضيح ل





Uninfected cc Cell rounding Syncytium

Problems with cell culture :

- Long incubation (up to 5 days) can be solved with rapid culture technique.
- Sensitivity is variable.
- Susceptible to bacterial contamination (عرضة للتلوث البكتيري).
- Some Viruses do not grow in cell culture e.g. HCV (hepatitis C virus).

★ **Rapid culture technique :**

Shell viral assay. It detect viral antigens within 1-3 days.

Antigens proteins appear stained

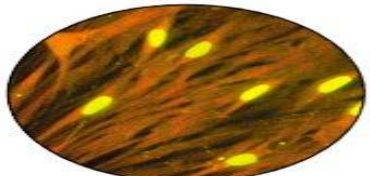


Fig. 2. CMV centrifugation culture fixed and stained 16 hrs after inoculation showing viral proteins in nuclei of infected human fibroblast cells

Serological tests

Antibody detection

- ★ Complement fixation test (CFT).
- ★ Immunofluorescence (IF).
- ★ Enzyme- linked immunosorbent assay (ELISA).

Antigen detection

It is a test that looks for (antibodies) from a sample to determine the availability of (antigen)

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

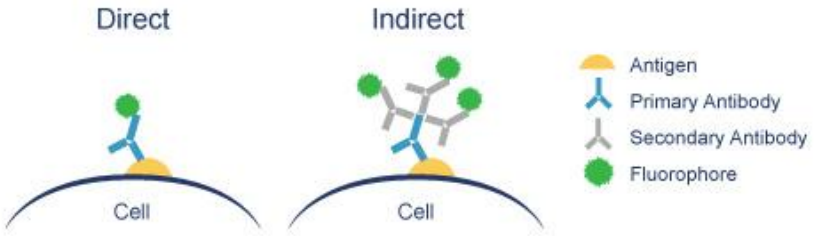
Serological Tests

★Immunofluorescence (IF)

- Uses fluorescent dye and fluorescent microscope to detect infection, either through antigens or antibodies.

★ Direct: antigen detection
-add antibodies labeled with fluorescent dye and look through a fluorescent microscope; if fluorescent then the result is positive.

★ Indirect: antibody detection
-add patient serum then add secondary-antibodies labeled with fluorescent dye.

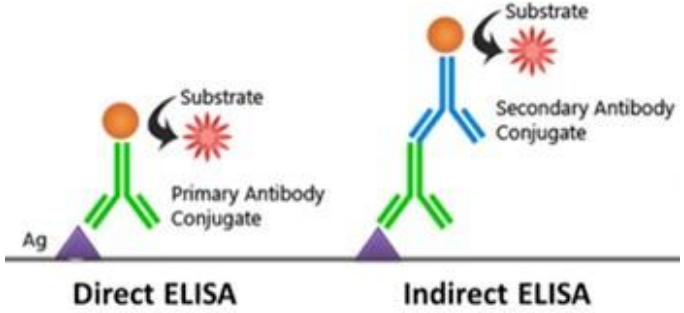


Serological Tests

★Enzyme-Linked Immunosorbent Assay (ELIZA):

Same principle as IF, but uses antibodies labeled with enzymes instead of fluorescent dye and is read through a spectrometer.

Direct: antigen detection
Indirect: antibody detection



★Molecular Test:

Detects the nucleic acid of the virus.
Uses polymerase chain reaction and amplification of viral nucleic acid and viral genome.

-Used for diagnosis.
-Is the only way to monitor a patient's treatment response by measuring viral load/concentration.

MCQs:

Q1: in virus replication, in which step the release of the viral genome either in the cytoplasm or nucleus?

a) release. b) adsorption c) uncoating. d) assembly.

Q2: which of the following is false about the viral?

a) all viruses must have capsid.

b) all viruses are haploid.

c) all viruses have either RNA or DNA can't be Both.

d) some of the viruses have an envelope.

Q3: Poxviruses are :

a) ds(DNA) env.

b) ds(DNA) non-env.

c) ds(RNA) env.

Q4: It's a protein coat for viruses?

a) envelope. b) Capsid. c) viral genome. d) the outer viral protein.

1/ c 2/b 3/a 4/b

SAQs:

1/ what the right step for (Nucleic acid and viral proteins assemble to make virions (an entire viral particle). Millions of virions are produced)?

Assembly

2/ Give me an example of complex viruses:

Poxviruses

Team Leaders:

- حنين الصميلي
- بدر القرني

Members:

- سارة يوسف الفليج
- أميرة الزهراني
- غادة السدحان
- نجود العلي
- جود الخليفة
- دينا عورتاني
- ريناد المطوع
- سارة الهلال
- طيبة الزيد
- لينا النصار
- ميسون آل تميم
- نورة المزروع
- سارة الخليفي
- رغد الخشان
- لينا العصيمي
- فيصل ع. الزهراني
- الوليد العازمي
- عبدالله الحوامدة
- عبدالله الداود
- عبدالرحمن البديوي
- فيصل القبلان
- عبدالله العثمان
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