

Microbiology

team 436



MEDICINE
KING SAUD UNIVERSITY

MICROBIOLOGY

Lecture : 2

VIRAL STRUCTURE CLASSIFICATION

IMPORTANT.
DOCTORS NOTES.
EXTRA INFORMATION.

Objectives-viral structure and classification:

- General characteristics of viruses
- Structure and symmetry of viruses
- Classification of viruses
- Steps of viruses replication
- Laboratory diagnosis of viral infections .

SHORTCUT YOU MIGHT NEED..

- Vs:Virus
- NA: Nucleic Acid
- INFs: Interferons
- CPE: Cytopathic effect
- HSV: Herpes Simplex Virus
- EBV: Epstein-Barr Virus
- HPV: Human Pappilomavirus
- HTLV: Human T-lymphotropic
•Virus
- IP: Incubation Period
- AB:Anti Body
- APC:Antigen Presenting Cells • NK: Natural Killer
- VICs:Virus Infected Cells
- IL: Interleukin
- CMI:Cell-MediatedImmunity • IR: Insulin Resistance
- CTCs: Cytotoxic T Cells
- IF: Immunofluorescence
- HAV: Hepatitis A Virus

PROPERTIES OF MICROORGANISMS

Characteristics	Parasites	Fungi	Bacteria	Viruses
Cell	Yes	Yes	Yes	NO
Type of Nucleus	Eukaryotic	Eukaryotic	Prokaryotic	-
Nucleic Acid	DNA <u>and</u> RNA	DNA <u>and</u> RNA	DNA <u>and</u> RNA	DNA <u>or</u> RNA
Ribosomes	Present	Present	Present	Absent
Mitochondria	Present	Present	absent	Absent
Replication	Mitosis	Budding or Mitosis	Binary Fission	Special 1V= million

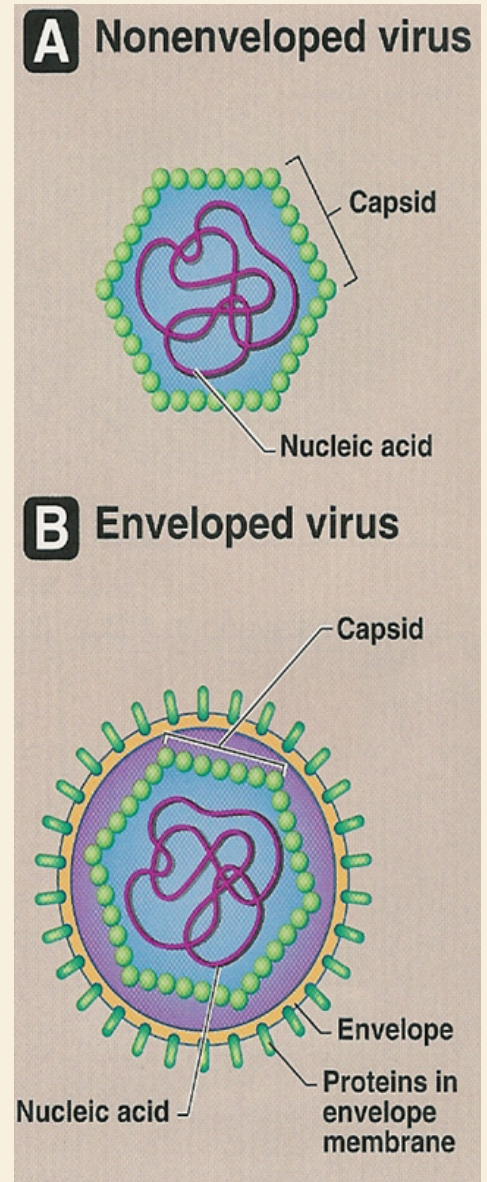
CHARACTERISTICS OF VIRUSES

- ◆ 1- **Acellular** organisms
- ◆ 2- Tiny particles
 - **internal core** (DNA/RNA)
 - **protein coat (capsid)**
 - **some** viruses have **lipoprotein membrane** (envelope)
- 3- **Obligate intracellular organisms**
- 4- **Replicate** in a manner **different** from cells (e.g. one virus replicate to Produce many viruses)

Taken from Host.
Not all viruses have envelope

Seen only by EM

note : viruses range in size from 20-300nm
{Extra info; way smaller then bacteria 2000-8000nm}



VIRAL STRUCTURE

- 1- Genome
- 2- Capsid
- 3- Envelope

- **I-Viral Genome**

Could be **RNA** or **DNA**

DNA: deoxyribonucleic acid

All **DNA** viruses are **double stranded** except for **Parvoviruses**.

Single molecule.

OR

RNA: ribonucleic acid

All **RNA** viruses are **single stranded** except **Reoviruses**.

Single/double.

Could have **positive** or **negative** polarity.

+VE POLARITY RNA = **Direct** protein synthesis (has mRNA → Ribosome at cytoplasm)

-VE POLARITY = **indirect** synthesis (go to host nucleus for protein synthesis)

Note: all viruses are haploid **except retroviruses are diploid**. Example, HIV

2-capsid

(Symmetry based on arrangement of capsomeres)

It's a protein shell enclosing the viral genome.
Capsids are build of a large Number of subunits called Capsomeres

Genome (NA)+capsid= nucleocapsid

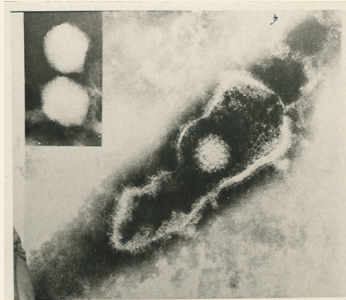
Function;
1-Protect Nucleic Acid
2-Facilitates its entry into cell

In Non-envelop viruses

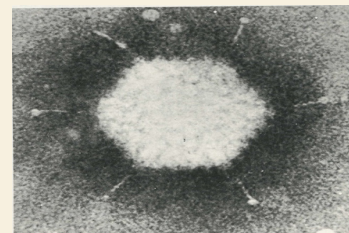
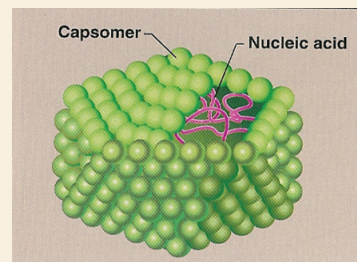
NA= nucleic acid

Capsomeres = subunits of capsid

Cubic symmetry (Icosahedral)



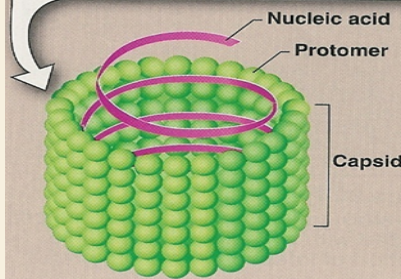
Herpes virus envelope



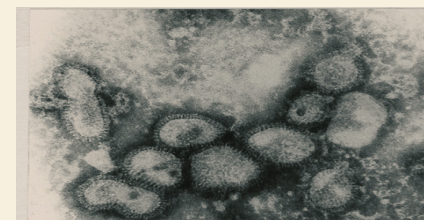
Adenovirus no envelop

Helical

Several rows of protomers have been removed to reveal nucleic acid surrounded by a hollow protein cylinder.

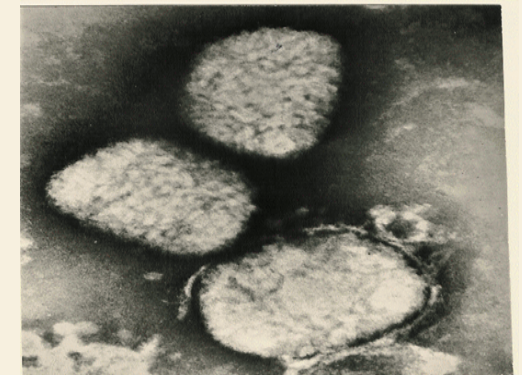


Elongated (filoviruses)



Pleomorphic (influenza v.)

Complex



Poxviruses

3-Viral structure

* It is **Lipoprotein membrane**
(**host lipid** + **virus specific protein**)

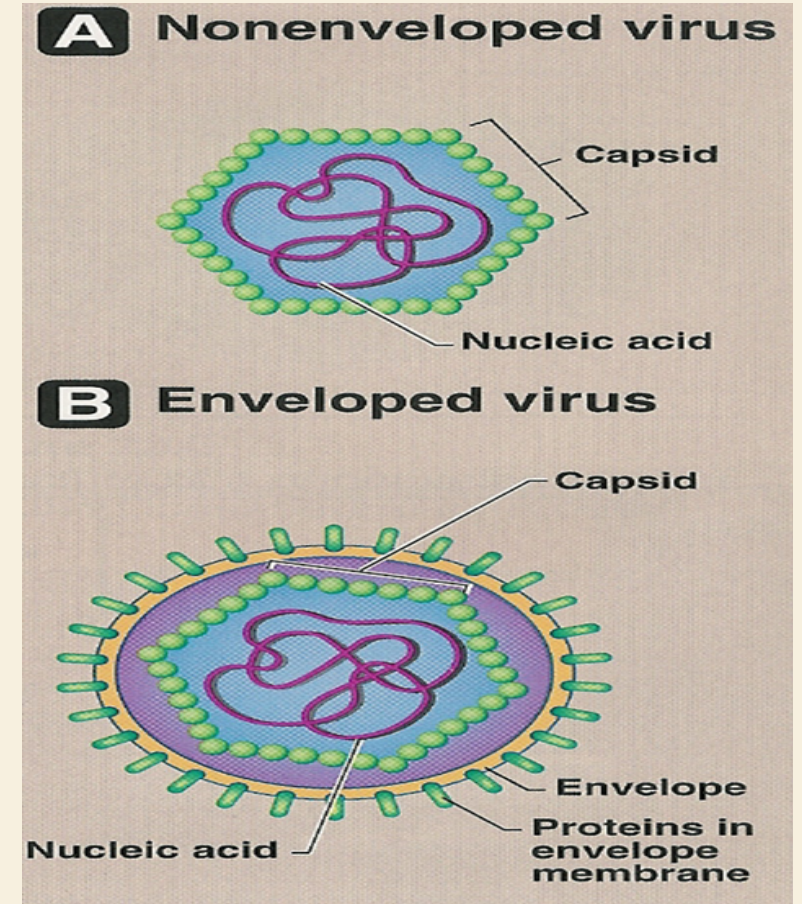
It also contain protein and glycoprotein

-**During viral budding :**

Envelope is derived from cell membrane
except herpesviruses from nuclear mb

-**Enveloped** Viruses are **more sensitive** to
heat, dry & other factors **than nonenveloped Viruses**

-Glycoprotein attaches to host cell receptor



Viral proteins

The outer viral proteins:

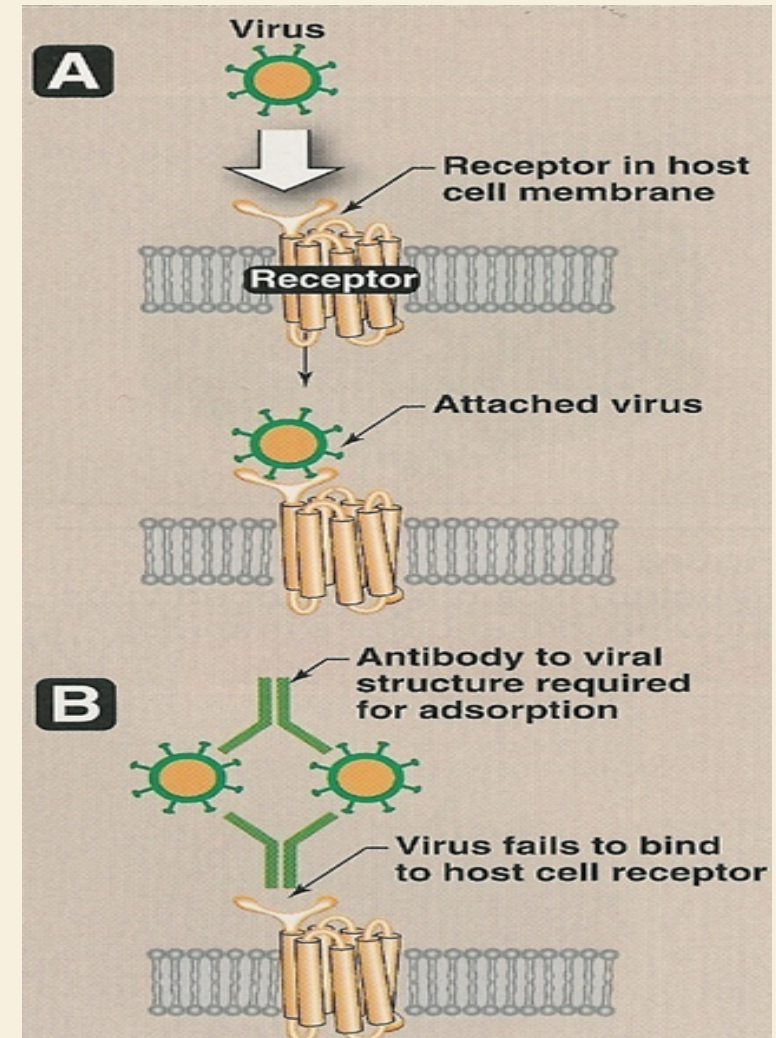
- Mediate **attachment to specific** Receptors
- Induce **neutralizing antibodies**
- Target of antibodies

The internal viral proteins:.

- Structural (capsid proteins of enveloped viruses)
- Nonstructural proteins(enzymes):

***All ssRNA viruses (-) polarity have transcriptase** (RNA dependent RNA polymerase) inside virions.

***Except RetroViruses & HBV** contain **reverse transcriptase.**



Classification of viruses

- ▣ **Type of Nucleic Acid** (DNA or RNA)
- ▣ The **number of strand**: Double **VS.** single-stranded
- ▣ The **polarity** of viral genome: Positive (+) or negative (-) stranded **RNA**
- ▣ The presence or absence of **envelope**
- ▣ **Type of symmetry**

Medically Important Viruses

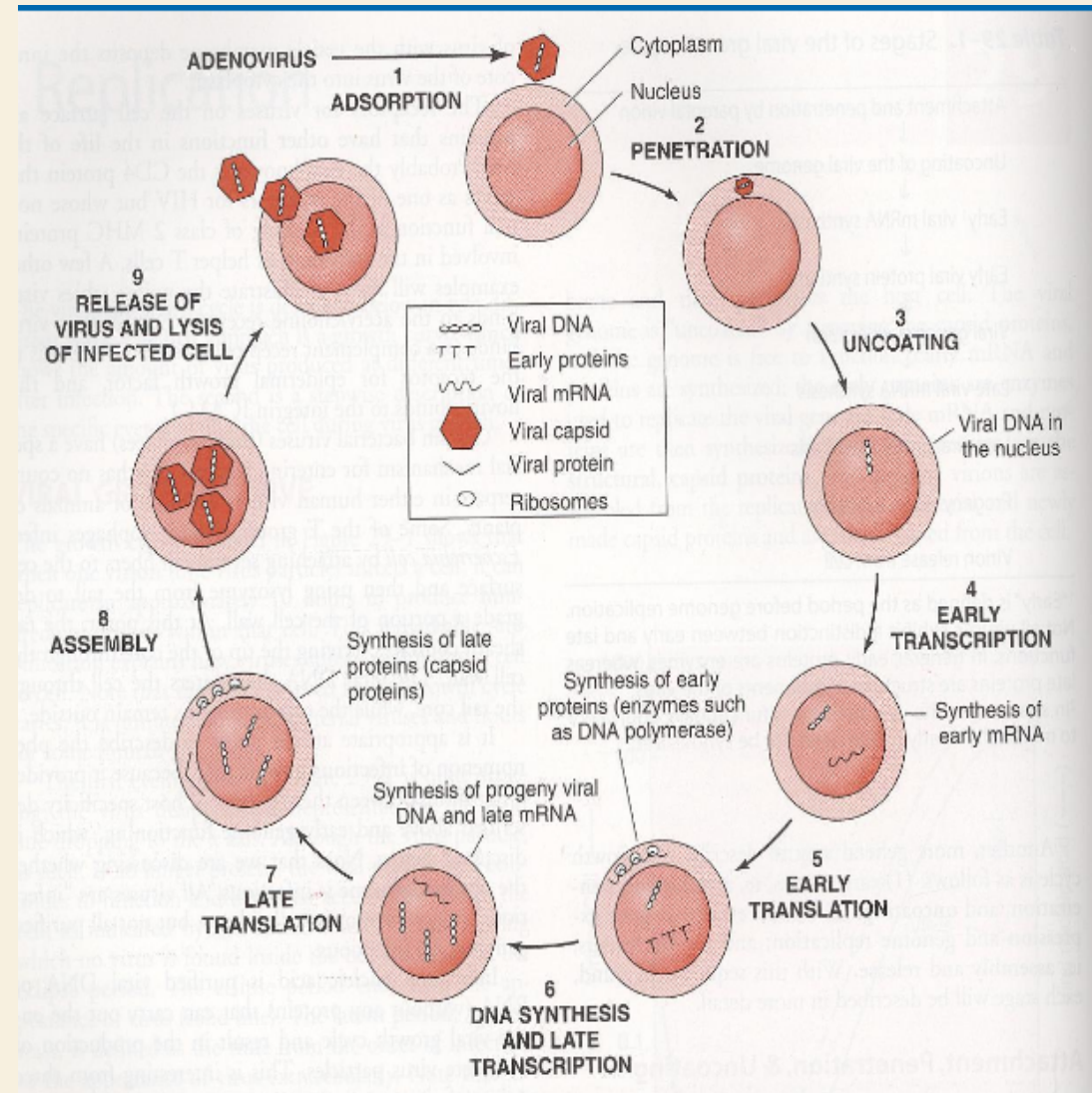
1- DNA			
Single Stranded	Double Stranded		
Non- Enveloped	Enveloped		Non- Enveloped
<u>Icosahedral</u>	<u>Complex</u>	<u>Icosahedral</u>	<u>Icosahedral</u>
Parvoviridae	Poxviridae	Herpesviridae	Adenoviridae

2- RNA			
Single Stranded			Double Stranded
Neg-Strand	Pos-Strand		Non-Enveloped
Enveloped	Enveloped	Non-Enveloped	<u>Icosahedral</u>
<u>Helical</u> Filoviridae	<u>Helical</u> Coronaviridae	<u>Icosahedral</u> Hepeviridae	Reoviridae
	<u>Icosahedral</u> Retroviridae		

Not important
BUT useful to
remember

Replication

- Adsorption
- Penetration
- Uncoating
- Synthesis of viral components
 - a) mRNA
 - b) Viral Proteins
 - c) Nucleic Acid (اختصارها NA)
- Assembly
- Release



1- Adsorption

- Glycoprotein (if enveloped virus)
- Folding proteins in capsid (if non-enveloped virus)

2- Penetration

- Fusion التحام: of host cell membrane and viral envelope (if enveloped virus)
- Endocytosis: Virus is swallowed by the cell and becomes in an Endocytotic Vesicle (both)
note: the virus tricks to the cell into thinking it is not harmful, so the cell swallows it (endocytosis)

Q- How does the virus get out of endocytotic vesicle?

A- Enveloped: Fuse with endocytotic vesicle membrane.

Non enveloped: Lyse تحلل the endocytotic vesicle membrane.

3- Uncoating

Release of genome in :

- Cytoplasm of host cell الخلية المتعرضة للهجوم (Direct synthesis of proteins = +ve RNA polarity)
- Nucleus of host cell (Indirect synthesis of proteins = -ve RNA polarity)

4- Synthesis of viral components

To make the viral proteins it needs +mRNA (قابل للترجمة)

<https://www.youtube.com/watch?v=ZGE4BLuAkuU&feature=youtu.be> للشرح

A) mRNA

If **-RNA virus** : viral genome transcription +mRNA (translatable)



If **+RNA virus** : no need for step (already have mRNA).

B) Viral proteins

+mRNA translation in host cell's ribosomes Viral Proteins



Directly

- enzymes - structural proteins

C) Viral Nucleic Acid

- The virus will replicate and synthesize its nucleic acids using the host cell's resources مواردها
- One virus can produce millions of viruses by continuation of this process (synthesis of viral proteins)

REPLICATION

- **Assembly:**
- The viral Proteins + Nucleic acid (DNA or RNA) assemble together = **virion***.
- Then the virion is released.

*What is a **virion**?

A virion is a complete virus particles, consisting of RNA or DNA surrounded by a protein shell.

Basically it is the infective form of a virus.

RELEASE

- It's the last step of the replication cycle of the viruses.

- **Enveloped viruses:**

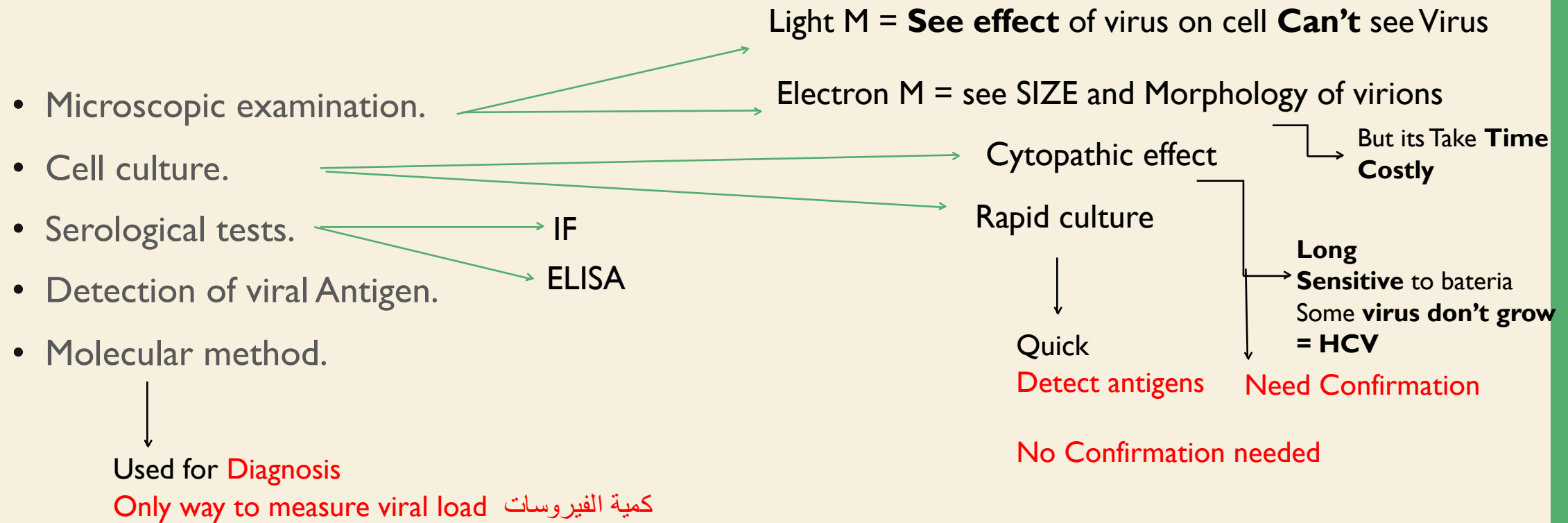
The **Virion** leaves the Cell and **creates its Envelope** by **budding** through the **host's cell membrane**.

Except Herpes V = budding host's nuclear membrane

- **Non-Enveloped viruses:**

Cell **lyses** or **rupture**

LABORATORY DIAGNOSIS OF VIRAL INFECTIONS:



MICROSCOPIC EXAMINATION:

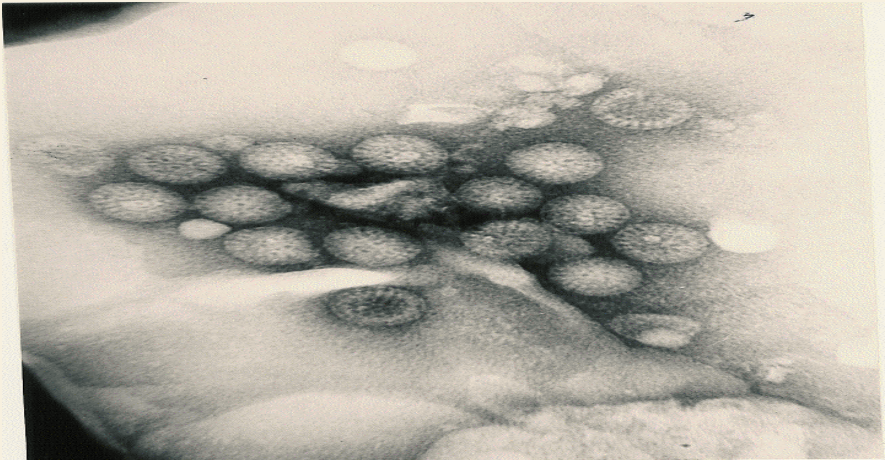
- Light microscopy:
 - Histological appearance “to see what the virus has done to the cell, without seeing the virus itself”

Ex. “Inclusion bodies”

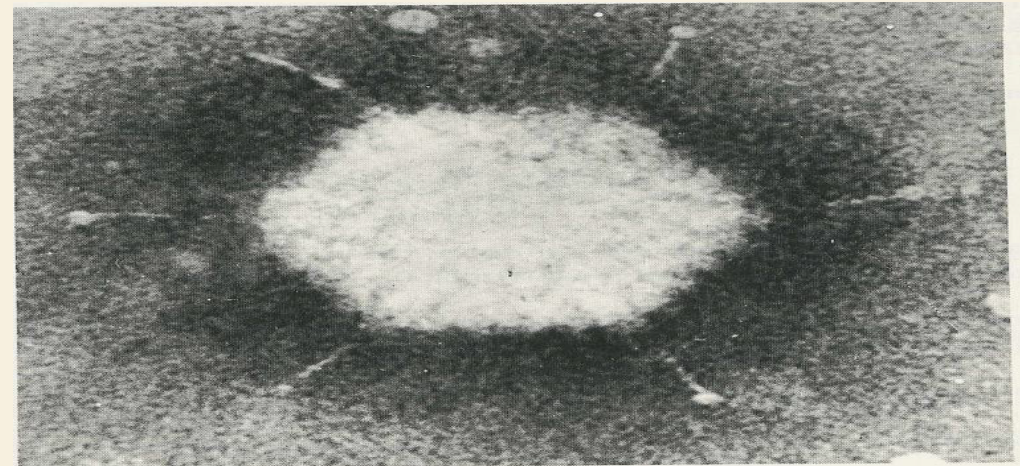
- Electron microscopy:
 - Morphology & size of virions . “ to see the virus particle itself”
 - Ex. Diagnosis of viral gastroenteritis such as Rota, adenoviruses. Diagnosis of skin lesion caused by herpes, or poxviruses.
 - It is replaced by Antigen detection & molecular tests

ELECTRON MICROGRAPHS:

Rotavirus



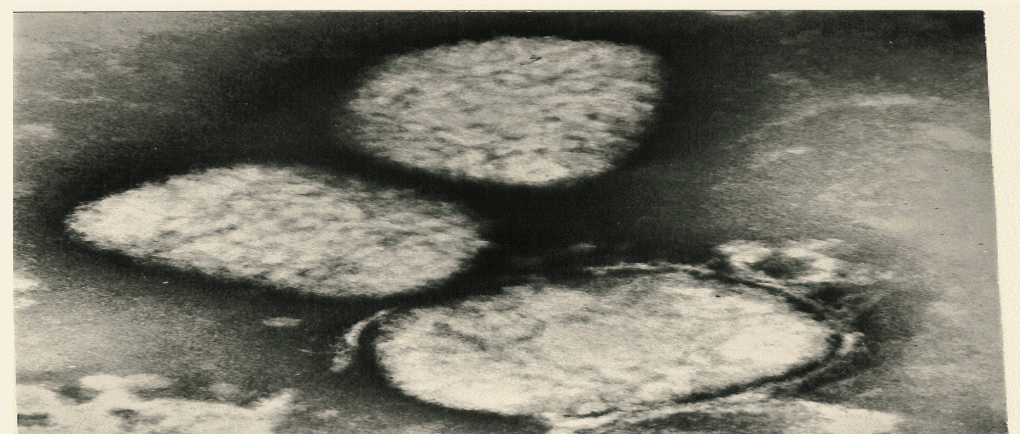
Adenovirus



Herpes virus



Poxvirus



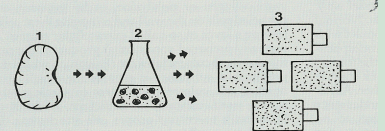
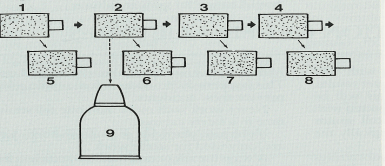
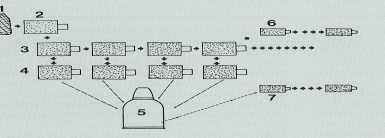
VIRUS CULTIVATION

- *Laboratory animals*
- *Embryonated eggs*
- *Cell culture*

CELL CULTURE

• هو تأثير الفيروس على الخلايا خارج بيئتها لتشخيص العدوى الفيروسية

- 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	What kind of viruses their are	picture
Primary cell culture	1 or 2(it lasts for very short period)	Usually RNA	
Diploid cell culture (semi – continuous)	20-50(it lasts for few weeks)	Usually DNA	
Continuous cell line	Indefinite		

Note : there are Variation of Sensitivity of cell cultures to infection by viruses

يعني الخلايا اللي في العبوات تختلف حساسيتها تجاه الفيروس ، وهذا الشيء اللي يحدد كونها

Primary or diploid or continues

DETECTION OF VIRAL GROWTH

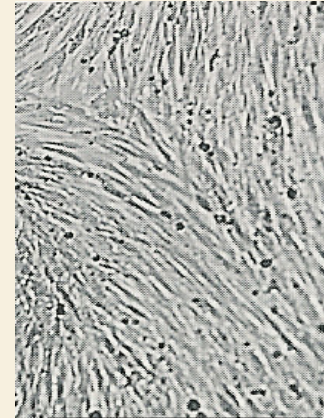
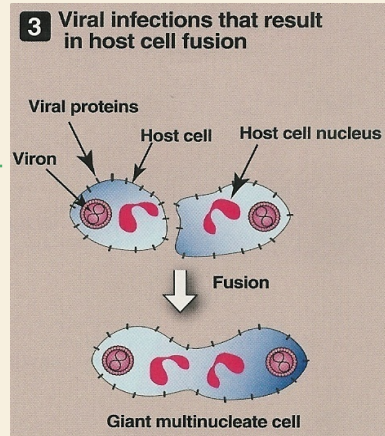
• كيف يمدينا نكتشف النمو الفيروسي ووش العلامات اللي تبان على الخلية من خلال العبوة اللي استخدمناها في cell culture

- **I- By the cytopathic effects**

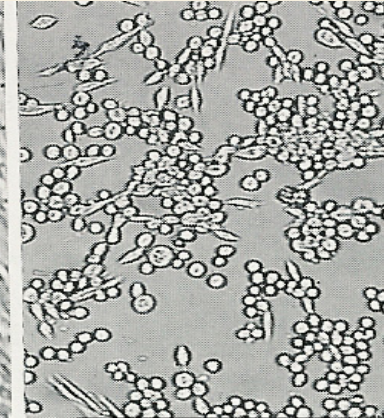
The affected cell will have “Rounding, shrinkage, aggregation, Syncytium(giant multinucleate cell) and lose of adherence.

- **by IF (Immunofluorescence)**

- **Other**



Uninfected



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 (عرضه للتلوث البكتيري)
- Some viruses do not grow in cell culture e.g. HCV

Rapid culture technique :

- **Shell vial assay**

It detect **viral antigens** within 1-3 days

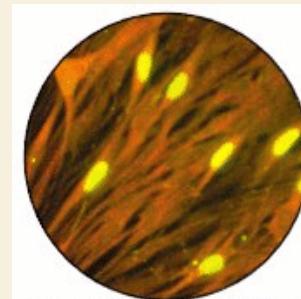
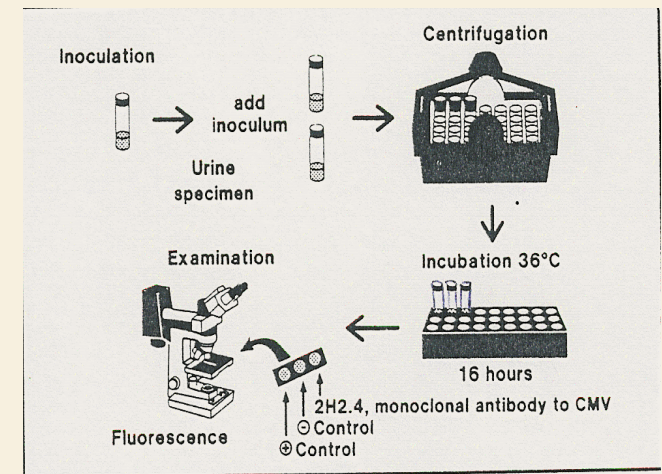


Fig. 2, CMV centrifugation culture fixed and stained 16 hrs after inoculation showing viral proteins in nuclei of infected human fibroblast cells

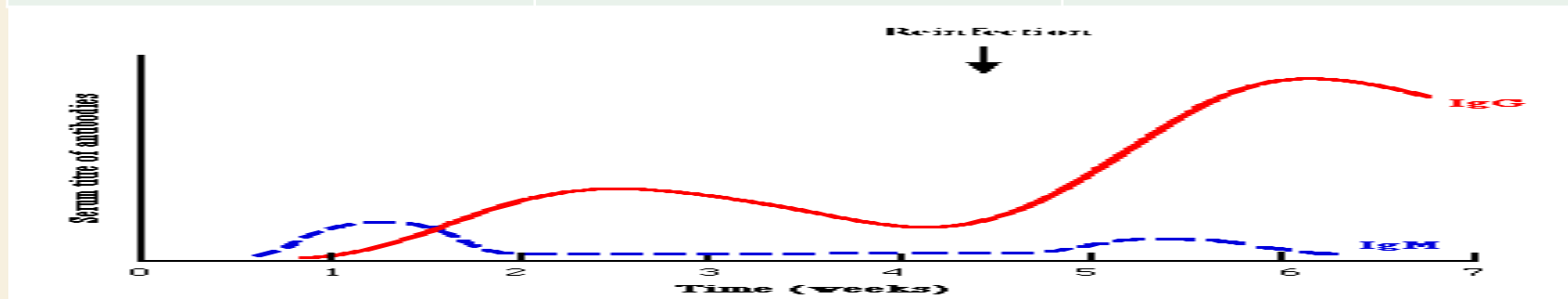


SEROLOGICAL TEST ANTIGEN DETECTION

- It is a test that looks for a (**anti bodies**) from a sample to determine the availability of (**antigen**)
- There are three techniques :
 - complement fixation test (CFT)
 - Immunofluorescence (IF)
 - enzyme-linked immunosorbent assay (ELISA)

ملاحظة: الطرق أحيانا تكون مباشرة وغير مباشرة ، اذا كانت غير مباشرة فهي عن طريق الأنتي بديز واذا مباشرة على طول تكون عن طريق الانتي جين

The sample	The test	The virus
Nasopharyngeal aspirate	Influenza V	IF
Skin scrapings	HSV	IF
Faeces	Rotavirus	ELISA
Blood	HBV(HBsAg)	ELISA



Serological tests

- Antibody detection
- Some techniques :
 - Complement fixation test (CFT)
 - Immunofluorescence (IF)
 - Enzyme-linked immunosorbent assay (ELISA)

Molecular Tests :

Polymerase chain reaction (PCR)

- NA amplification technique.
- Viral genome

Uses;

- Diagnosis**
- Only way to measure viral load** and monitor response to treatment

Immunofluorescence (IF):

using a *fluorescent dye*
And can be viewed by **fluorescence microscope** .

- Direct Ag detection; Sample (Ag)
- Indirect Ab detection; Sample (Ab)

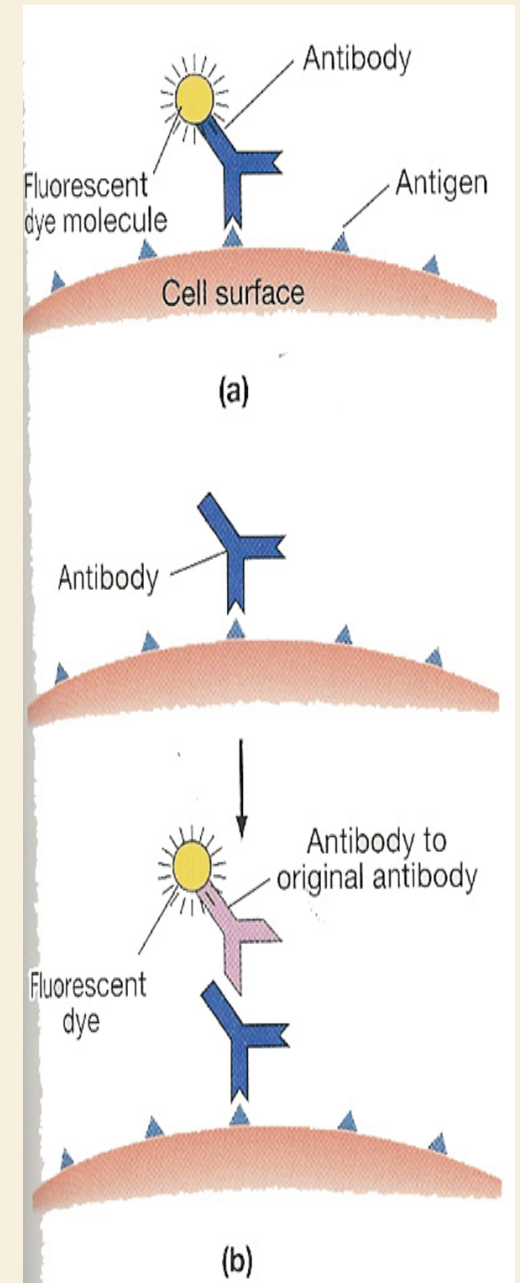
Enzyme-linked immunosorbent assay (ELISA)

Sample is then viewed using spectrometer.

-**Indirect** ELISA for Ab detection ;

coloured wells indicate reactivity

-**Direct** ELISA for Ag detection or specific IgM, IgG :



QUESTION 1

- **Which of the following has DNA or RNA ?**
- Parasite
- Fungi
- Bacteria
- virus

QUESTION 2

- **Which of the following replicate by budding or mitosis?**

- Parasite
- Fungi
- Bacteria
- virus

QUESTION 3

- **What is the range of size of a virus?**

- 200-300 nm
- 20-300 nm
- 2-300 nm
- 20-23 nm

QUESTION 4

- **All viruses are except retroviruses are**

- Deploid ,haploid

- Haploid ,deploid

QUESTION 5

- **Which of the following is helical symmetry**

- Proxvirus
- Filoviruses
- Adenivirus
- Herpes virus

QUESTION 6

- **All of the following can be used to classify viruses except:**
- Type of symmetry
- Number of strands
- The average count of ribosomes
- The polarity

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