Biology department
Fourth stage
Raghad Ibrahim
2016-2017

Practical Virology

Lab(1) Laboratory Rules:

1- You must always wear medical cloves, mask and coat.
2- Don't allow to smoke, drink and eat inside the virology lab.
3- Wash your hands before and after working inside the lab.
4- Keep your hands away from nose, eyes and mouth.
5- Keep your bench clean and tidy. Disinfect the bench before and after working.
6- Don't leave any contaminated objects around.
7- All glass wear should be sterilize by autoclave or by oven before and after working.
8- Mouth pipetting is so dangerous, so you should use a balloon with the pipette.
9- All the virological tests should be done inside the safety cabinet.
10- When you deal with animals , you should be very careful and try to reduce the noise because a lot of noise may be distrube the animal and make it nervous or tension.
11- When you deal with antisera, you should be very careful specially with those samples of highly infectious (HIV or HBV) because it consider as a source of infection.

☐ *General Properties of Viruses*

↳ *Introduction to Viruses*

Viruses are the smallest infectious agents (20 -300 nm in diameter), containing only one kind of nucleic acid (RNA or DNA) as their genome. The nucleic acid is encased in a protein shell, which may be surrounded by a lipid – containing membrane. The entire infectious unit is termed a virion. Viruses are inert in the extra-cellular environment. They replicate only in living cells, being parasites at the genetic level.

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Definition in Virology

Capsid: *The protein shell or coat that encloses the nucleic acid genome. Empty capsids may be byproducts of the replicative cycle of viruses with icosahedral symmetry.*

Nucleocapsid: *The Capsid together with the enclosed nucleic acid.*

*Structural units**:** The basic protein building blocks of the coat. They are usually a collection of more than one non identical polypeptide.*

Capsomers: *Morphological units seen in the electron microscope on the surface of icosahedral viral particles. Capsomers represent clusters of polypeptides, but the morphological units do not necessarily correspond to the chemically defined structural units.*

Envelope: *A lipid – containing membranes that surrounds some viral particles. It is acquired during viral maturation by a budding process through a cellular membrane. Virus – encoded glycoproteins are exposed on the surface of the envelope.*

Virion: *The complete viral particle, which in some instances (adenoviruses, papovaviruses, picornaviruses) may be identical with the Nucleocapsid. In more complex virions (herpesviruses, orthomyxoviruses), this includes the nucleocapsid plus a surrounding envelope. This structure (the virion) serves to transfer the viral nucleic acid from one cell to another* of viruses

Comparison between viruses and bacteria

No.	Property	Viruses	Bacteria
1	Size	20-300 nm	1000 nm
2	Genome (type of nucleic acid)	DNA or RNA but not both	DNA and RNA
3	Cell wall	Envelope present in some viruses	Cell wall
4	Ribosomes	No ribosomes	Ribosomes
5	Multiplication by binary fission	_	+
6	Sensitivity to antibiotics	_	+
7	Growth in culture media	Grow only in living host cell	Grow in culture media

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The structure of viruses:

1. Viral nucleic acid:

The viral nucleic acid is located internally and can be either single- or double- stranded RNA or DNA. The nucleic acid can be either linear or circular. The DNA is always a single molecule, the RNA can exist either as a single molecule or in several pieces (segmented).

- Some RNA viruses are positive polarity and others are negative polarity.
- Positive polarity is defined as an RNA with same base sequence as the mRNA.
- Negative polarity has a base sequence that is complementary to the mRNA.

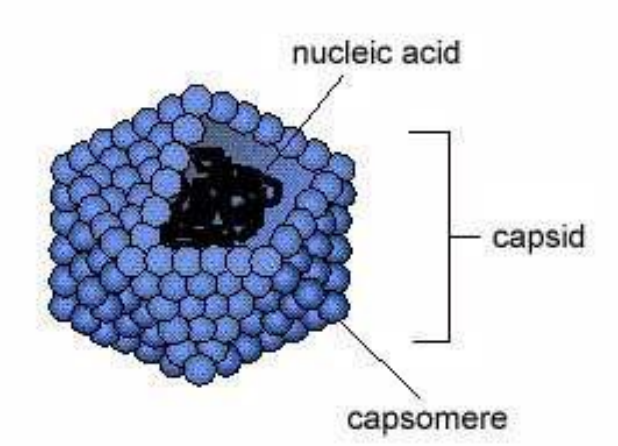
2. Capsid:

The protein shell, or coat, that encloses the nucleic acid genome and mediates the attachment of the virus to specific receptors on the host cell surface.

3. Capsomeres:

Morphologic units seen in electron microscope. Each capsomere, consisting of one or several proteins.

Naked viruses are composed of nucleic acid + capsid (nucleocapsid)



4. Viral envelope :

The envelope is a lipoprotein membrane composed of lipid derived from the host cell membrane and protein that is virus- specific.

Practical Virology

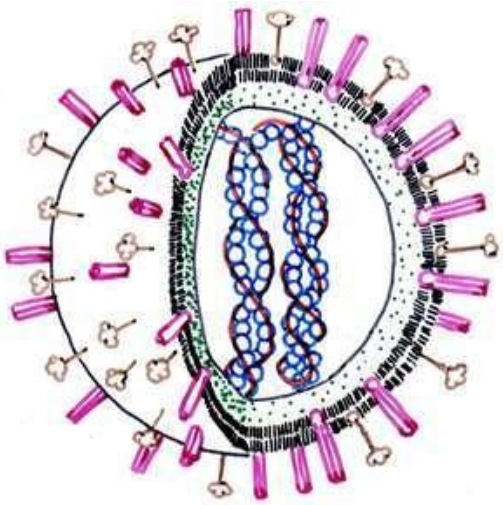
Furthermore, there are frequently **glycoproteins** in form of spike-like projections on the surface, which attach to host cell receptors.

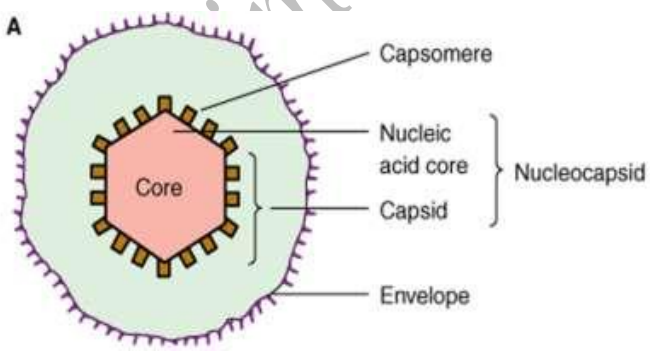
Matrix protein mediates the interaction between the capsid proteins and envelope.

The presence of an envelope confers instability on the virus.

Enveloped viruses ⟶ NA + capsid + envelope

The whole virus particle is called **virion**.



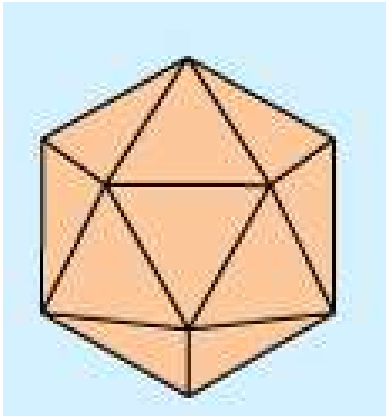


Types of symmetry of virus particles:

1. **Icosahedral symmetry**

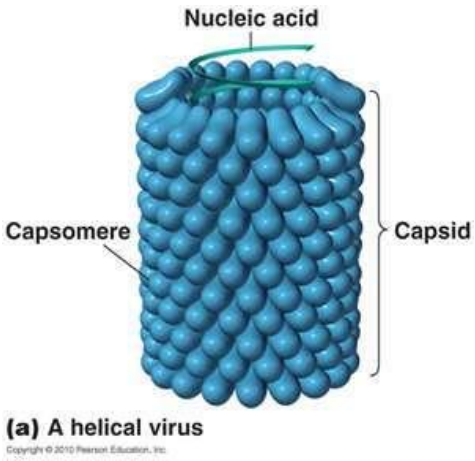
Composed of 12 vertices, has 20 faces (each an equilateral triangle) with the approximate outline of a sphere.

e.g. Herpesviruses , Adenoviruses



2. **Helical symmetry**

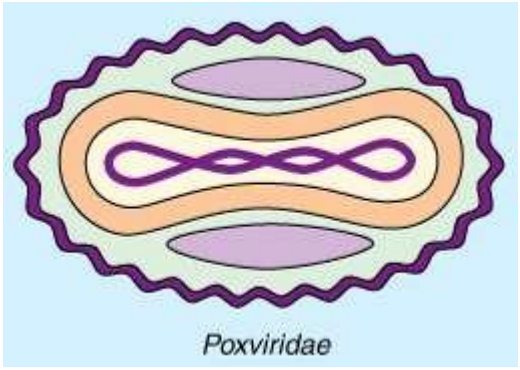
In which the capsomeres are arranged in a hollow coil that appears rod- shaped. The helix can be either rigid or flexible.



e.g. Influenza viruses

3. **Complex structures**

e.g. Poxviruses



CLASSIFICATION OF VIRUSES

◈ Basis of Classification

The following properties listed in order of importance, have been used as a basis for the classification of viruses. The amount of information available in each category is not uniform for all viruses. For some agents, knowledge is available for only a few of the properties listed **:-**

1. Nucleic acid type RNA or DNA**:** single – stranded or double – stranded; strategy of replication.
2. Size & morphology, including type of symmetry, number of Capsomers, and presence or absence of membranes.
3. Susceptibility to physical and chemical agents, especially ether.
4. Presence of specific enzymes, particularly RNA and DNA polymerases concerned with genome replication, and neuraminidase necessary for release of certain viral particles (influenza) from the cells in which they were formed.
5. Immunologic properties.
6. Natural methods of transmission.
7. Host, tissue, and cell tropisms.
8. Pathology; inclusion body formation.
9. Symptomatology.

◈ Classification by Symptomatology

A) Generalized Diseases.

B) Diseases Primarily Affecting Specific Organs.

1. Diseases of the nervous system – polio myelitis
2. Diseases of the respiratory tract – Influenza, para influenza.
3. Localized diseases of the skin or mucous membranes – Herpes simplex type 1.
4. Diseases of the eye**:** Adenovirus (conjunctivitis), herpes (krato conjunctivitis).

5. Diseases of the liver – Hepatitis type A (infectious hepatitis)
6. Diseases of the salivary glands – Mumps and cytomegalovirus
7. Diseases of the gastrointestinal tract – Rotavirus.

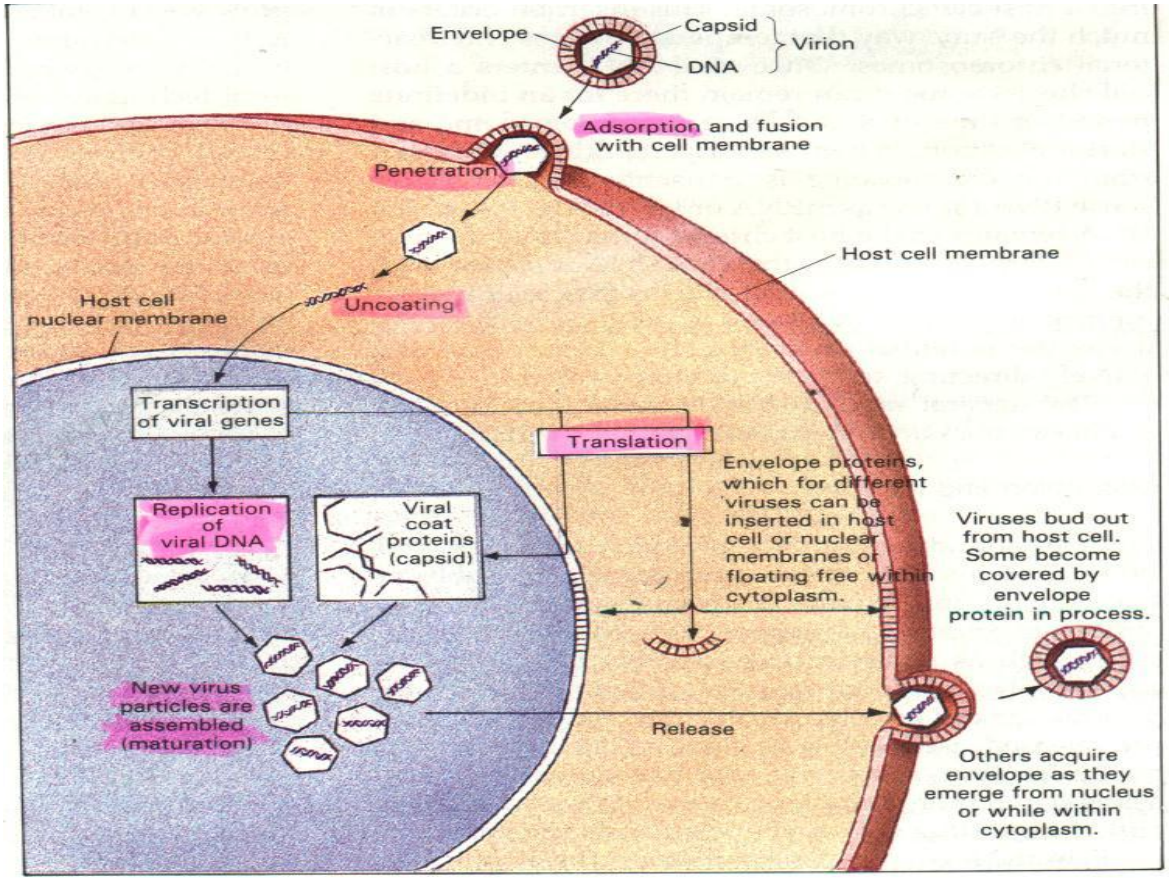
8. Sexually transmitted diseases – Herpes simplex virus.

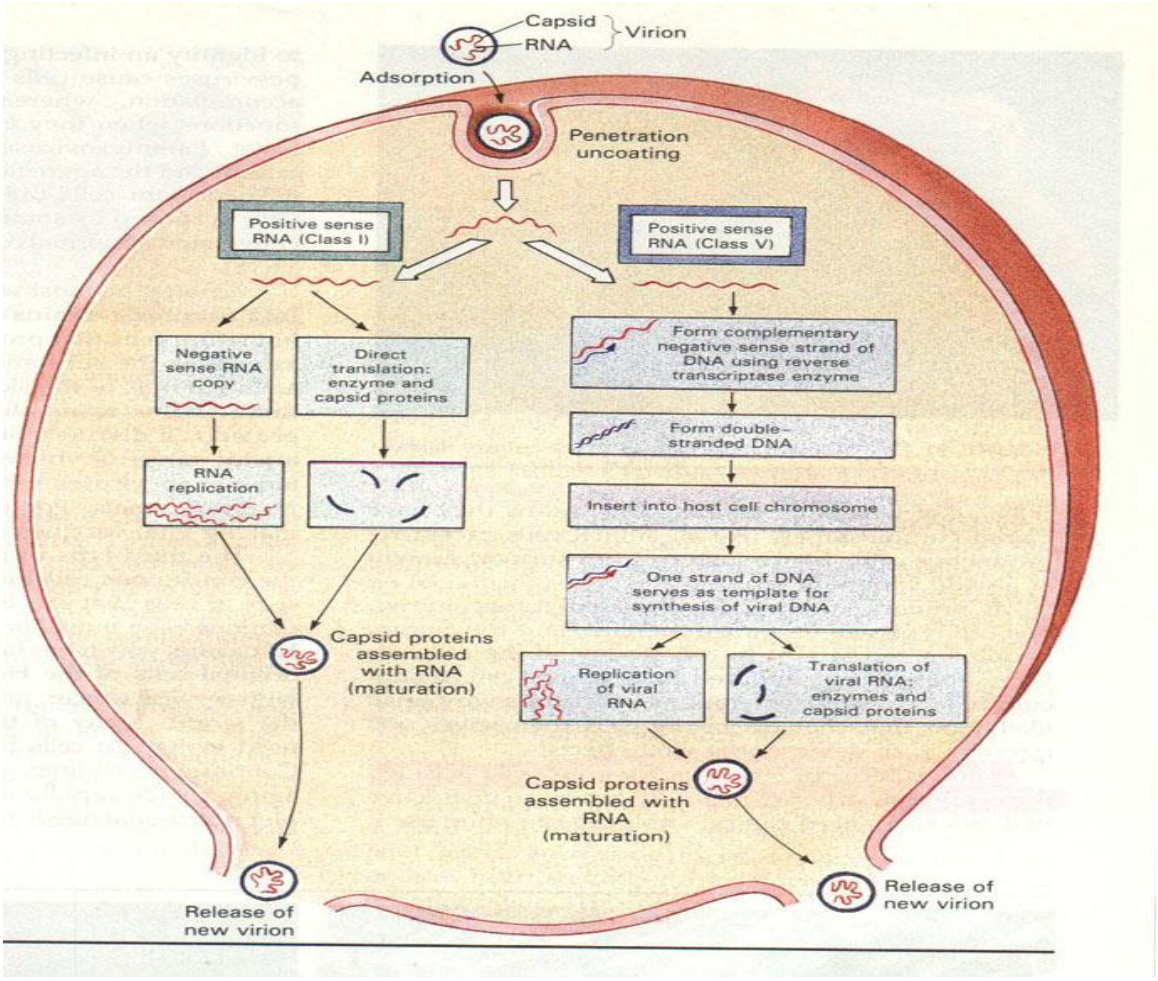
Nucleic Acid Core	Capsid Symmetry	Virion: Enveloped or Naked	Ether Sensitivity	Number of Capsomeres	Virus Particle Size (nm)[a]	Size of Nucleic Acid in Virion (kb/kbp)	Physical Type of Nucleic Acid[b]	Virus Family
DNA	Icosahedral	Naked	Resistant	32	18–26	5.6	ss	Parvoviridae
				72	45	5	ds circular	Polyomaviridae
				72	55	8	ds circular	Papillomaviridae
				252	70–90	26–45	ds	Adenoviridae
		Enveloped	Sensitive	180	40–48	3.2	ds circular[c]	Hepadnaviridae
				162	150–200	125–240	ds	Herpesviridae
	Complex	Complex coats	Resistant[d]		230 × 400	130–375	ds	Poxviridae
RNA	Icosahedral	Naked	Resistant	32	28–30	7.2–8.4	ss	Picornaviridae
					28–30	6.4–7.4	ss	Astroviridae
				32	27–40	7.4–8.3	ss	Caliciviridae
					27–34	7.2	ss	Hepeviridae
					60–80	16–27	ds segmented	Reoviridae
		Enveloped	Sensitive	42	50–70	9.7–11.8	ss	Togaviridae
	Unknown or complex	Enveloped	Sensitive		40–60	9.5–12.5	ss	Flaviviridae
					50–300	10–14	ss segmented	Arenaviridae
					120–160	27–32	ss	Coronaviridae
					80–110	7–11[e]	ss diploid	Retroviridae
	Helical	Enveloped	Sensitive		80–120	10–13.6	ss segmented	Orthomyxoviridae
					80–120	11–21	ss segmented	Bunyaviridae
					80–125	8.5–10.5	ss	Bornaviridae
					75 × 180	13–16	ss	Rhabdoviridae
					150–300	16–20	ss	Paramyxoviridae
					80 × 1000[f]	19.1	ss	Filoviridae

Growth cycle of viruses

Take place in 7 stages as following**:**

(Attachment ,pentration ,and uncaotingTranscrption, synthesis of viral components, Assembly, Release)


Envelope
Capsid
DNA
Virion
Adsorption and fusion with cell membrane
Penetration
Host cell membrane
Host cell nuclear membrane
Uncoating
Transcription of viral genes
Translation
Envelope proteins, which for different viruses can be inserted in host cell or nuclear membranes or floating free within cytoplasm.
Viruses bud out from host cell. Some become covered by envelope protein in process.
Replication of viral DNA
Viral coat proteins (capsid)
New virus particles are assembled (maturation)
Release
Others acquire envelope as they emerge from nucleus or while within cytoplasm.


Capsid
RNA
Virion
Adsorption
Penetration uncoating
Positive sense RNA (Class I)
Positive sense RNA (Class V)
Negative sense RNA copy
Direct translation: enzyme and capsid proteins
Form complementary negative sense strand of DNA using reverse transcriptase enzyme
Form double-stranded DNA
RNA replication
Insert into host cell chromosome
One strand of DNA serves as template for synthesis of viral DNA
Capsid proteins assembled with RNA (maturation)
Replication of viral RNA
Translation of viral RNA: enzymes and capsid proteins
Capsid proteins assembled with RNA (maturation)
Release of new virion
Release of new virion

Lab 2 Collection of samples

1. Sampling

The aim of the examination (i.e. diagnosis) determines the type of sample,

Type of sample

- 1- **Swabs**: from Throat, Nasal, Eyes, Swabs used for respiration infection (Influenza virus), the swab stick is broken off aseptically in to the fluids and the cap is tightly closed.
- 2- **Nasopharyngeal aspirate**specially in children, and **Mucus sucked** by using Vacuum Pump.
- 3- **Stool samples**: 1-4 gm of feces must be taken for Rota virus, Coxsackie virus, Adeno virus, Reo virus.
- 4- **Urine samples**: For cytomegalovirus, Mumps, Measles, Papova viruses.
- 5- **Blood samples**: withdrawn aseptically by vein puncture in to a vacutainer contain heparin for HIV, Arbo virus, Rota virus from Leukocyte.
- 6- **Skin scraping** in case of vesicles or other skin lesions for small pox, Herpes virus.
- 7- **Biopsy and autopsy** may be taken by needles or knife (must not placed in formalin or any fixative)
- 8- **C.S.F**. in case of meningitis for Arbo virus, Entero virus, Mumps
- 9- **Saliva**: For Rabies infection.

2.Specimens preparations:

1- **Transported of swabs for virology diagnosis**:

Sufficient amount of materials collected from lesion can be done by swabbing. For example; when samples taken from skin, Always scrape the

epithelium because the viruses are intracellular parasites, don't pick up just mucus or secretions. If blisters were founded, open these blisters and pick up the materials from deep lesion, then put the swab in the Glycerol media and send it to the lab.

2- **Stock of Glycerol medium**:

Glycerol medium is an isotonic buffer solution contain minerals, albumin, gelatin, yeast extract and antibiotics. This medium is useful for : a- prevent drying out of the samples. b- inhibition of bacterial growth c- diluting antibodies which may come with the samples.

3- **Making a slide preparations**:

Slide preparation made in case of direct diagnosis by staining the slide using of immunoflorescent stain, after rolling the swab over the slide, staining, examing under immunoflorescence microscope to show the reaction that occur between viral Ags and Abs.

4- **Making a fecal suspension**:

A 20% of fecal suspension prepared by collect about 1gm of feces + 4ml of saline put in special screw capped bottles. Shake the bottle by vortex and Then centrifuged 3500 rpm to obtain clear samples.

5- **Urine samples**:

Urine samples are collected as a (mid stream method). After washing the genitalia, leave the first and last drops of urine and collection done from mid stream by sterile container. Some time urine samples buffered by adding buffer solution.

6- **Blood samples**:

Fine system for blood sample collection is by the use of vaccum system, in which the blood sample is drown in to an evacuated tube. Blood must be always considers as a contagious material.

7- **Tissue culture inoculation**:

Materials from patients are inoculated in to the tissue cultures to assay the pathogenic effect that caused by the virus infection. Safety cabinet should be used to avoid the contamination of the tissues culture and prevent lab. Acquired infection.

8- **Sterilizing glass pipette**:

All the virology objects should sterilizing by heating sterilization methods (oven or autoclave) . Stainless steel boxes or glass tubes can be used as a container. For individual pipette, paper-wrapped and then sterilized by dry heat or plastic wrapped by gas sterilization.

There are different method use to cover the opening:

- **Aluminum foil caps** : by using a folded of aluminum foil to close the opening of the glass ware and to prevent entry and exit of the air.
- **Cotton, wool plugs and papers**: the plug wrapped with paper to avoid contaminated of cotton wool plug with dust and sterilization for a long time.

Figure 2.3 *Cell culture flasks, dishes and plates.* Photographs of TPP cell culture products courtesy of MIDSCI.

Figure 2.4 *Cell culture work.* Precautions to avoid contamination include working in a sterile cabinet and wearing gloves and mask. Photograph courtesy of Novartis Vaccines.

Reaction to physical and chemical agents:

1. **Heat and cold**

Viral infectivity is generally destroyed by heating at 50-60 C^0 for 30 mint., hours at 20 C^0, days at 4 C^0. Viruses can be preserved at -90 C^0 or -196 C^0 (liquid nitrogens).

2. **PH**

Viruses can be preserved at physiological PH (7.3).

3. **Ether susceptibility :**

   Ether susceptibility can be used to distinguish viruses that possess an envelope from those that do not.

4. **Detergents:**

   Nonionic detergents solubilize lipid constituents of viral membranes. The viral proteins in the envelope are released. Anionic detergents also solubilize viral envelopes; in addition, they disrupt capsids into separated polypeptides.

5. **Salts**

Many viruses can be stabilized by salt in concentrations of 1 mol/L. e.g. $MgCl_2$, $MgSO_4$, $Na2SO_4$.

6. **Radiation**

Ultraviolet, X-ray, and high-energy particles inactivate viruses.

7. **Formaldehyde**

Destroys viral infectivity by reacting with nucleic acid.

8. **Antibiotics**

Antibacterial antibiotics have no effect on viruses.

Measuring the sizes of viruses

The following methods are used for determining the size of viruses and their components:

A) Direct observation in the electron microscope

The electron microscope uses electron rather than light waves and electromagnetic lenses rather than glass lenses. The electron beam obtain has a much shorter wavelength than that of light, so that the objects much smaller than the wave length of visible or ultraviolet light can be visualized.

B) Filtration through membranes of Graded Porosity

The approximate size of any virus can be measured by determining which membranes allow the infective unit to pass and which hold it back. The size of the limiting APD (average pore diameter) multiplied by 0.64 yields the diameter of viral particle.

C) Sedimentation in the Ultracentrifuge

If particles are suspended in a liquid, they will settle to the bottom at a rate that is proportionate to their size. The relationship between the size and shape of a particle and its rate of sedimentation permits determination of particle size.

Lab 3 Cultivation of viruses(Propagation)

Many viruses can be grown in cell cultures or in fertile eggs under strictly controlled conditions. Growth of virus in animals is still used:

1. For the primary isolation of certain viruses.
2. For studies of the pathogenesis of viral diseases and of viral oncogenesis.

➔ **There are three basic types of cell culture** :

A) Primary cultures (ex:- monkey kidney &chick embryo)

Are made by dispersing cells (usually with trypsin), from freshly removed host tissue. In general they are unable to grow for more than a few passages in culture.

- Advantage :-
  1. The best experimental model for in vivo situation
  2. The may express the characteristic which are not seen in cultured cells
  3. Boarded range of viral susceptibility
- Disadvantage
  1. Latent viruses may be present especially in primary monkey kidney cell
  2. Un able to grow or many than few passage(once or twice)
  3. Some are very expensive and difficult to obtain a reliable supply

B) Secondary cultures(ex:-human embryonic kidney &HMC5(human lung fibroblast)

Diploid cell lines are secondary cultures which have undergone a change that allows their limited culture (up to 50 passages) but which retain their normal chromosome pattern.

- Advantage :-The cell widely used to study viruses in vitro and for vaccine production
- Disadvantage:-they don't divided indefinitely and eventually they physical characteistics may change after which the cell will eventually

C) Continuous cell lines

Are cultures capable of more prolonged, perhaps indefinite growth that has been derived from diploid cell lines or from malignant tissues. They invariably have altered and irregular numbers of chromosomes.

- Advantage : may be the most easy to handle
- Dis advantage :-the range of viruses supported limited.

NOTE: The type of cell culture used for viral cultivation depends on the sensitivity of the cell to a particular virus and found virus can detected by many mode.