VIROLOGY

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- We are interested in studying virology because a large percentage of viruses are pathogens that cause different diseases in humans.
- One of the defining features of viruses is that they **obligate intracellular parasites**. Viruses can't have metabolic functions outside of a host cell, therefore it can't replicate. Instead, they are metabolically inactive or considered as inert objects (جمادات).
- There is a wide variability in the host range for viruses. Viruses can infect any type of living cell (bacteria e.g., bacteriophages, archaea, parasites, plants, animals, humans)
- The first virus ever discovered was a plant virus called *tobacco mosaic* virus.

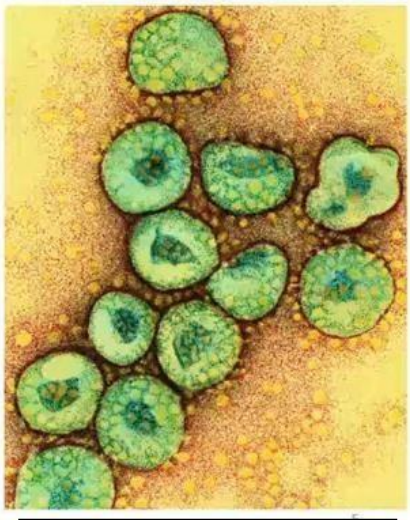
General properties of viruses:

1. **The smallest infectious agents.**
   There are infectious agents that are smaller than viruses but are inconspicuous (غير واضح) in terms of their features (prions and viroids) but considering the larger groupof pathogens including (parasites, fungi, bacteria, viruses), the smallest are viruses.
2. **They contain one kind of nucleic acid.**
   Either DNA or RNA never both. Unlike bacteria which has it's DNA genome associated with different types of RNA (rRNA, tRNA, mRNA, etc.) within the same bacterial cell as well as humans and other organisms.
3. **The entire infectious unit is termed a virion.**
   Virion: complete viral particle that can initiate an infection. Parts of the viral nucleic acids and protein may be found in a cell and not the structure (invader: complete viral particle) and such cell is not termed to have a virion infection but rather a viral/virus infection.
4. **The universe of viruses is rich in diversity.**
   Viruses exhibit diversity in terms of shape (20nm-300nm or more in diameter), genome (DNA, RNA, ss, ds), structure (enveloped, unenveloped).

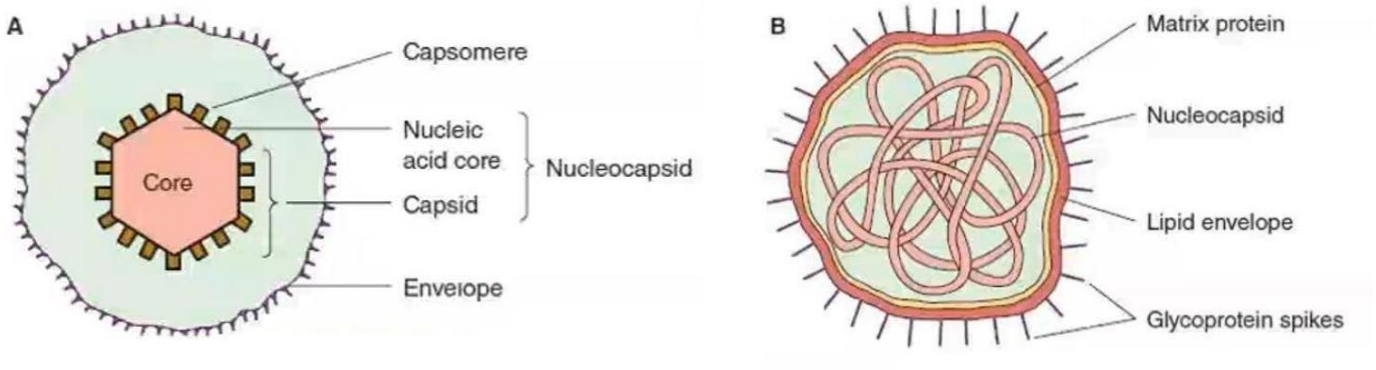
Terms and definitions in Virology:

- **Virion: The complete virus particle.**
- **Envelope: A lipid-containing membrane that surrounds some viruses.** (Not a constant common feature that is present in all viruses) The basic structure of a virus is a single type of nucleic acid surrounded by a protective protein coat. Any other feature may be present in some viruses and not all. Some viruses may have an envelope that is taken from the cellular membrane or the nuclear membrane (e.g., herpesvirus) or Golgi apparatus or the EndoplasmicReticulum.

- **Capsid: the protein coat that encloses the viral genome.** The word capsid comes from a ***F***rench word that means *wallet.* A capsid functions in enclosing the viral nucleic acid and protecting it as the nucleic acid of the virus (its genetic material) is necessary for the production of proteins and the replication of the virus, so it needs to be protected.
- **Capsomere: morphologic units seen in the electron microscope on the surface of icosahedral viruses.** Unlike bacteria, viruses can't be seen under a light microscope because they are very small (smallest infectious agents) and need an EM to be visualized. Electron micrograph examination of icosahedral viruses shows capsomeres.
- **Defective virus: a virus particle that is functionally deficient in some aspect of replication.** (can't replicate efficiently or can't replicate at all within its target cell on its own) In some cases, a defective virus enters the cell but is unable to create a viral progeny (produce other viruses within the cell). And in other cases, a defective virus may be able to replicate in the presence of a helper virus ***e.g., Hepatitis D*** is caused by the hepatitis delta virus (HDV, delta agent) one of the hepatitis viruses. Hepatitis D does not occur when delta agents are present in the body on their own because they're defective. Hepatitis D infects patients who are already infected with hepatitis B (have chronic infections) or if both viruses entered the body together. Hepatitis B virus functions as a helper virus for the defective delta agents. It provides for the deficiency delta agents have, which is ***hepatitis B surface antigen*** that will be incorporated in its envelope aidingthe delta agent to recognize its target cells and initiate infection.
- **Peplomers: virus-encoded glycoproteins that are projected from the envelope.**
- **Nucleocapsid: the protein-nucleic acid complex.** (Nucleic acid attached to capsid) The nucleocapsid is present in different types of viruses but is mostly evident (ظاهر) in helical viruses. In the process of creating helical viruses, the nucleic acid is incorporated in the capsid proteins forming the nucleocapsid.
- **Subunit: a <u>single</u> folded viral polypeptide chain.**
- **Structural units: the basic protein building blocks of the coat. They are usually a collection of <u>more than one</u> non-identical protein subunit. The structural unit is often referred to as a protomer.** (The simplest unit of structure to be studied and may contain <u>multiple</u> types of polypeptides)

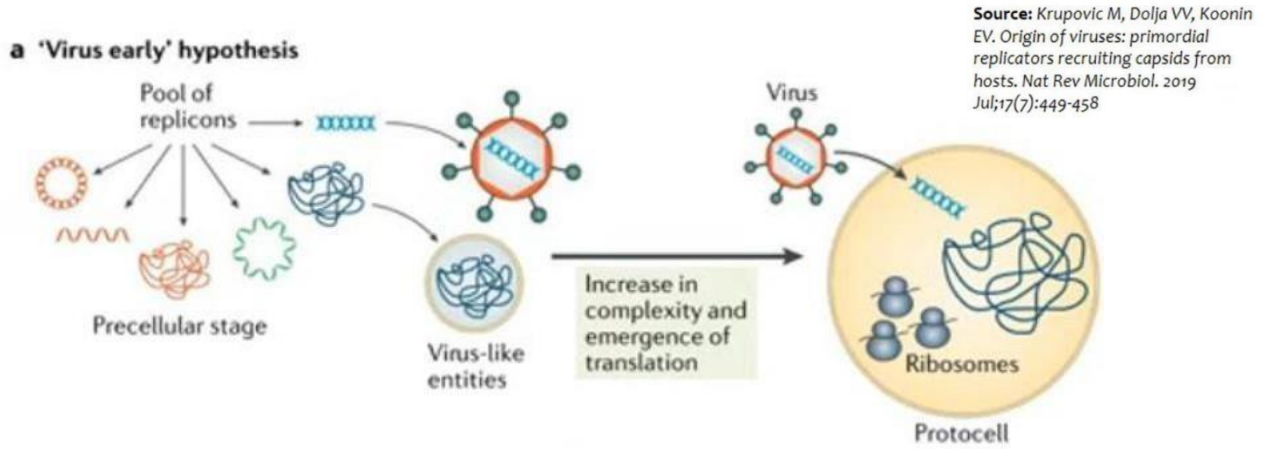


Parainfluenza virus
The yellow structures protruding from the envelope are the peplomers.



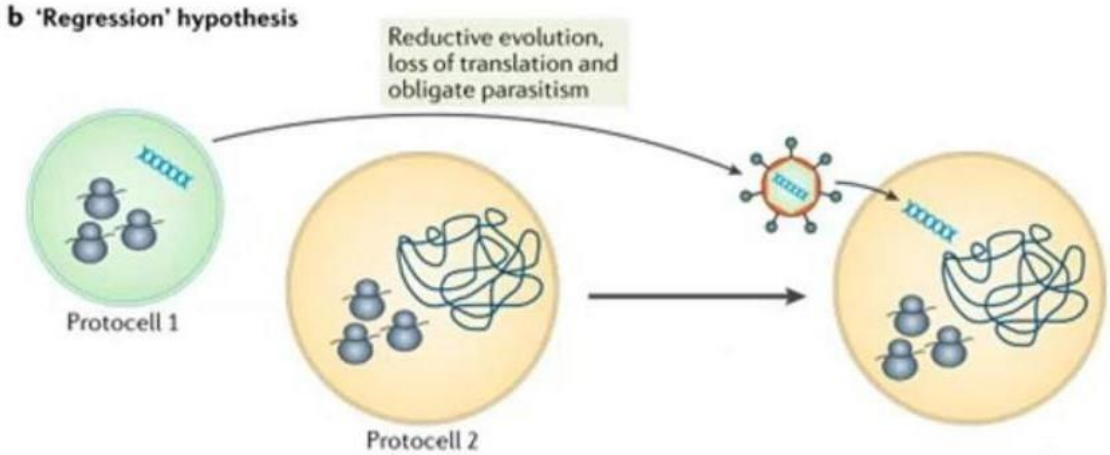
Evolutionary origin of viruses:

Where did viruses come from? How can they show such diversity?
There are multiple hypotheses that discuss the origin of viruses, none of which has enough evidence to become a theory of the origin of viruses.

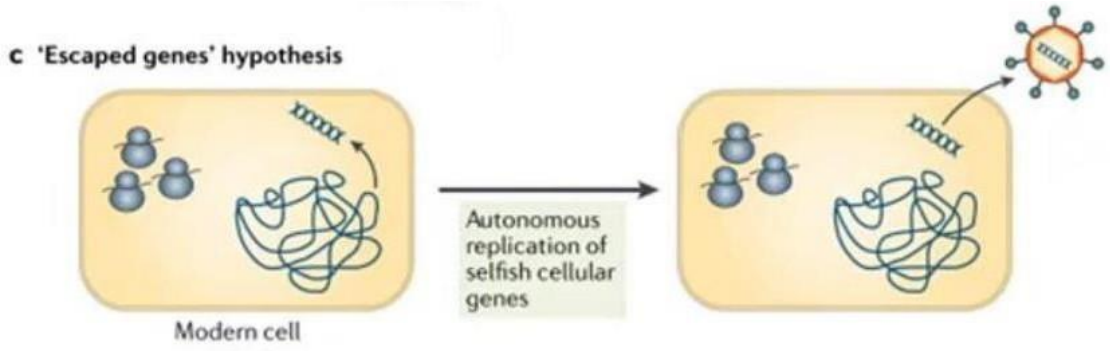


Source: *Krupovic M, Dolja VV, Koonin EV. Origin of viruses: primordial replicators recruiting capsids from hosts. Nat Rev Microbiol. 2019 Jul;17(7):449-458*

-Virus early hypothesis: Before there was any life on Earth, the primary components of viruses were created and when the first living cells appeared, viruses had the ability to infect them.
Viruses existed first.



-Regression hypothesis: parts of the genome of already existing cells escaped these cells and infected others.



-Escaped genes hypothesis: genes escaped cells and were able to support themselves and infect the same type of cells they escaped.

- As mentioned earlier, not enough conclusive evidence has been provided to support any of these hypotheses and the subject is still open to further studies. However, it is possible that all three hypotheses are reasons for the presence of viruses and their diversity and not necessarily one hypothesis.

- Virus origin in humans and all forms of life remains ambiguous, (غامض) there is no clear delineation of the evolutionary history of the presence of viruses on Earth.

لا يوجد تفسير واضح لوجود الفيروسات على الأرض و تطورها

Virus Classification:

There are many types of viruses, so classification of viruses is very important in studying viruses and making inferences (استنتاجات). **The standardized method of classification** that is adopted by the international committee on taxonomy of viruses ICTV is the ***ICTV classification system.*** Viruses are not classified the normal way of classifying organisms into kingdoms, phylums, etc., since there is some debate on whether they are living organisms or not. They are classified somewhat similarly. The highest classification in the hierarchy of the classification of viruses is the ***order*** which is not present in all viruses as most viruses are classified on the level of ***families*** adding the suffix **-viridae** e.g., Poxviridae, Parvoviridae, Herpesviridae then into ***subfamilies*** adding the suffix **-virinae.** Members of the same subfamily are more related to each other (similar/share common features) than they are to other viruses within the same family but a different subfamily. The next level of classification is the ***genus*** *(plural of genera)* suffix **-virus** and finally ***species***.

The ICTV system is revised regularly and in the case of identifying and characterizing novel viruses these viruses are named and added to the system to update it.

Other methods of classification:

- **Virion morphology (size, shape, symmetry, etc.).** This system was used earlier and grouped viruses of similar shapes together e.g., helical, icosahedral, complex.

- **Virus genome properties.** e.g., DNA viruses, RNA viruses, ssDNA viruses, dsDNA viruses, etc.

- **Genome organization and replication.** (discussed below) depends on Baltimore classification system which is conceived and developed by David Baltimore, a Nobel laureate) حاصل على جائزة نوبل who studied retroviruses and designed this system which classified viruses into 7 classes.

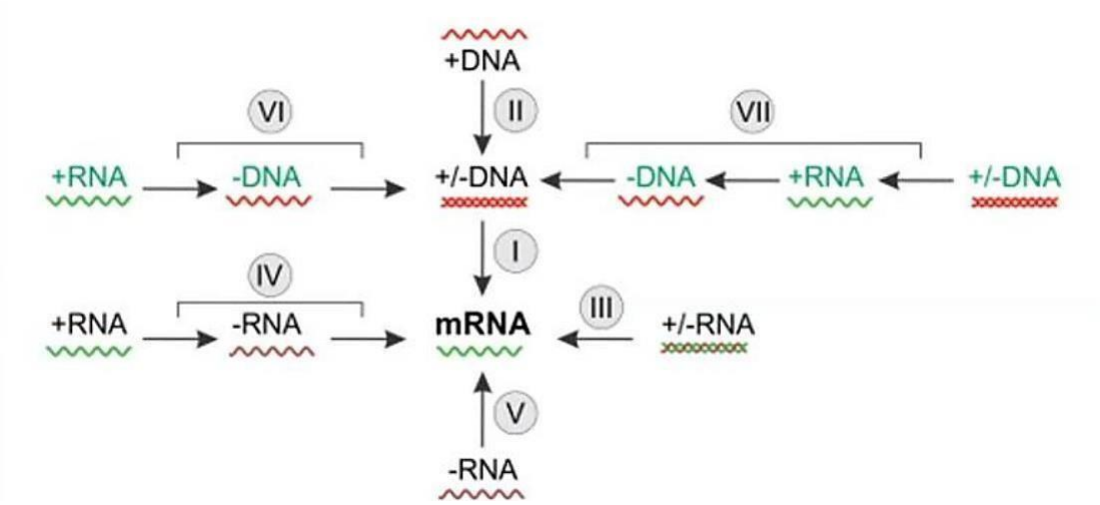
- **Virus protein properties.** depending on the amino acid composition of structural proteins that reflect as antigenic differences. This system is now obsolete, (مهمل) because studying the differences in amino acids does not give the same accuracy as studying genetic differences in the genome itself ***because*** in the case of synonymous mutations, the amino acid sequence won't change but the genome will, and we have 1 stop codon and 63 codons that encode for 20 amino acids i.e., there are amino acids that are encoded by multiple codons. So, proteins may appear similar when studying them on the amino acid level but are genetically different.

- **Antigenic properties.**

- **Physicochemical properties of the virion (T, pH, ether, etc.)**

- **Biologic properties.**

Baltimore Classification System:



DNA viruses (Classes I & II):

- **Class I:** dsDNA viruses.

- **Class II:** ssDNA viruses.

RNA viruses (Classes III, IV & V):

- **Class III:** dsRNA viruses.

- **Class IV:** positive (+) sense ssRNA viruses. (+) sense means that RNA can act like a messenger RNA (mRNA) that is identified by the ribosomes and translated when it enters the cell.

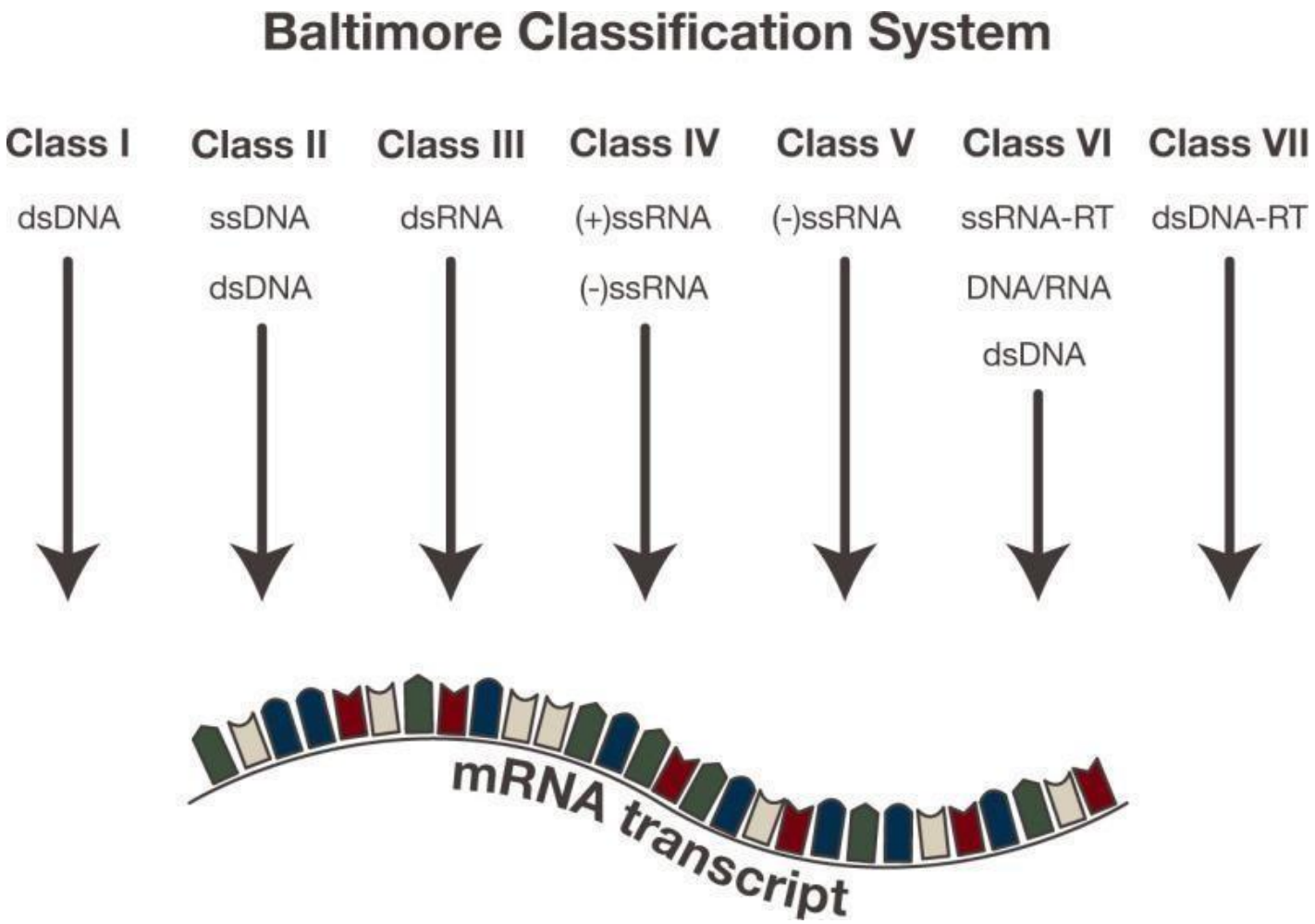
- **Class V:** negative (-) sense ssRNA viruses. (-) sense means that RNA acts as a template for the enzyme *RNA polymerase* to produce + sense RNA that acts as mRNA.

Viruses that replicate through intermediates (Classes VI & VII):

- **Class VI:** positive (+) sense ssRNA diploid genome viruses that replicate through a DNA intermediate. RNA enters the cell and gets converted to DNA by the enzyme *reverse transcriptase,* producing dsDNA that gets integrated in the host's nucleus as a provirus which then gives mRNA and the viral genome.

- **Class VII:** dsDNA viruses that replicate through a ssRNA intermediate. DNA gives RNA and reverse transcriptase converts it to DNA. e.g., **Hepatitis B virus**.

Another figure to help you understand:



Summary of the different features between RNA and DNA viruses:

How can structural differences in viruses lead to differences between them?
1.**The presence or absence of envelopes**. Naked viruses appear to be resistant to harsh environmental conditions like extreme temperatures, pH values and desiccation جفاف. While enveloped viruses are sensitive and can't resist such conditions. That is mainly because envelopes are made of lipids that aren't as stable as proteins. Also, glycoproteins that are embedded in the envelope help in the entry or binding and fusion of the virus to target cells, so when the lipid composition is disturbed (although the capsid is still there), the glycoproteins are affected, and the virus won't be able to enter cells and is less resistant. Also, naked viruses are resistant to ether while enveloped viruses are sensitive to it.

2.Variability in genome size. For example, **hepatitis B virus** with approximately 3,200 bps is the smallest genome (we say approximately because there are length differences in the genotype). The size of the genome in DNA viruses may be as big as 200,000-300,000 bases or more (like **herpesviruses** and **poxviruses)**. RNA viruses show variability, for example **hepatitis D virus** which has 1700 bases, and **picornaviruses** 4000-5000 bases, and the largest RNA viruses are **coronaviruses** with a genome of 32,000 bases. The variability in the genome size is higher in DNA viruses than RNA viruses.

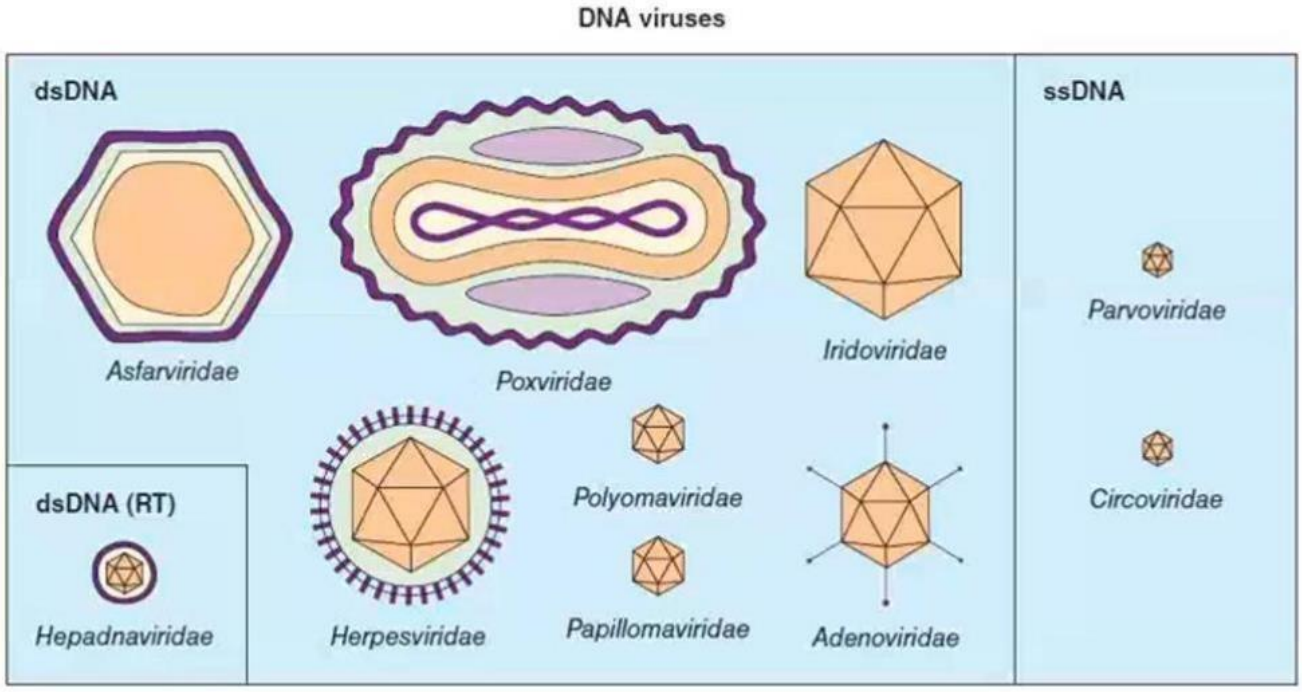
Universal System of Virus Taxonomy

TABLE 29-1 Families of Animal Viruses that Contain Members Able to Infect Humans

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
					30	2.0–3.9	ss circular	Anelloviridae
				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
					35–40	4	ds segmented	Picobirnaviridae
					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

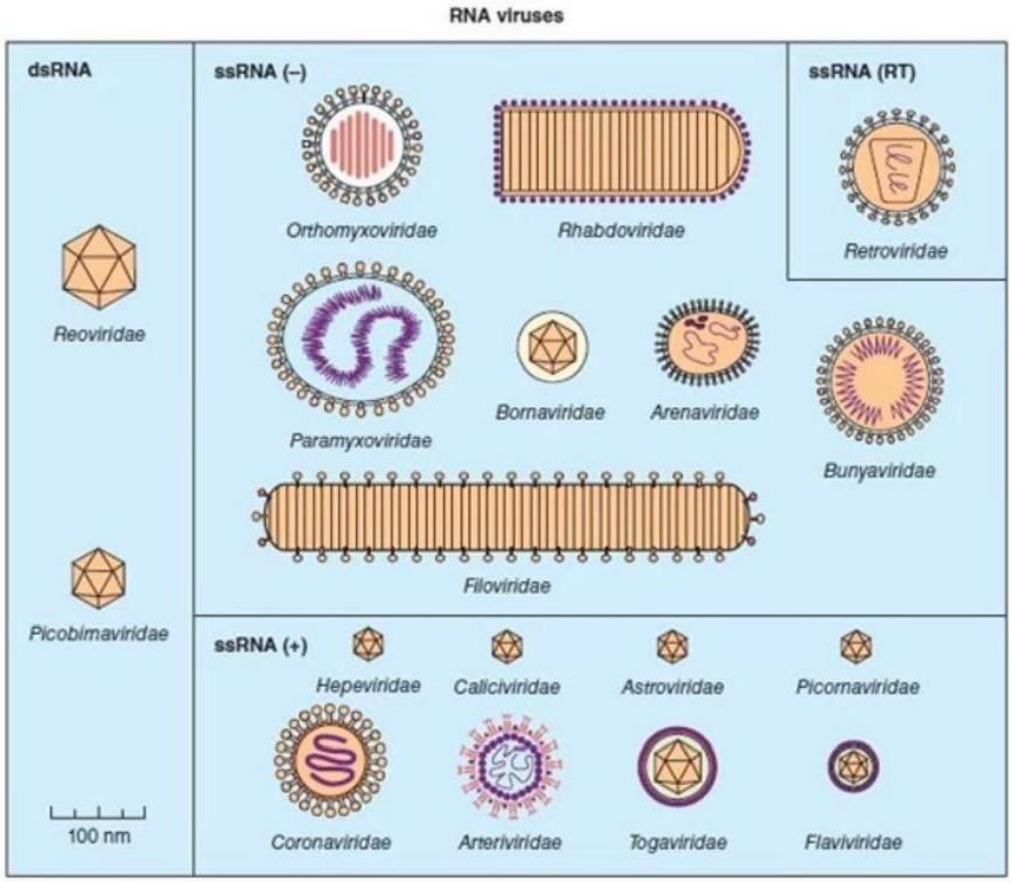
Shapes and relative sizes of animal DNA viruses of families that infect vertebrates:

More variability. Smallest are parvoviruses.



Shapes and relative sizes of animal RNA viruses of families that infect vertebrates:

Less variability. Smallest are picornaviruses.



Other infectious agents:

The basic structure of viruses includes nucleic acid and protein. If nucleic acids could cause infections on their own they're considered viroids.

- **Viroids:** small infectious agents that cause diseases of **plants**. They are composed of ss, ccc-RNA consisting of about 360 nucleotides and with a highly base-paired rod-like structure. Viroids replicate by an entirely novel mechanism (by chloroplasts).
  Viroid RNA does not encode any protein products and the devastating plant diseases induced by viroids occur by an unknown mechanism.
  To date, viroids have been detected only in plants; none have been demonstrated to exist in animals or humans.

- **Prions:** infectious particles composed of <u>protein with no detectable nucleic acid</u>.

  These proteins exist naturally in our bodies but changes in the secondary structure of the prion protein changes its properties:
  - They are highly resistant to inactivation by heat, formaldehyde and other sterilizing agents, and ultraviolent light that inactivate viruses.
  - Causes prion proteins to recruit normal proteins and misfold them causing diseases in the CNS.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Monday, January 25, 1999 Published at 17:47 GMT

UK

One random meal could have caused CJD

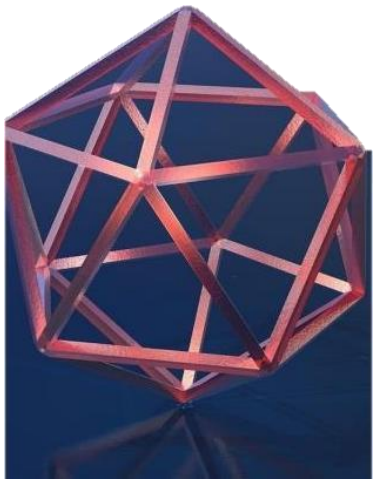
Stephen Churchill occasionally ate beefburgers and sausages

Britain's first teenage victim of new-variant Creutzfeldt-Jakob Disease may have caught the illness from just one random meal of contaminated meat, an inquest heard on Monday.

  The prion protein is encoded by a single cellular gene.

  Prion diseases, called "transmissible spongiform encephalopathies," include scrapie in sheep, mad cow disease in cattle, and kuru and Creutzfeldt-Jakob disease in humans.

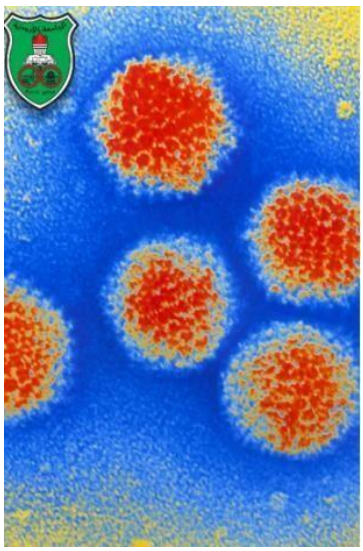
Types of symmetry of virus particles :



- Methods used for fine resolution of the basic virus morphology include: EM, cryo-EM, and x-ray diffraction techniques.
- All cubic symmetry observed with animal viruses is of the icosahedral pattern, the most efficient arrangement for subunits in a closed shell.
- The icosahedron has 20 faces (each an equilateral triangle), 12 vertices, and fivefold, threefold, and twofold axes of rotational symmetry. The vertex units have five neighbors (pentavalent), and all others have six (hexavalent).
- In icosahedral viruses the capsid is first formed then the nucleic acid enters so some icosahedral viruses are devoid of nucleic acids. Therefore, can't cause productive infections but appear under the microscope as viral particles. Unlike helical viruses which are all nucleocapsids because during replication of nucleic acids as these viruses are formed the capsid subunits are incorporated.

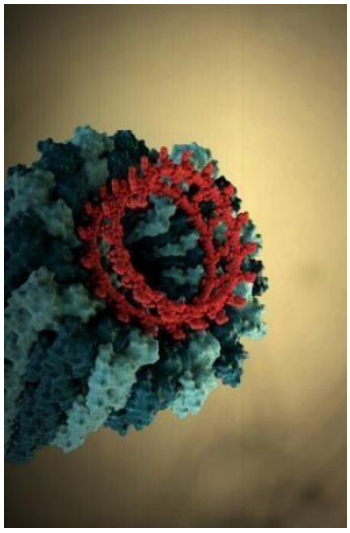
Icosahedral Symmetry:

- There are exactly 60 identical subunits on the surface of an icosahedron.
- To build a particle size adequate to encapsidate viral genomes, viral shells are composed of multiples of 60 structural units.
- The use of larger numbers of chemically identical protein subunits, while maintaining the rules of icosahedral symmetry, is accomplished by sub-triangulation of each face of an icosahedron.
- Most viruses that have icosahedral symmetry show the physical appearance of a sphere rather than an icosahedron.
- Note: enveloped viruses appear as spheres, the symmetry shows when the envelope is removed (helical, icosahedral as spheres or regular structures).



Adenovirus: icosahedral naked virus, appears like a sphere or a cube.

Helical Symmetry



Tobacco mosaic virus mentioned earlier

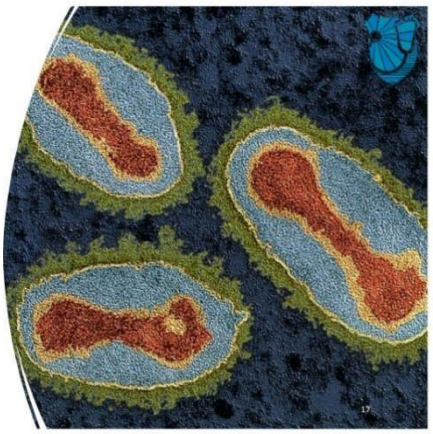
▪ Protein subunits are bound in a periodic way to the viral nucleic acid, winding it into a helix.

▪ The nucleocapsid is then coiled inside the envelope. Thus, there is a regular, periodic interaction between capsid protein and nucleic acid in viruses with helical symmetry.

▪ It is not possible for "empty" helical particles to form.

▪ All known examples of animal viruses with helical symmetry contain RNA genomes and, except for rhabdoviruses, have flexible nucleocapsids that are wound into a ball inside envelopes

Complex Symmetry



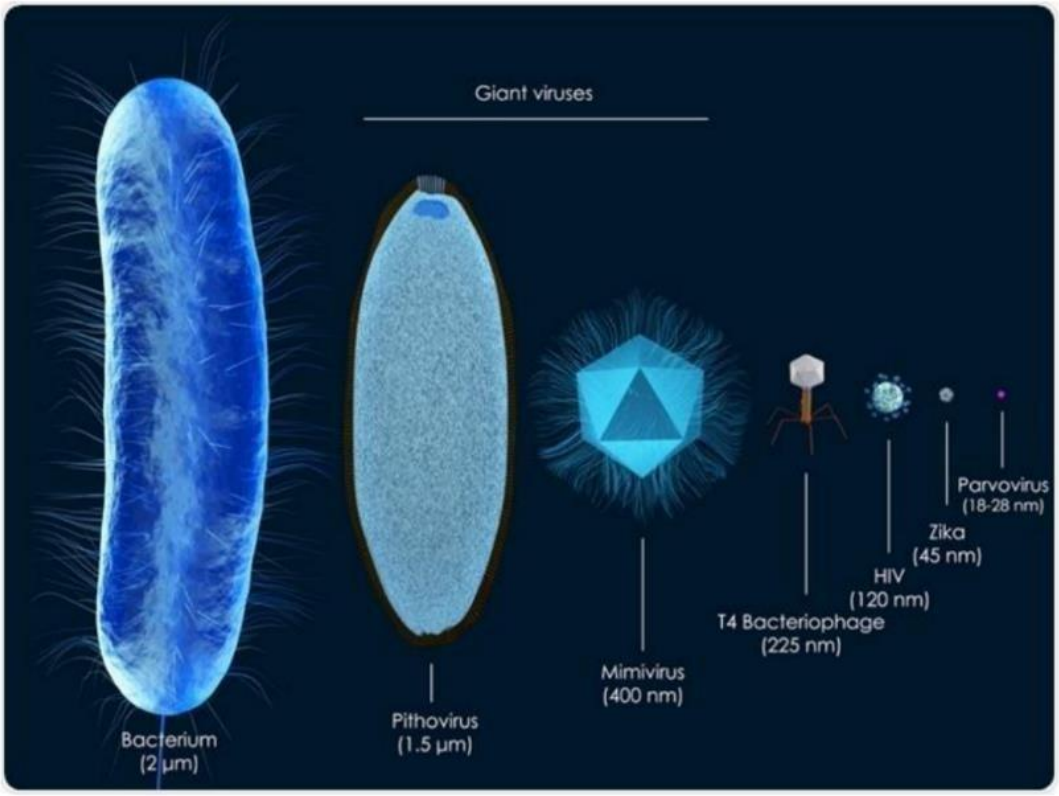
▪ Some virus particles do not exhibit simple cubic or helical symmetry but are more complicated in structure.

▪ For example, **poxviruses** are brick shaped, with ridges on the external surface and a core and lateral bodies inside.

Measuring the Sizes of Viruses

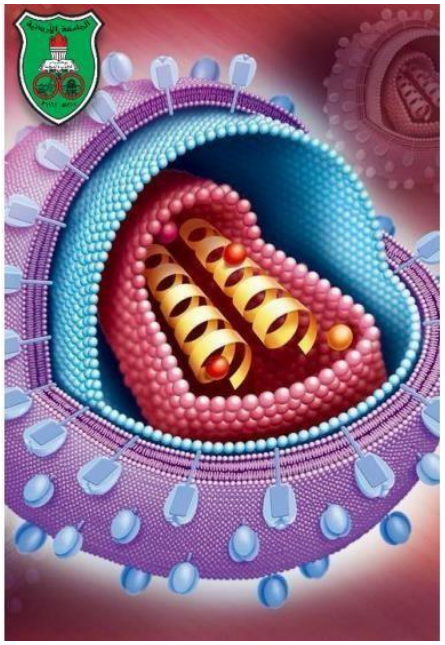
Direct observation in the electron microscope is the most widely used method for estimating particle size.
Some viruses are similar in size to bacteria, but these viruses do not cause infections in humans.

▪ For comparisons, Staphylococcus species have a diameter of about 1000 nm (1 µm). (2) Bacterial viruses (bacteriophages) vary in size (10–100 nm). Some are spherical or hexagonal and have short or long tails. (3) Representative protein molecules range in diameter from serum albumin (5 nm) and globulin (7 nm) to certain hemocyanins (23 nm). (4) Eukaryotic ribosomes are about 25–30 nm in size, with mitochondria being much larger (1–10 µm). (5) Red blood cells are about 6– 8 µm in diameter. (6) The width of a human hair is about 100 µm.



Chemical composition of viruses

proteins:

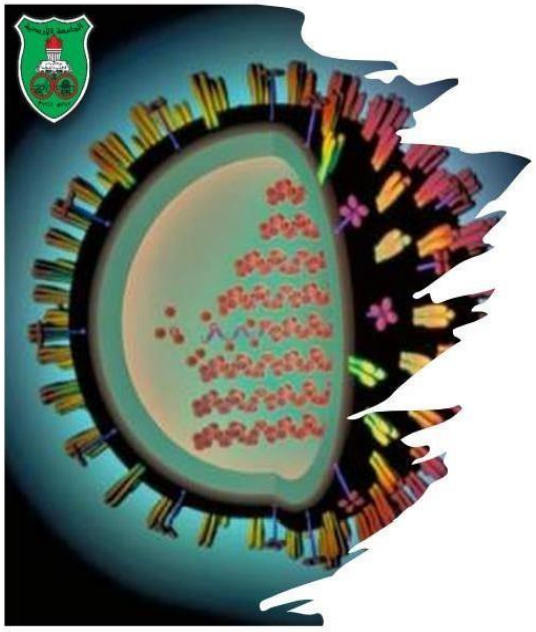


▪ **The structural proteins** of viruses (present in capsids or envelopes) facilitate transfer of the viral nucleic acid from one host cell to another, protect viral genome against inactivation by nucleases, and participate in the attachment of the virus particle to a susceptible cell.

▪ The proteins determine the antigenic characteristics of the virus.

▪ Some viruses carry **enzymes (which are nonstructural proteins)** inside the virions. The enzymes are present in very small amounts and are essential for the initiation of the viral replicative cycle when the virion enters a host cell (initiation and regulation of viral replication). Examples include an RNA polymerase carried by viruses with negative-sense RNA genomes

Nucleic acids:

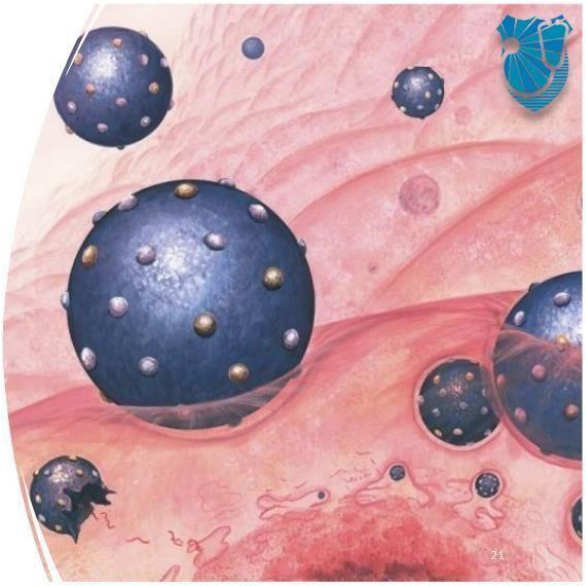


▪ Viral genome may be ss or ds, circular or linear, and segmented or non-segmented (segmented: the presence of multiple unidentical molecules of nucleic acid and each segment gives one or two viral proteins e.g., infleunzaviruses the segmentation allows such viruses to cause different outbreaks and pandemics -every 30 to 40 years or less. The reason for that is when a cell gets infected by two different types of viruses: a human virus and an animal virus, exchange of nucleic acid segments can occur and create a novel virus that no one is immune to and that will be transmitted rapidly. The last infleunza pandemic was swine flu H1N1 virus انفلونزا خنازير in 2009. People who are infected with the human influenza virus and are regularly around animals act as pools where this random event of exchange of nucleic acids resulting in a pandemic can happen.

▪ For DNA viruses, genome size ranges from 3.2 kb (hepadnaviruses) to 375 kb (poxviruses). The size of the viral RNA genome ranges from 4 kb (picobirnaviruses) to 32 kb (coronaviruses).

▪ The RNA may be a single linear molecule (e.g., picornaviruses). For other viruses (e.g., orthomyxoviruses), the genome consists of several segments of RNA. The isolated RNA of viruses with +sense genomes (i.e., picornaviruses, togaviruses) is infectious, and the molecule functions as an mRNA within the infected cell. The isolated RNA of the -s RNA viruses, such as rhabdoviruses and orthomyxoviruses, is not infectious.

Envelopes:



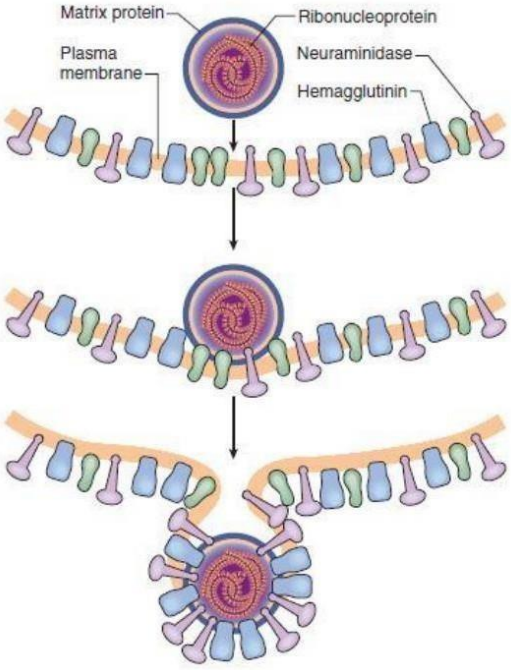
▪ A few different viruses contain lipid envelopes as part of their structure.

▪ There are always viral glycosylated proteins protruding from the envelope and exposed on the external surface of the virus particle.

▪ Lipid-containing viruses are sensitive to treatment with ether and other organic solvents, indicating that disruption or loss of lipid results in loss of infectivity.

▪ Non-lipid-containing (naked) viruses are generally resistant to ether.

- Enveloped viruses leave infected cells by budding from their membranes enclosed by parts of the membranes without killing the cell. While naked viruses kill the infected cell. However, cells infected with enveloped viruses eventually die because the virus takes over the cellular activities necessary for the survival of the cell and directs them towards the synthesis of viral proteins and nucleic acids.



Glycoproteins:

▪ In contrast to the lipids in viral membranes, which are derived from the host cell, the envelope glycoproteins are virus encoded.

▪ However, the sugars added to viral glycoproteins often reflect the host cell in which the virus is grown rather than the viral encoded enzymes.

Cultivation and assay of viruses

If a certain disease is suspected to be caused by a virus the golden standard method is to isolate the virus from each case by cell culture to characterize the virus. Cell culture is the best approach despite the presence of molecular techniques because pure isolates are needed to follow up with sequencing and downstream applications to characterize the virus. So viral cultures are the first step in characterizing novel viruses.

In bacteriology the medium used in cultures contains nutrients, suitable pH values, and no other cells. That is not the case for viruses because they obligate intracellular parasites and need cells in the media where they're grown.

▪ Cells grown in vitro are central to the cultivation and characterization of viruses.

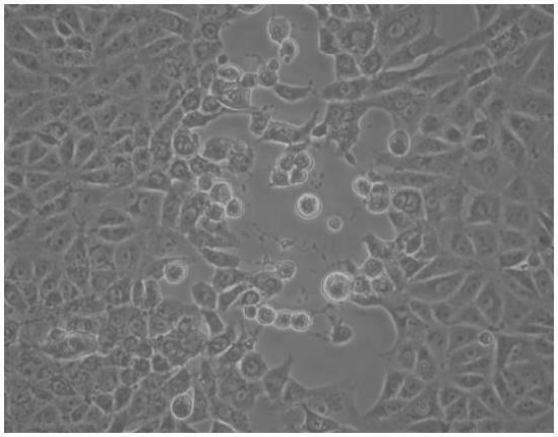
▪ Primary cultures are made by dispersing cells (usually with trypsin) from freshly removed host tissues, and they are unable to grow for more than a few passages.

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

▪ Continuous cell lines are cultures capable of more prolonged— perhaps indefinite—growth that have been derived from diploid cell lines or from malignant tissues. They invariably have altered and irregular numbers of chromosomes. The type of cell culture used for viral cultivation depends on the sensitivity of the cells to a particular virus.

Multiplication of a virus can be monitored in a variety of ways:

1. Development of cytopathic effects (i.e., morphologic changes in the cells). Types of virus-induced cytopathic effects include cell lysis or necrosis, inclusion formation, giant cell formation, and cytoplasmic vacuolization. Most viruses produce some obvious cytopathic effect in infected cells.



2. Appearance of a virus-encoded protein, such as the hemagglutinin of influenza virus. Specific antisera can be used to detect the synthesis of viral proteins in infected cells. Other methods used include enzyme immunoassay, immunofluorescence assay.

3. Detection of virus-specific nucleic acid. **Molecular-based assays** (most common now) such as polymerase chain PCR (components: target sequence, primers, bases, enzymes) reaction provide rapid, sensitive, and specific methods of detection (using fluorescent dyes, agarose gel electrophoresis). Detection can be done in real time like real time PCR using specific probes.

4. Adsorption of erythrocytes to infected cells, called hemadsorption, caused by the presence of virus-encoded hemagglutinin (parainfluenza, influenza) in cellular membranes.

5. Viral growth in an embryonated chick egg may result in death of the embryo (e.g., encephalitis viruses), production of pocks or plaques on the chorioallantoic membrane (e.g., herpes, smallpox, vaccinia), or development of hemagglutinins in the embryonic fluids or tissues (e.g., influenza).

GOOD LUCK