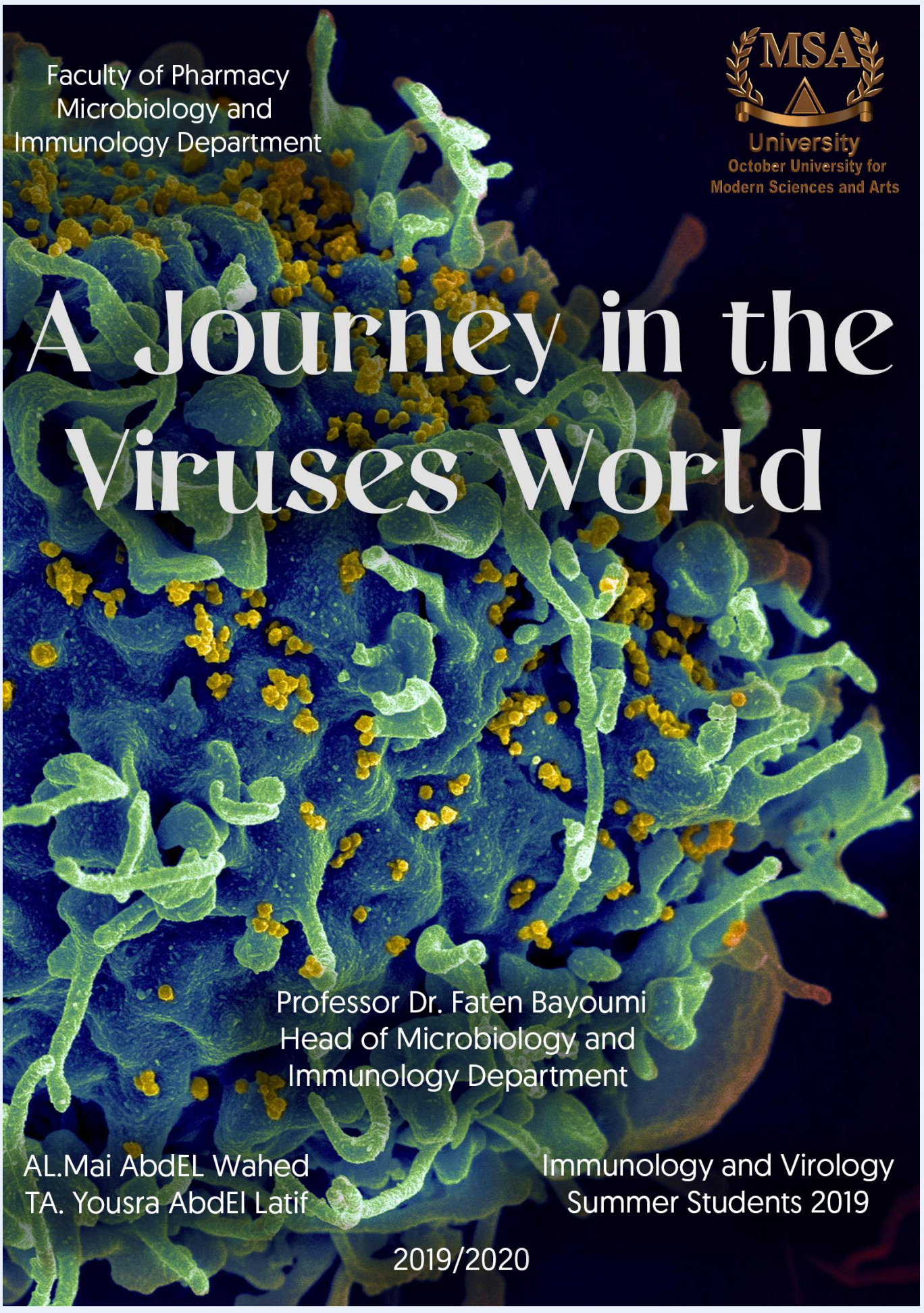
Faculty of Pharmacy
Microbiology and
Immunology Department

MSA
University
October University for
Modern Sciences and Arts

A Journey in the Viruses World



Professor Dr. Faten Bayoumi
Head of Microbiology and
Immunology Department

AL.Mai AbdEL Wahed
TA. Yousra AbdEl Latif

Immunology and Virology
Summer Students 2019

2019/2020

Immunology and Virology (PM212) course student names

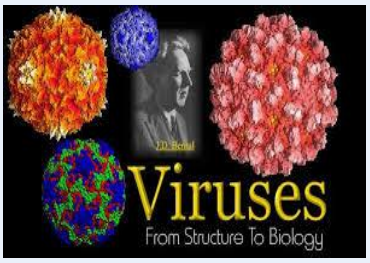
Summer 2019

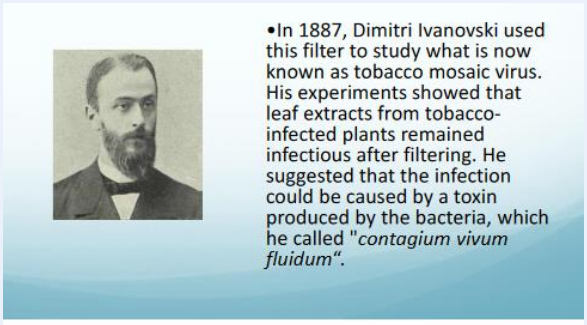
Abdelfattah Mohamed Gabal	Marina Waleed
AbdelrahmanMohamed Deyaaeldin	Michael Makram
Abdullah Hamdi	Mohamed Akram Mohamed
Ahmed Alaaeldin Fangary	Mohamed Hassan
Ahmed Sameh Maher	Mohamed Hossam Eldin
Amira Hamdy Hassan	Mohamed Mahfouz
Bassam Osman Labib	Mohamed Salah AbdoAllah
Bassel Tarek	Mostafa Ahmed Mostafa
Dina Aboalkassem Hamdy	Omar Abdelaziz Ahmed
Donia Adel Elbanna	Omar Ashraf Ahmed
Eiad Mohamed Abdelmoneam	Rawan Magdy
Gerges Sabry Shehata	Rawda Ashraf Naguib
Hatem Fouad Kamel	Sarah Khaled Khalifa
Ibrahim Nabawy	Shahdan Ehab
Khaled Salah	Shorouk Gamal Ali
Kholoud Nasser Mohamed	Soheib Samy Mohamed
Manar Mohamed Othman	Tasneem Magdy Ahmed

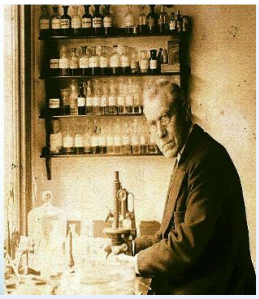
Table of contents

Discovery of Viruses

Viruses were first described due to infection of tobacco leaves in the end of the year of the 19th century. The name of the viruses was coined from Latin word meaning poison or slimy liquid. Two scientists contributed to the discovery of the virus, tobacco mosaic virus. In 1892, lvanoski reported that extracted from infected leaves were still infectious after filtration through chamber land filter candle. In 1898, Beijerinck was the first showed that the instant was able to migrate in agar gel, so being an infectious soluble agent. After the development of porcelain filter, viruses were the first discovered that filter named the chamber land Pasteur filter was that can remove any visible bacteria in the microscope from any liquid sample.







Tobacco mosaic virus could transmit the disease even after filter by chamber land Pasteur filter has removed all viable bacteria from extract. In 1886, Adolph Meyer showed that tobacco mosaic disease, from a diseased plant (tobacco plant) could be transmitted to a healthy one through liquid plant extracts. Another attempt to discover the virus was reported in 1901 by the US army physician, Walter Reed. Johan Kunkel small discovered that in 1869-1938 insect could act as vectors and transferred virus plants.

The virus was spread by mites called a ‘ceria tulip’. In 1948, by wheat streak mosaic virus 70% of the wheat crop was destroyed. Francis Holmes, a pioneer in plant virology, revealed that 129 of viruses that caused disease of plants. Intensive agriculture provides a rich environment for many plant viruses. Joseph Atabekov revealed that a lot of plant viruses could infect only a single species of the host plant.

•In 1884, Charles Chamberland invented a filter that has pores smaller than a bacterium. Thus, he could pass through the filter a solution with bacteria and eliminate them completely from it.

How can technology help virus complete discovery:

Technologies have aided for the discovery of several viruses of all types of living organisms .Particles of a single stranded viruses are very small, about 20-250 nanometers in diameter. There wasn't a good vine of the structure till the development of electron microscope.

The surface structure of virions could be detected by both scanning and transmission electron microscopy, but the virus internal structure can only be detected in images from transferred electron microscope.

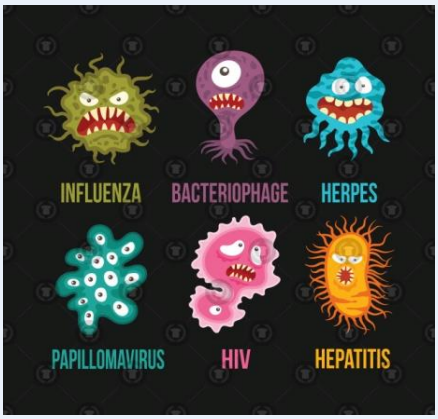
Viruses were categorized by the type of nucleic acid they contained. Nucleic acid was single or double stranded RNA or DNA.

Viruses are parasitic entities, a cellular that aren't categorized in any kingdom unlike most living organisms, viruses can't divide and aren't cells.

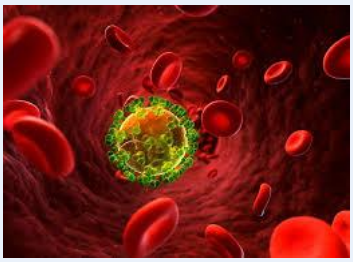
Viruses infect a host cell and used host cell for its replication processes to yield identical progeny virus particles. They are finding between nonliving and living organism. Living things produce, grow and metabolize as for viruses replicate but to do, viruses depend on their host cells. They don't grow or metabolize, but are accumulated in their mature form. Moreover, no one knows exactly when virus they come or emerged, since viruses don't leave historic foot prints as fossils.

- ✓ 1901: Walter Reed - (the first human virus) yellow fever virus.
- ✓ 1906: Vario La Virus (Negri)
- ✓ 1892: Dimtrii lvanovsky - observed that agent of tobacco mosaic disease passes through porcelain filter that retain bacteria.
- ✓ 1903: Rabies virus – Rifffat bay, Remlinger
- ✓ 1898: Paul Frosch and Feriedich Loffler - the first animal virus

- ✓ 1911: Reyton Rous - sarcoma virus
- ✓ 1933: Smith - Human influenza virus
- ✓ 1908: Poliovirus, chicken leukemia virus. 1931: Shope - Swine influenza virus
- ✓ 1915: Felix Dherelle Frederik Twort.- Bacteriophages.

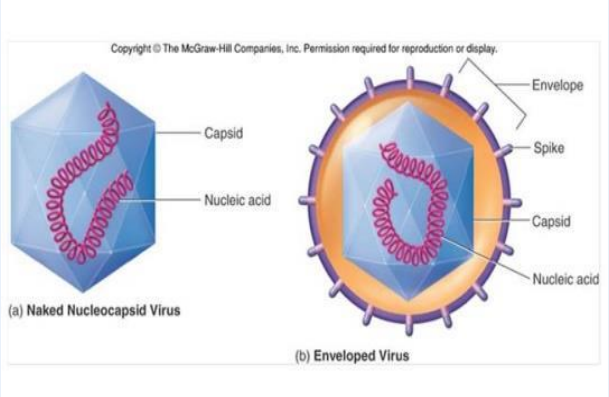


Classical methods used for the identification of known viruses include molecular detection methods as polymerase chain reaction (PCR), cell culture based assays and immunological assays. When studying filterable characteristic of the virus and found that virus is an obligate parasites. This means that virus is unable to live on its own. In 195 chase and Hershey showed that it was nucleic acid portion that was responsible for the infectivity and carried genetic material. In1949, Iwaff found that virus could behave like bacteria gene on the chromosome and also found the operon model for gene repression and induction. Crick and Matson in 1954 found that exact structure of DNA, and in 1949 Frederick Robbines,



Johan, Tomas Wellar and Enders grew. The Polio virus in cultured human embryo cells, the first virus to be grown without using solid animal eggs or tissue. This enabled Jonas Salk to make an effective polio vaccine. In 1931 Max Knoll and Ernst Ruska found electron microscopy that enabled the first image of viruses.

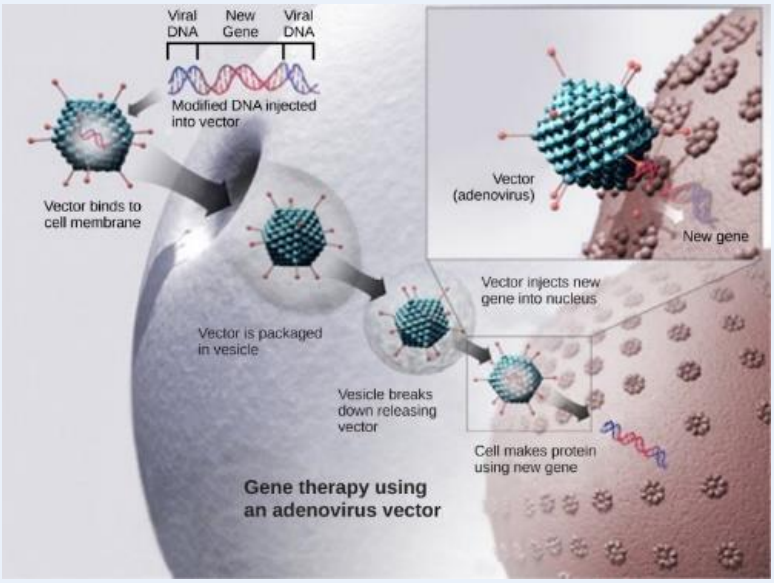
→Structure of Viruses



Viruses are considered to be the smallest known microbe ever; viruses are very small that it said that 500 million viruses that cause common cold could be placed on the head of a pin. Viruses unlike the other microorganisms it cannot live by its own, viruses need a host cell to start replicating and multiplying itself and continue its cycle, so because of that viruses are unique microorganisms. Viruses mainly are formed of genetic material which can be either DNA or RNS that are enveloped by a shell that is known as capsid that is comprised of protein mainly, in some types of viruses there is another layer that serves as protective coat called envelope it's a spikey coat that protect the virus.

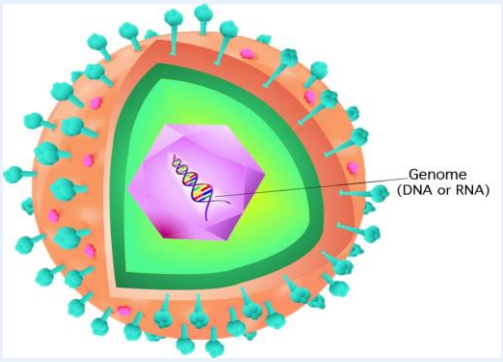
→Viral Multiplication

The main purpose of the virus's existence is to make another copy of them. Viruses attach itself to the host cell and then start to penetrate it after that the viruses use the host cells as a machinery to make copies of them and their genetic material after finishing its job viruses leave the host cell by the whole bursting of cell by lysis or by budding either way the host cell is damaged.



→Classification: DNA Viruses & RNA Viruses

Viruses is classified according to ICTV [international committee on taxonomy of viruses]



Baltimore classification:

Viruses were placed in groups according to the type of genome in the virion by the virologist David Baltimore. He subdivided the viruses according to the nature of the nucleic acid into DNA viruses and RNA viruses.

DNA or "deoxyribonucleic acid"

DNA is composed mainly of three components: sugar, phosphate and nitrogen base. The sugar is deoxyribose sugar and is attached to a phosphate group. Together they create the sugar backbone of the DNA. Nucleic acid is formed from molecules called nucleotides.

Every single nucleotide contains three basic things considered as the columns of the nucleotides and they are:

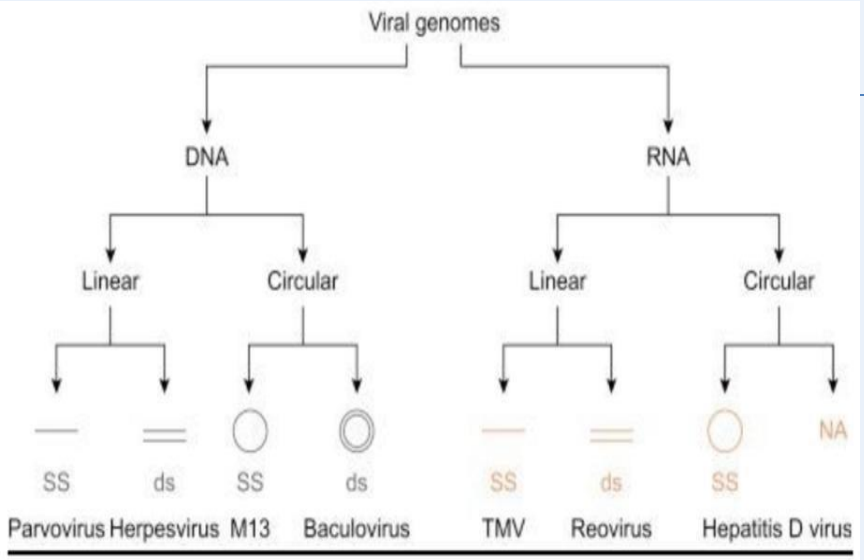
The Phosphate base:

Each nucleotide has only one phosphate group which yields the nucleotide.

The Nitrogen base: (two bases)

- Purine bases: Adenine (A) and Guanine (G)
  Xanthine and Hypoxanthine
- Pyrimidine Bases: Thymine (T) and Cytosine (C) –T is in DNA only,
  Uracil (U) – only in RNA.

(Continued next page)



DNA viruses' genomes

The DNA of DNA viruses is small if it compared to the host cells. These viruses can use the host cell to replicate their own genome, their DNA virus's genome is considered as small genome as their base pairs range is between 10 kilo base pairs to 30 kilo base pairs. This type of DNA viruses is divided into double strand DNA viruses (ds-DNA viruses) and single strand DNA viruses (ss-DNA viruses).

Double strand DNA Viruses

Double strand DNA viruses are considered large genome among viruses as it has up to 30 kilo base pairs. They have the properties that the DNA does not enter the host cell therefore virus's genome does not combine with the host genome that reduces the dangerous of mutagenesis Example of this type is Adenoviruses. Adenoviruses are commonly infect the cardiac cell and the common effect of this virus that they reduce the transducer of cardiac cell and affect the immune response of the body.

Another example of this virus is the bacteriophage which is also double strand DNA, they are viruses that infest bacteria as virus T4 that infect *Escherichia coli* bacteria. For example, Variola virus is a cause of smallpox. It is an extremely destructive disease with high death and spreading rate. Fecundation with vaccinia virus has high protective property against infection with Variola virus. Another example the inflammation of the liver caused by hepatitis B virus (HBV). Hepatitis B can be transmitted by exposure to infected blood, saliva, vaginal fluid or other fluid in the person body. An infected woman can transfer the disease to fetus during pregnancy or at birth. More than 90% of infected infants will be chronically infected. On the other hand, there is kind of people have no symptoms at all. HBV usually get well on its own or it may lead to chronic HBV Chronic HBV lead to liver cancer or failure and may lead to cirrhosis.

Single strand DNA viruses

Single strand DNA viruses are simply differentiated into linear and circular non-envelope genome. Circular genomes include like Spiraviridae, Anelloviridae and Bacilladnavinus, while linear genomes include Bidnaviridae and Parvoviridae. This type of viruses (single strand DNA viruses) found in aqueous media (fresh water and sea water) and sediments. This type of virus was first discovered by Sinshemer 1959; as he was working on bacteriophage cale Phi X 174, he discovered that it was single strand DNA virus.

However, this was a new discovery because the scientists believed that there are only double strand DNA viruses until very recent times. There is very famous family of this type of viruses called Spiraviridae; it is famous because it is one of only two families that single stranded DNA viruses infect Archea.

RNA viruses

RNA viruses can be arranged for positive and negative senses or ambisense RNA diseases with the polarity or meaning of their RNA. Positive-sense, viral RNA is probably mRNA and can therefore be transcribed rapidly by the host cell. Negative-sense viral RNA is integral to mRNA and in this way should be changed over to positive-sense RNA via a RNA-dependent RNA polymerase prior interpretation. As such, purified RNA of a positive-sense virus can legitimately cause disease however it might be less irresistible than the entire infection molecule. Refined RNA of a negative-sense infection isn't irresistible without anyone else as it should be translated for positive-sense RNA; every virion can be interpreted to a few positive-sense RNAs. Ambisense RNA infections look like negative-sense RNA infections, with the exception of the likewise decipher qualities of the negative strand.

There are 6 types of viruses:

- The DNA viruses form types I and II, while The RNA viruses constitute the other types.
- Type III viruses contain a double-stranded RNA genome which has from one to twelve different RNA molecules, each one of them code for one or more viral proteins.
- Type IV viruses contain a positive single-stranded RNA genome, the genome acts as mRNA (messenger RNA).
- Type V viruses contain a negative single-stranded RNA genome used as a template for mRNA synthesis.
- Type VI viruses contain a positive single- stranded RNA genome and uses DNA to replicate so they cannot be considered as RNA viruses.

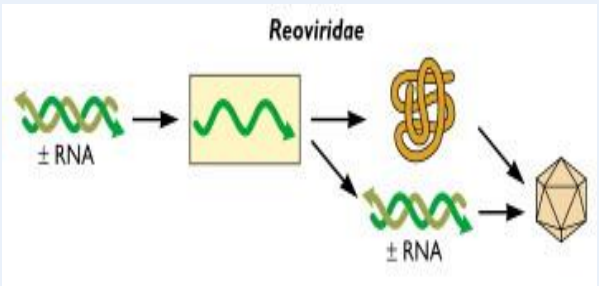
Replication and expression of the viral genome:

Viral genomes having DNA undergo replication in the host cell nucleus while viral genomes having RNA replication occurs in the cytoplasm. The gene expression in both conditions is used to produce viral proteins in the cytoplasm where the host can use synthetic protein machines. The primary requirements for replication of these genomes are just as important for infected cells. These requirements include short nucleic acid primer with a free 3′-OH group which have to be available for the extension by using nucleic acid polymerase.

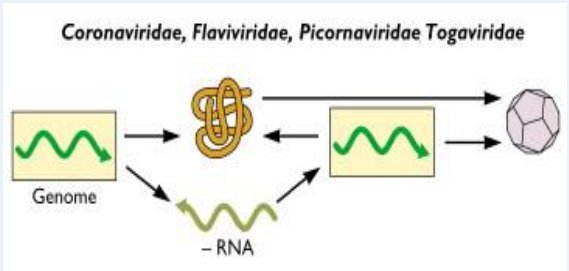
How RNA viruses maintain their genome integrity:

In order to protect the integrity of the genome, RNA viruses have developed different processes. These include mechanisms to enhance replica fidelity, recombination operations that permit sequence exchange among distinct RNA templates, and repaired genome termini processes.

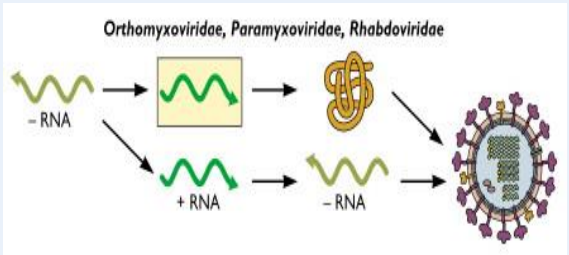
The genome of RNA is a mold used to synthesize extra strands of RNA. The genome, the DNA layer (Copy Genome), and the mRNAs must at least be synthesized in three types of RNAs. The use of the virally-encoded RNA-dependent polymerase RNA [RdRp] is to reproduce your genomes. These RdRp with other proteins that are needed to synthesis the viral genome are called the replicase complex. The replica complex consists of a number of proteins needed for the production of the infectious genomes. Negative -RNA viruses with genomes functioning as mRNAs. This form translates genomes to the RdRp, which is required to synthesize the extra viral RNAs, quickly after entry to the host cell. The RNA viruses on the positive strand are used to reproduce larger complexes of cell membranes.



Double-stranded RNA genomes



Single-stranded (+) RNA genomes



Single-stranded (-) RNA genomes

Investigate aspects of viruses

Viruses can be found almost everywhere as it considered the most huge type of biological entity ever exist.

A virus is an obligate intracellular parasite; meaning it will only replicate when inside a living host cell. This is due the fact that viruses contain no cellular contents and organelles to perform the necessary functions, such as protein synthesis, hence no enzymes.

There are numerous ways to identify and extract viruses. Laboratory diagnosis of viruses includes electron microscope, haemagglutination inhibition, immunofluorescence, and tissue culture.

→(1) Serologic test for viruses

Serologic test are tests which take place in the labs in order to check for the antibodies present in the blood serum of the patient which indicates the immune system response towards a specific type of infection to produce these antibodies against certain foreign antigen which the body has exposed to. Also, other substances which are known as antibody like substances may appear in the serum as response of this effect. The following table shows several serologic test that are being used .Some of them are new technique tests while the other tests are the classical serological test:

Table (1): serologic tests for viral identification

Classical tests	New methods
Complement fixation tests	Particle agglutination
Immunofluorescence techniques	Western blot test
Neutralization test	Enzyme linked immunosorbent
Hemagglutination test	Radioimmunoassay

The principal of the test:

The main reason behind performing these serological tests is to identify the antigen particle that is not easily identified such viral antigen and the bacterial antigens, in addition to parasitic infections.

The concept behind these serological techniques is to confirm the presence of certain antibodies in the patient serum by collecting a blood samples from the patient by the doctors and sent to the lab to make the desired serology test to confirm the presence certain antigen in the serum by finding out its specific antibody.

Positive and negative serologic test results indication:

The result obtained from the serologic test varies, as it might give positive readings, as well as, negative readings.

Starting with the negative results: It shows that the absence of the antibody is assumed to be found in the patient serum. This indicates that the patient is not infected by any antigen as the antibodies for that certain antigen is absent from the patient serum.

In case of the result shows positive indications: it means that the antibodies are present in the serum of the patient for a certain antigen.

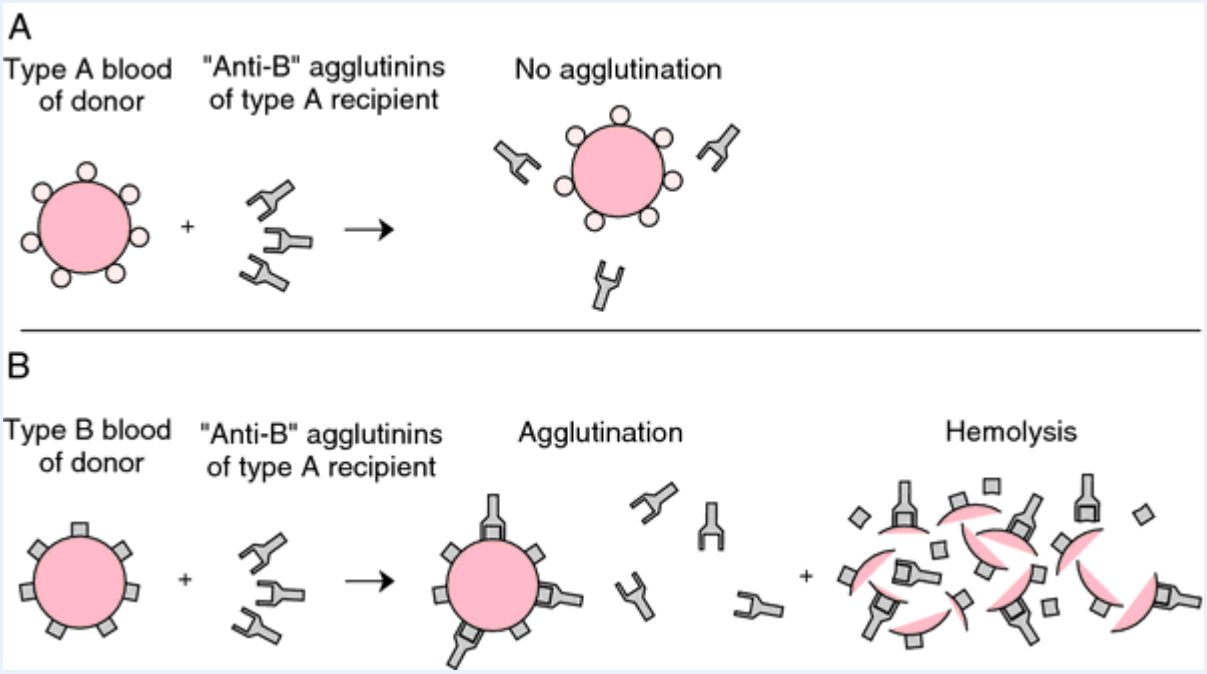
Results of the serologic tests:

The result of a serologic test might be either normal or abnormal. The meaning that the test is normal that the test indicate no antibody in the serum blood and this in turn indicates that there is no infection and the blood sample is totally normal and free from infection.

But the meaning of abnormal result that the test has indicated the presence of antibodies in the blood sample taken as this indicates also that the body has responded to a certain infection that stimulate the immune response to take an action against that antigen.

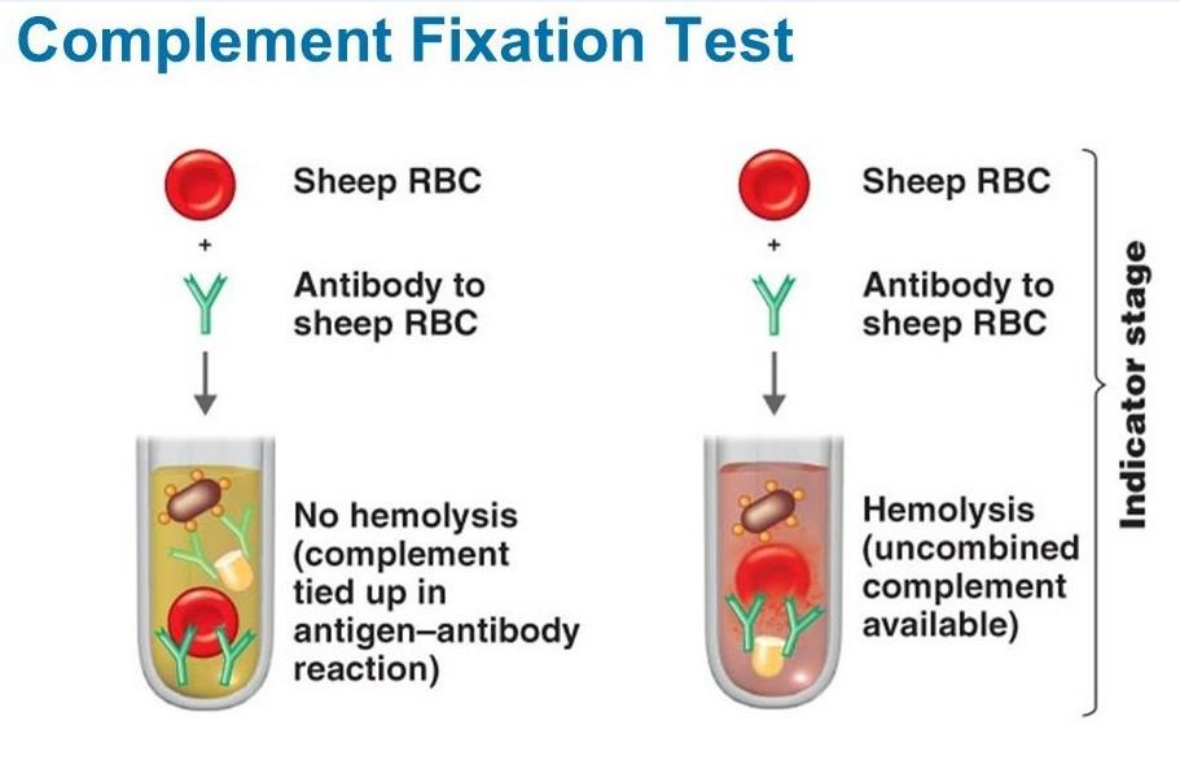
Types of serology tests used:

1- **Agglutination tests:** the agglutination test relies on the ability of antibody to agglutinate certain antigen as the antigen in this case is detected by agglutination or the clumping of the antigen. This serologic test could be used as a qualitative test to determine the presence of either the antigen or antibody, by mixing the antibody with the particular antigen and if agglutination occurs, this indication of positive agglutination test and if negative result is obtained this means that there is no reaction occurred between the particular antibody and antigen. As well also this test could be as quantitative test in order in measuring the antibody level for certain antigen.



2- Complement fixation test:

This test is considered an immunological medical test that mainly search for infection evidence as it is working by test for the presence of either specific antigens or antibodies in the serum.



3- Western blot test:

This test is mainly used in the diagnosis of the HIV. This method can be done by taking first the blood sample from the patient serum which used in order to detect the antibodies that are specific for the HIV (supposed virus causes the disease).

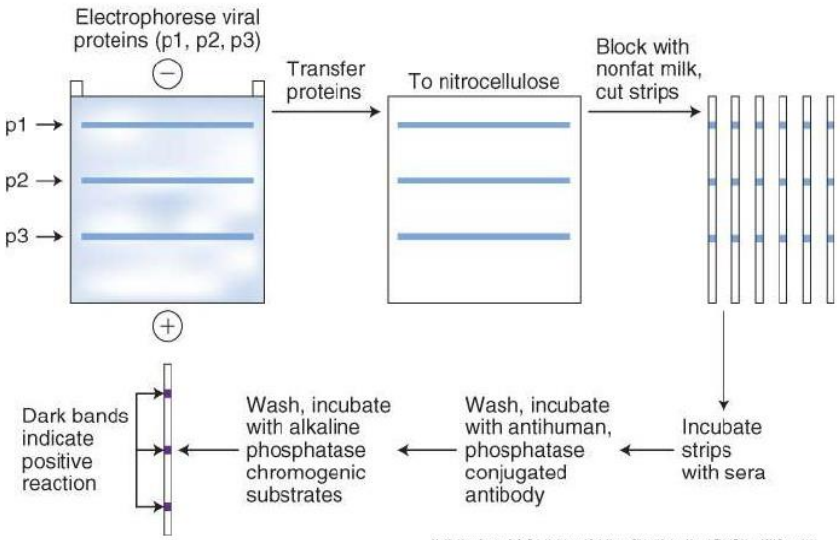


Figure 5.21a: The basic principles behind the Western blotting procedure.

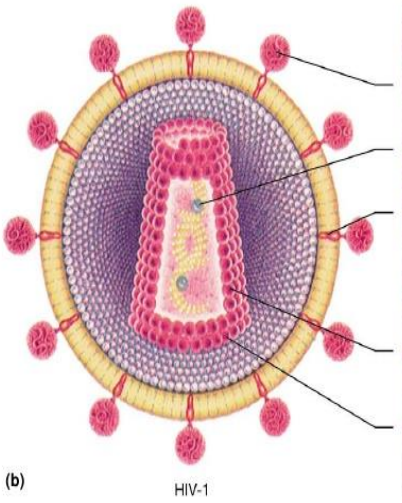


Figure 5.21b: The structure of HIV-1.

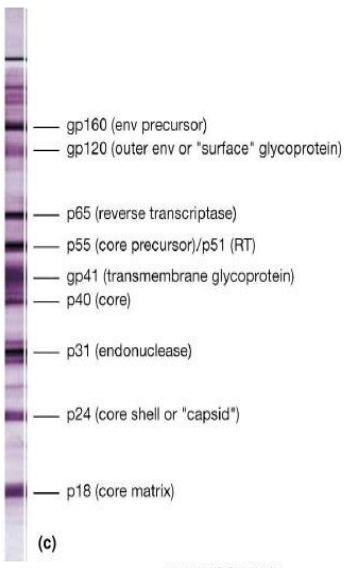


Figure 5.21c: The typical results of a Western blot testing patient serum for HIV-1 antibodies.

4- Virus neutralization serology test:

Used to measure the capacity of antibodies to neutralize the antigen's infectious proprieties. This method involves the dilution of specific antibodies for the certain antigen to be identified, then these antibodies are mixed after being diluted with a suspension of the antigens.

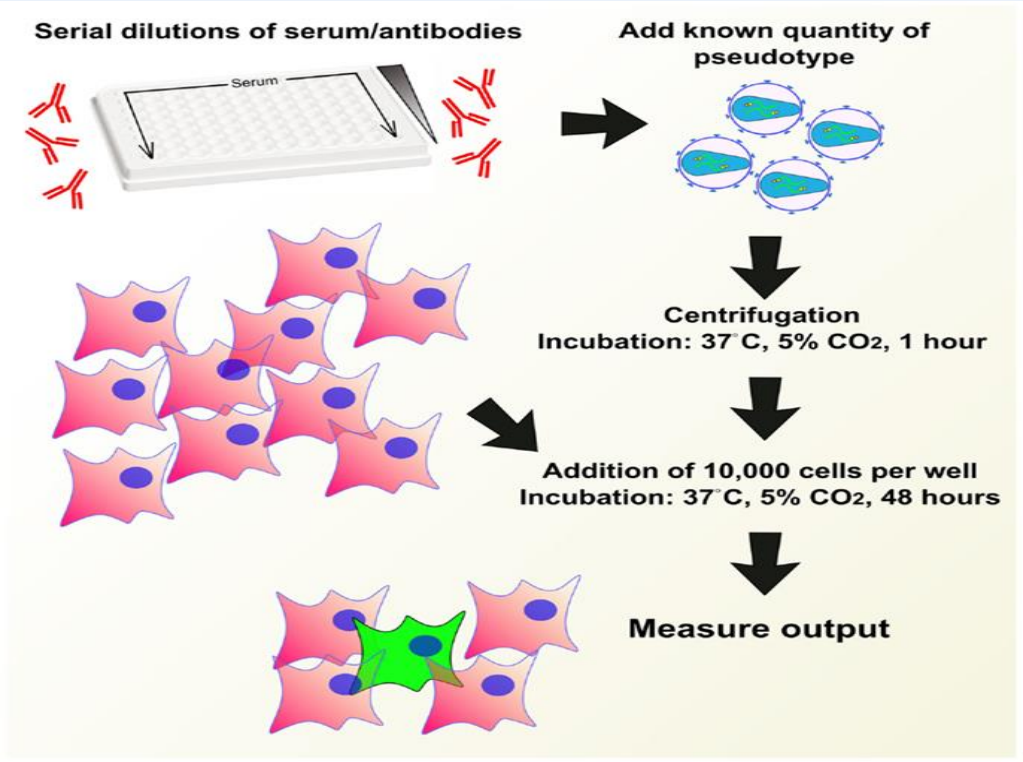


Table (2): Examples of tests used in the viral serology

CMV IgG	This antibody is tested to indicate if the person was infected previously with CMV
Hepatitis A IgM antibodies	These antibody is used in the detection acute hepatitis A virus infection
Hepatitis A IgG antibody	The IgG antibodies are used to determine whether there is an antibodies against HAV in the sera or not
Rubella IgG	Is used in order to determine that if there is an antibodies present for the rubella virus in the serum or not
Varicella zoster virus IgG	Used in order to determine if the person has been exposed previously to varicella zoster virus or not.
Measles IgM	Used in order to determine the measles acute infection, but sometimes the IgM antibodies might give a false negative result after the onset of rash by 3 days.

→(2) Tissue culture

What is tissue culture?

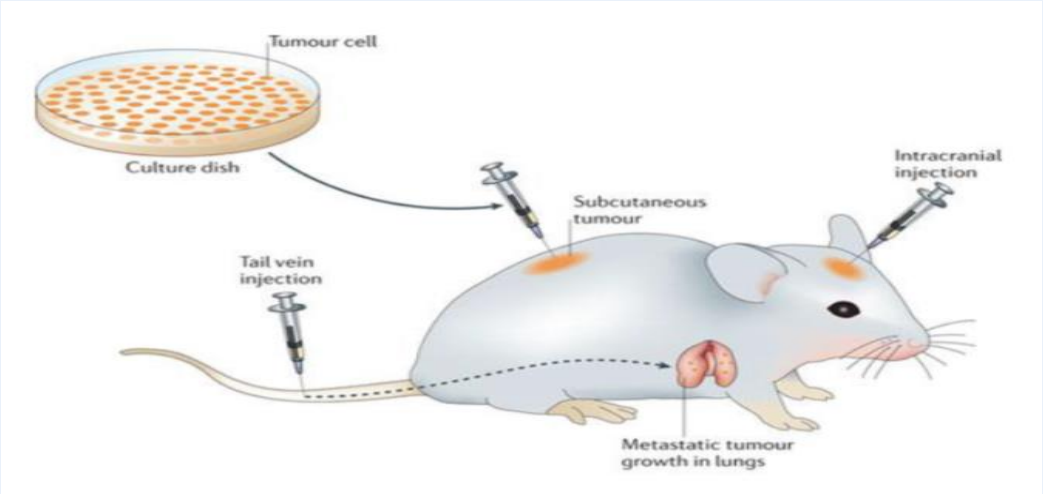
Tissue culture is a beneficial technique for cultivating medical samples that may have a virus. This technique enables scientists to isolate, detect and identify of viruses inside the laboratory. Tissue culture includes developing animal cells in flasks by using various broth mediums and afterwards contaminating these cells with virus. This typically starts with a primary culture aiming to reach confluence; that is formation of monolayer of cell in a flask. When achieving a confluence, the cells afterward pass from primary to secondary and finally to tertiary, until a line of cell is set up. The virus isolation in a cell culture consumes time.

How does it identify the virus?

Remember saying that viruses are obligate intracellular parasites? This means they need living host cells or tissues in order to replicate and reproduce to give a cytopathic effect (CPE) and cause infection. The living host cells are in the form of culture, and may be animal inoculation, embryonic egg inoculation or cell culture.

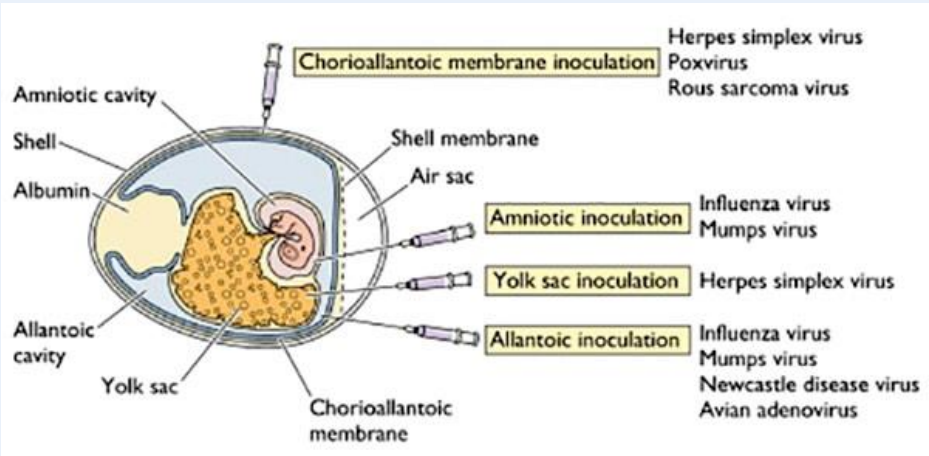
(1) Animal inoculation (of healthy animals): most commonly used are suckling mice (less than 48hrs old) that are affected by coxsackie and toga viruses by intranasal or intracerebral inoculation. Other animals such as guinea pigs, hamsters or rabbits may also be used, with other routes of inoculation such as intraperitoneal and subcutaneous.

Once the virus causes its infection, it's isolated and purified from the animal tissues and examined for identification. The first live inoculation used on human volunteers was yellow fever virus (Aryal, 2018). The pros of animal inoculation are that diagnosis, pathogenesis and clinical indications are identified, as well as one can study the immune responses and production of antibodies. Also, primary isolation of specific viruses can be carried out. The cons, however, are that this procedure is costly and choosing correct and healthy animals is difficult, nevertheless maintaining them. Moreover, there may be issues with human viruses reacting differently in human cells than in animal cells, leading to generation of escape mutants.



(2) Embryonated egg inoculation: this method was first put to test in 1931 by using embryonic hen's egg (other egg types may be used depending on examination). A 7-12days old chick egg is used, and using small sterile drills the virus are introduced to the embryo (surface of egg is disinfected with iodine beforehand). After that, the opening left by the penetration is sealed with gelatin or paraffin. The egg is left to incubate for 2-3 days for 36 degrees Celsius. Once incubation period is over, egg shell is broken, and virus is secluded from its tissue. CPE is show on egg embryo in the form of its death, damage, or by forming typical pocks or lesions on egg membranes.

The viral penetration can occur in various parts of the embryonic egg as shown in the diagram above, depending on the virus that is being isolated and identified. It may chorioallantoic membrane, allantoic cavity, yolk sac or amniotic sac. This procedure is better and more widely used than animal as it's cheaper and easier to maintain the embryonated eggs. They are also more readily available and have wider ranges of tissues and fluids. However, inoculation sites vary for each virus for their growth and replication.



(3) Cell culture (AKA tissue culture): may be

(a) Organ cultures – performed when certain organs are infected with highly specialized parasites. For instance, coronavirus identification using tracheal ring culture.

(b) Explant culture – rarely performed.

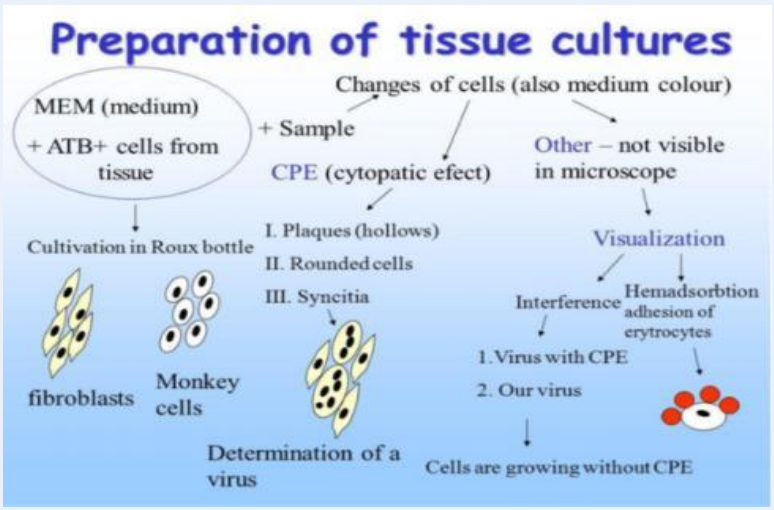
(c) Cell culture – most widely used culture for identification and isolation. There are 3 types of cell culture:

Primary cell culture	Diploid cell culture (Semi-Continuous)	Heteroploid cultures (Continuous cell lines)
The use of animal or human cells for a limited period of time, and for primary isolation of viruses and for making of vaccines. For example: Monkey Kidney cell culture.	Diploid cells so have same chromosomal number as the parents', used in cultivation of fastidious viruses and in vaccine production. For example: Human embryonic lung strain culture.	A consequential from cancer cells and may be serially cultured without limit (unlike Diploid cell culture) and so it's continuous. To ensure its maintenance, it is stored at -70 degrees Celsius or serially sub cultured. However, unsuitable for vaccine production due to its derivation from cancerous cells. For example: Human Carcinoma of Cervix cell lines (HeLa).

How tissue culture is carried out to identify viruses (generally):

Sample is first collected by centrifuging the liquid media to allow all microbes except viruses to remain at bottom of tube, while viruses remain spread out in liquid. Then, arrange a serial dilution of viruses in 6 labeled sterile microcentrifuge tubes, and add the growth culture medium to the tubes. The tubes are then incubated for 90 minutes at 35 degrees Celsius with the presence of 5% CO2. Then inoculum (cell culture growth medium) is removed and a fresh medium is introduced again into each of the 5 tubes, leaving 1 tube for control test. Incubation is continued till virus begins to grow, and period varies depending on virus type.

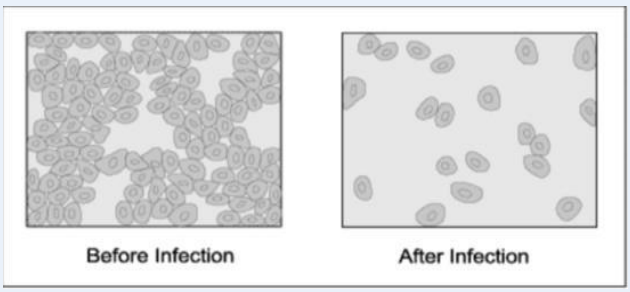
Virus exhibit cytopathic effect on culture medium as indication for infection, and scientists observe the changes that occurred on monolayer cells using light inverted microscope. Changes may be shrinkage or swelling, syncytium formation, or any other observable change. This may be an introductory identification of the virus.



Most commonly, CPE shows up after incubation of 5-10 days, although an exception to this is Herpes Simplex Virus (HSV) which shows CPE after 24hr incubation only, while other viruses such as Cytomegalovirus (CMV) show CPE after 10-30 days incubation period.

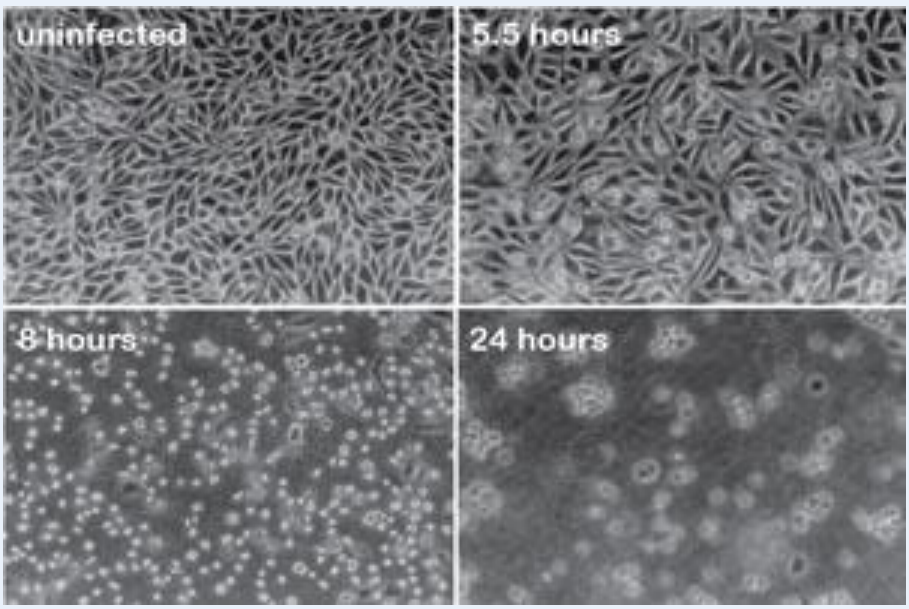
These changes include the degradation of monocyte cells because of viral infection and swelling or shrinkage of cells. Cytopathic effects include:

- ✓ Clumping
- ✓ Rounding
- ✓ Fusion (syncytium formation)
- ✓ Detachment (plaques)
- ✓ Ballooning (Giant cell)
- ✓ Inclusion body formation



So to conclude, the CPE shown depends on several factors, which are tissue culture, specimen/virus type and period of incubation.

Pictures of CPE examples



→ (3) Polymerase Chain Reaction (PCR)

It is invented in 1985 by Kary Mullis .It is used to make many copies of DNA or gene in molecular biology, it is called in many ways “molecular photocopying," the (PCR) is a simple technique used to "amplify" - copy - small segments of DNA. As many amounts of DNA are necessary for used in many different ways like molecular and genetic analysis.. This way provides generating of millions of images of a specific DNA sequence

The principle of PCR technique:

Is that the double-stranded DNA molecules bind to ethidium bromide which emit fluorescence that can be detected, and DNA quantified. The synthesis of genes by PCR and the role of PCR in site-directed mutagenesis are described elsewhere

Technique of PCR:

The essential requirements for PCR are listed below:

1. A target DNA (100-35,000 bp in length).

2. Polymerase enzyme

In this reaction the polymerase enzyme which is used is called Taq polymerase and it is a DNA polymerase enzyme which is stable in high temperatures as it is isolated from a type of bacteria which is thermophilic and it is called thermos aquaticus. This enzyme can withstand at a temperature up to 95° C (i.e., thermo-stable).

3. Two primers (forward and reverse):

They are short sequence of nucleotides, and contain usually from 8 to 60 pairs of bases. They are complementary to certain part of DNA or RNA which will be copied during the reaction. Two short DNA strands are designed to bind to the start and end of the target sequence used in PCR.

4-Deoxyribonucletide triphosphates:

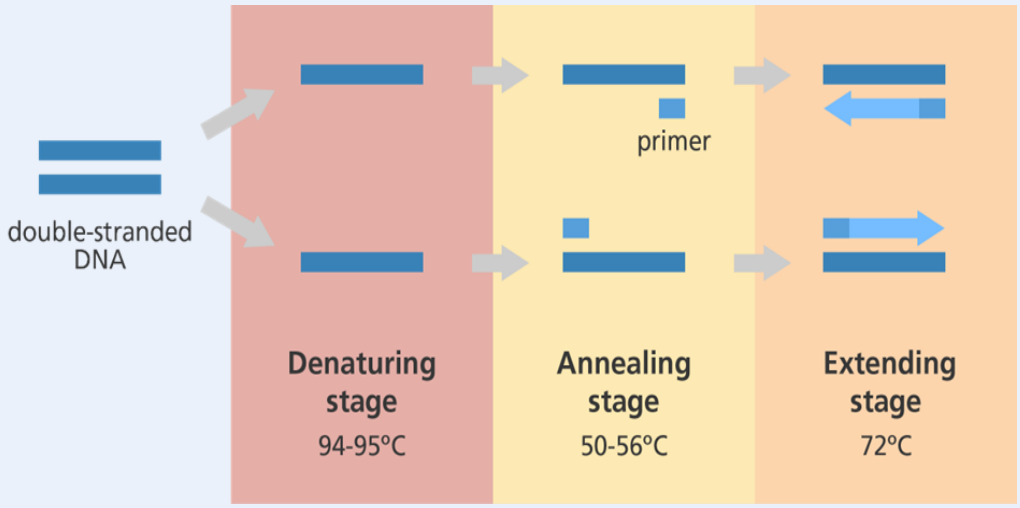
They are four and considered as building blocks for DNA replication. Their names are: adenine, guanine, cytosine and thymine. Polymerase enzyme will add bases which will complement the new growing strand. They are four deoxyribonucleotides (dATP, dCTP, dCTP, dTTP).

5-PCR buffer:

It is used to keep the pH of the system stable. Sometimes it consists of MgCl2 which act as a co-factor for polymerase enzyme and affects activity of enzyme.

The process of PCR:

Components that are mentioned before are mixed and placed in the machine then many repeated cycles of DNA formation are occurred in passing through three main steps which are: 1) denaturation: it is the first step in the reaction. It includes raising the temperature to 90-98 degrees for 20-30 seconds. This leads to breaking bonds of hydrogen that connect between bases of double strands, and separate them to two single strands. It takes four stages to work which are:



Denaturing stage

In this stage, the temperature is raised to 94-95C, as the DNA and all other ingredients are heated. This high temperature leads to the broken of hydrogen bond between two DNA strand. Therefore, this will give us two single strand of DNA that acts as templates for new DNA production. So, in this stage it is important to keep maintain the temperature for long time to make sure the DNA is completely separated, and this usually takes from 15 to 30 seconds.

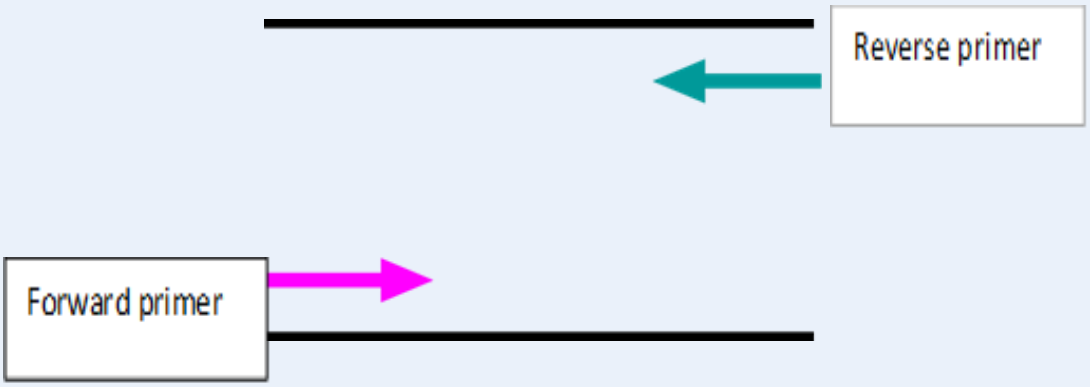
Annealing Stage

In this stage, we ensure coolness from 50 to 60C which help the primer to attach to single stranded DNA on special location by hydrogen bonding. Primers may be DNA or RNA and its sequence may be from 20 to 30 bases in length. They are complementary in sequence to complete the short section of DNA on each end.

The primers are the starting point in PCR reaction, once the primer attach to the DNA and the polymerase enzyme attach to the primer, these will make new DNA strand by completing from the loose DNA.

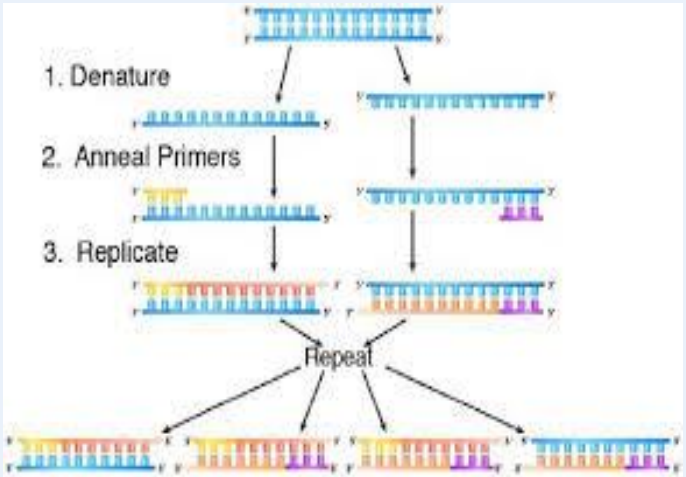
DNA complementary separated strands are opposite from each other, so there are reverse primer and forward primer. This process usually takes from 10 to 30 seconds.

When sequences of primers matches to the single strand of DNA. Polymerase enzyme binds to primer and DNA single strand and the formation of DNA begins.

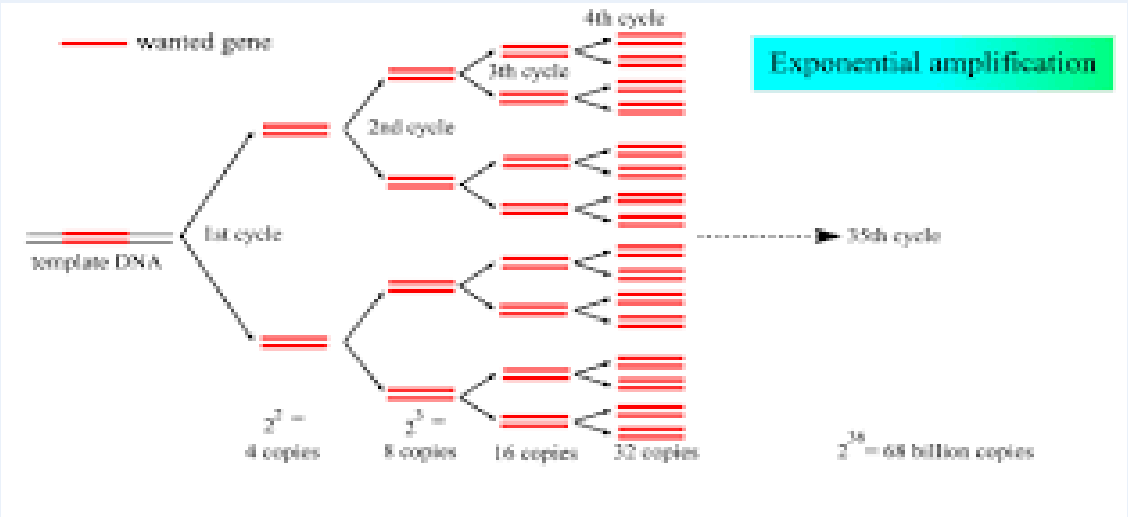


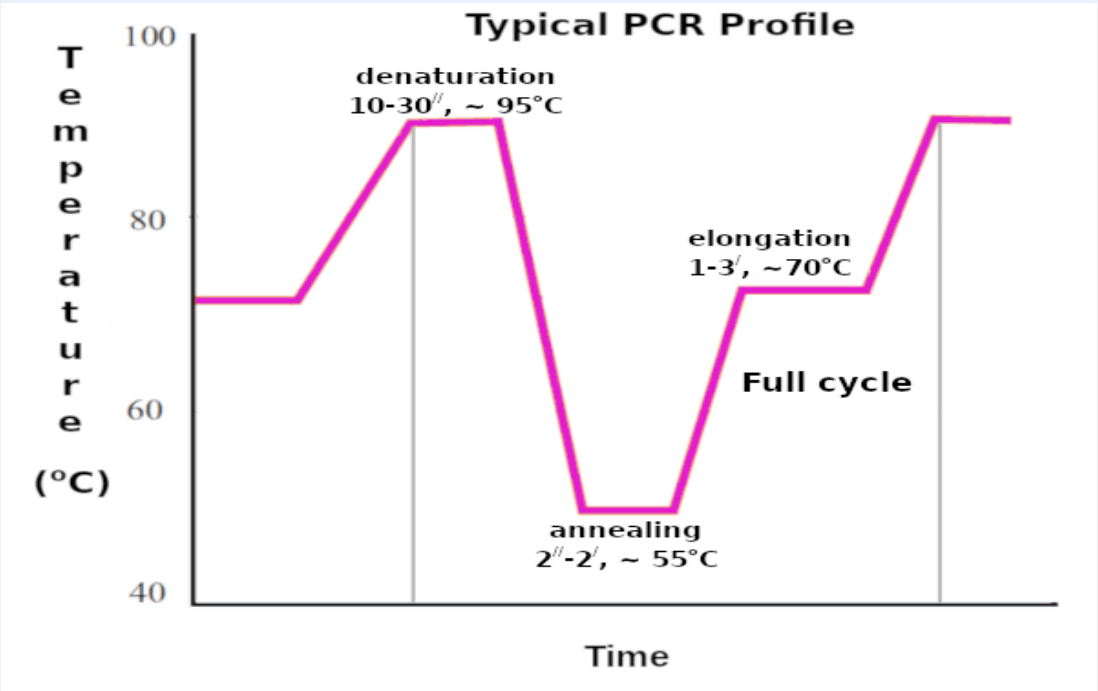
Extending Stage

In the final step, to make the new DNA by using a special taq DNA polymerase enzyme that add DNA bases to complete the reaction, so heat is increased to 72C. This taq DNA polymerase enzyme is extracted from thermophile bacteria known as *thermus aquaticus*, this bacteria lives in hot spring and can increase temperature higher than 80C. So, this means that this DNA polymerase has high stability and can withstand the higher temperature needed to break the double DNA strand in the denaturing stage. Therefore, DNA from other bacteria cannot withstand this high temperature; for example, human polymerase works at 37C. In this stage, the result is new strand of DNA and the duration of this step depends on how the DNA length sequence being amplified and it takes around one minute to copy 1000 DNA bases.



During this step polymerase enzyme works on forming a complementary strand to the template of DNA by adding nucleotides in the direction 5→3.





Applications of PCR:

1) Detection of infectious diseases:
- Detection of human immunodeficiency virus, even it is from the most difficult viruses to be detected. Also used for detection of organisms that causes diseases as tuberculosis.
- Can detect some types of cancer. For example, leukemia.
- Used for detection of DNA of viruses and some sub-types. For example, viruses that causes epidemics.

2) Applications in researches in molecular biology:
- Sequencing of DNA, cloning of DNA and gene expression.

3) Applications in medical field:
- It is used in analyzing of the presence of genetic disease mutations.

Therefore, to detect whether the virus has DNA or RNA it depends on identification of viral protein and nucleic acids. To know this, we must extract the DNA/RNA to ensure this but the extraction of DNA differs from RNA, as RNA is more degradable than DNA.

However, in case of DNA virus, the same and simple method of the PCR is used. On the other hand, the reverse transcriptase polymerase chain reaction is a specific and rapid method used to detect PRRSv in all of the following conditions: serum, oral fluids, lung, lymph nodes, spleen and environmental samples. This method is done by extracting the viral RNA and converting it into DNA by reverse transcriptase, amplification with PCR and detection of the DNA amplified. This method is used in case of RNA virus. Finally, it is seen that PCR is useful in many applications and can detect whether RNA virus or DNA virus.

Fig (IIIa): **The device used for performing PCR technique – Thermocycler**

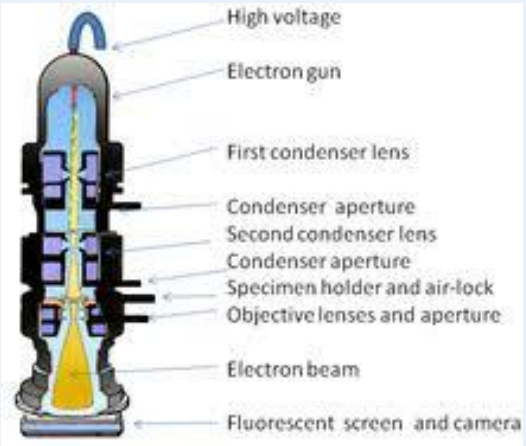
➔ (4) Electron microscope (EM)

There are many types of microscope and an electron microscope is a one type of them it uses electrons to know a specimen and create a magnifying glass. Electronic microscopes have a much larger resolution than optical microscopes and can achieve much higher magnification. Some electronic microscopes can magnify samples up to 2 million times, while the best optical microscopes are limited to magnification 2000 times. Both electronic and optical microscopes have accuracy limits imposed by wavelength. The biggest reason for the accuracy of the electron microscope is amplified to the wavelength of the electron, where the length of the de Broglie wave, much smaller than the photon length of the light, electromagnetic radiation. Virologist use electron microscope in widely way because viruses are usually too tiny to be scanned directly by using microscope's light. And it is necessary to analyze morphology of viruses to diagnose a virus in specific clinical cases or to analyze the introduction and compilation of the virus.

In addition, the characteristic control of the virus particle is necessary if the virus is spread in the cell culture, especially if the virus genome is altered. In most events, the methodology's basic for scanning electron microscopy such as: negative staining and ultrasound division, is sufficient to give pertinent information about the virus infrastructure. Furthermore, the description of the fixed protocols for negative coloring and the net resonance division is complemented by passive immunoglobulin protocols and rapid resonance division.

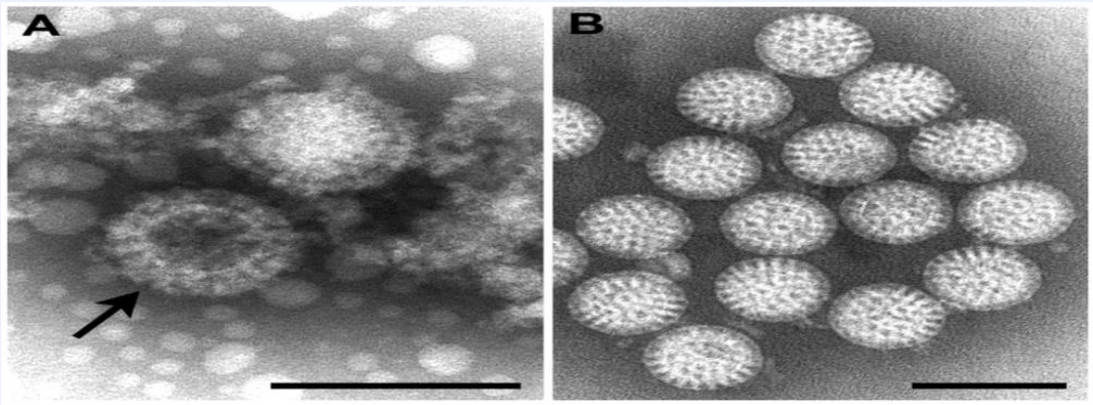
- **Types of electron microscope:**

Electron microscope has two main type froms; its - EM transmission (TEM) and EM scan (SEM). The (SEM) is used to display tender specimens (tissue, sections, molecules.) out of which electrons can generate a projection picture. TEM is similar to the traditional light microscope (composite) in many ways.



Transmission Electron Microscope

TEM functions are many; to depict the inner part of cells (in tender sections), protein molecules' structure (contrasting with metal shading), organization of molecules in viruses and cytoskeletal lines (prepared by negative staining technique), and configuration of protein molecules in cell membranes by breaking the freezing). Viruses are very small and can only be seen by TEM (electron microscope for transmission).



- **Current use of EM in diagnostic virology:**

Body fluids are the samples that are very often received for viral testing in the EM diagnostic laboratory, especially feces and urine samples; despite of all fluids samples (CSF, bronchial liquid, tears, bubbles or sludge) can be processed by negative staining and displayed within few time (60 sec). In fecal samples case, generally gastrointestinal viruses cannot grow in tissue cultures maintained by routine viral culture laboratories, and molecular or immunological reagents are not present to all gastrointestinal viruses. Thus, EM is the diagnostic method most likely to infect all these factors if they are present in sufficient numbers.

With regard to urine samples, a lot of concepts still make EM a more useful test method, although PCR tests are more sensitive. For instance, many adults (~ 90%) are exposed to multiple tumor viruses, and are likely to produce a positive urine test PCR. EM, on the other hand, is not sensitive, and it seems that finding morphological evidence for polymaphyls in urine indicates and raise in the important virus titer in monitoring the re-activation of the multiple-tumor virus in bone marrow patients and kidney transplant patients.

- **Negative staining:**

Negative coloring is a quick step used to display small property, such as viruses in liquids. For sample conversion, large property (such as cell or bacteria wreckage) ought to be rejected at down rapitidy (e.g., at 2000 x g) to few minutes (3to5). The supernatant shall be placed immediately on a net and a negative coating (only 10 to 15 minutes required) or, whether sufficient size is provided, the supernatant is extremely centrifuged, the resulting pellet is suspended in a small microliters of water and after that patched poorly (1 to 1.5 H). The super centrifugal oblige should be 100,000 ×, and the type of tool used and the time counts on the specimen quantity.

For instance, an Airfuge rotates from 50 µl in the electron microscope - 90 rotor or from 200 to 400 µl in tubular rotor, causing damage for pellet virus in 30 minutes. To include that the viruses are pelleted in huge sizes (e.g., 2 to 4 ml) in an ultrafiltrial centrifuge, 50 minutes is enough. If enough sample size is provided, we advise that all fluid samples (especially urine and CSF) be concentrated by super centrifugal.

- **Thin section:**

Thin division of tissues and cells is always required to help electron beam penetration and avoid thick ones which cannot be fully permeated. Any method for firming tissues for the thin section is appropriate for screening / detecting viruses. This involves installation at 2 - 4% of the buffered glutaraldehyde, washing in the buffer, installation / coloring using 1 % of stored storage, washing, and often (but not necessarily) fixing block / patching with 0.5 to 1 % Temporarily or hydraulically. In a group series of acetone or ethanol solutions and then incorporated into epoxy resins is followed by dehydration.

- **Fast technologies:**

Negative staining is a fast procedure; it takes only fifteen minutes for immediate preparation (liquid placement directly on net). The longer step is concentrating the sample by centrifugation or other procedure. The super centrifugal capacity is 100000 x g for one hour, and the aerobic fungi takes half hour at 100,000 x g (30 lbs. of air pressure). Super centrifuges must be used for all fluids, including feces samples if sufficient quantity is sent. Aerobic fungi can be used for washing and concentration more pellets that are obtained using an ultrafilterial centrifuge

Treatment from viral infection

Treatment of virus

As known viruses are not similar to bacteria as viruses mainly rely on mimicking their host cells metabolic functions, and also antibiotics have no significant effect against viruses at all. It's very difficult to find out chemical substances that have the ability to kill the viruses without harming the cell of the host, however scientists have developed an antiviral agents that for certain types of viruses such as influenza virus. The mechanism that these drugs rely on is targeting a viral enzyme known as neuraminidase. These enzyme inhibitors are highly recommended to be taken as prophylactics or within the first hours of the exposure that should not exceed the 30 h maximum to give the desired action and limit the spread of the viruses.

Chemical substances that are used in management of viral infections and have proved its ability to specifically targeting the replication of the viruses without causing a huge harm to the cell replication these drugs are acyclovir, zidovudine and ribavirin. These drugs have shown a great effect on improving patients with viral infections and also curing them without causing toxic side effects to the host cells.

It was shown that zidovudine has a great effect on prolonging the patient lives that are infected with AIDS or HIV.

Interferons are considered as natural cell products that are formed by the cells that exert an antiviral activity. Interferons are proteins that mainly synthesized by the cells of the vertebrates.

Interferons might be formed without stimulation of the cell or intrinsically or can be formed in a response of a viral infection or even to immune reaction as well. --Interferons play a big role in inhibiting the multiplication of viruses. In general interferons are specific for species; this means that the interferons will only inhibit the viral infection in the cells that have produced these interferons.

Interferon have shown a great results and success in treating viral diseases including rhinovirus that cause cold, herpes virus in addition to papillomaviruses that cause warts and benign tumors. As it can cause protein synthesis inhibition in the cells of the host taking a large amounts of interferons could be very harmful.

→Immunization

What immunization means:

Immunization is the process by which an individual becomes resistant to an infectious disease, typically through vaccine administration (Vaccination). Vaccines stimulate the body's own immune system to secure the individual from infection or disease afterwards. Immunization is a proven tool for controlling and eliminating life-threatening infectious diseases and an estimated annual average of 2 to 3 million deaths.

Immunity through immunization occurs without the effect of illness and without the danger of life-threatening disease complications. Once a person is immunized, specific immune cells called memory cells will prevent re-infection in the future once again. Not all vaccines, however, provide immunity for a lifetime. For example, tetanus vaccine requires booster doses to maintain immunity for adults every 10 years.

There are two types of immunization:

-Passive immunization:

Passive immunity is where the pre-synthetic elements of the immune system are transferred to the body so that the body does not need to produce these elements by itself. Currently, antibodies can be used for passive immunization. This method of immunization begins to work very quickly, but it lasts for a short time because antibodies decompose naturally, and if no B cells produce more antibodies, they disappear. When antibodies are transported from the mother to the fetus during pregnancy, passive immunization can occur physiologically to protect the fetus before and shortly after birth. Passive immunization (manual) is usually administered by injection and if a particular disease has recently erupted or used as an emergency treatment for toxicity, like tetanus. Antibodies produced in animals, known as serum therapy despite anaphylactic shock is likely to occur due to immunity to the serum of the animal itself.

Thus, if available, cell culture is used to humanize antibodies in vitro.

Active immunization: can occur naturally when a person comes into contact with, for example, a microorganism. The immune system will eventually produce antibodies and other defenses against microorganisms. The next time, the immune response to this microbe can be very effective; this is the case with many childhood infections.

A person will only contract once, but will be immunized later. Artificial active immunization refers to the place where microorganisms or parts of them are injected into the body before they can be naturally ingested.

When use intact microorganisms are, they are pretreated. Immunization is very important to great that the control Disease's centers and Prevent named it one of the (Top Ten Public Health Achievements of the 20th Century) .

The live attenuated vaccines pathogenicity is reduced. Their effectiveness depends on ability of the immune system by triggering and replicating a response similar to an infection naturally .A single dose is usually effective. Examples of live attenuated vaccines: MMR, yellow fever, rotavirus.

Active immunity	**Passive immunity**
Active immunization involves your body's direct response to an unknown pathogen. This reaction is an antibody that produces an antigen specific for a particular pathogen.	Passive immunization is an immune response involving antibodies obtained in vitro.

Economics of immunization:

Socially optimal outcome:

The size of the difference depends on the value of society for different immunization. Many times, immunization does not reach a socially optimal amount sufficient to destroy the antigen. Instead, the number of societies they reach allows the optimal number of patients. The most common immune infection in United States still exist in very small amounts, with occasional large out breaks. The good example of a disease is Measles whose best social conditions leave enough room for out breaks in United States, often leading to a small number of deaths like smallpox.

In these cases, the marginal benefits of society are so great that society is paying cost of reach level of immunity, which makes impossible to spread and 5 survival of the disease. Although the severity of certain diseases varies, the ratio of the cost of immunization to the benefit of marginal of society mean that complete eradication is not usually the ultimate immunization goal. Although it is difficult to say that the best results in society are, we know that it is not eradicating all diseases that are vaccinated.

Positive externality:

Immunization imposes so-called social consumer externalities. As well providing individuals against certain antigens by protection , it also provides protection in society with other individuals by group immunity. Since this additional protection does not considered transactions of market of immunization, we believe that the marginal benefit of each immunization is underestimated. It is caused by making decisions individual based on marginal benefits rather than social marginal gains. Underestimation of Society of immunization: means that by transactions of normal market, our final number is lower than the optimal amount in society.

Internalizing the externality: means payments equivalent to marginal benefits should be paid. In some countries such as United States, its payments are provided in form of government subsidies. Prior to 1962, the immunization program in US was conducted at the local and state government levels. Inconsistent subsidies have led by some areas of US reaching the best number in society, while others have no subsidies and are still at the level of private marginal immunity. Since 1962 and the Vaccination Assistance Act, it is very difficult saying when the social optimization had been achieved. As well determine the hardships of true social marginal benefits of immunization; we also see that civilization movements have changed the marginal benefit curve.

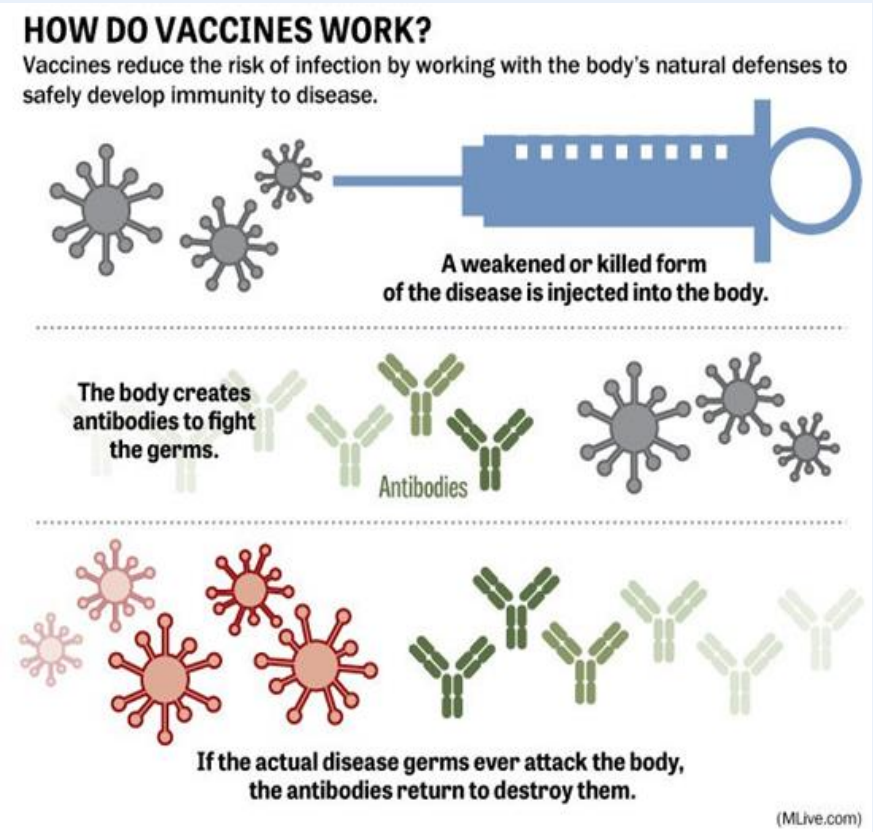
Vaccination dispute, it has variation the way private citizens consider the marginal benefits of immunization. When someone A believes that there is a significant risk of health, which may be greater than antigen oneself, and is immune, they can be reluctant for paying or receive immunization. By fewer and fewer participants, marginal gains, and social optimization, it is very difficult for governments achieving this by subsidies. In addition to government intervention by subsidies, can also bring society to the best results in society to provide free immunization to developed regions. Without ability for beginning immunization, societies of developing cannot achieve the amount determined by marginal benefits. By implementing a six immunization programs, organization can turn privately under-vaccinated communities into social optimization.

→Vaccination

How vaccines work:

Vaccines help the body to defend itself when it invaded by germs like virus:

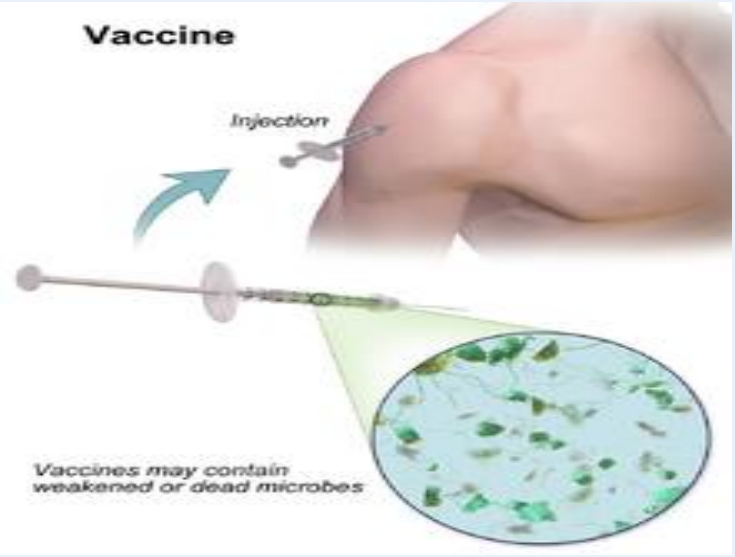
1. They expose the body to a very tiny, very secure quantity of weakened or murdered viruses or bacteria.
2. If you are exposed to it later in life, your immune system will then learn to recognize and attack the infection.
3. You won't get ill as a result, or you might have a milder infection. This is a natural way of dealing with diseases of infection.



Types of vaccines:

- ✓ **Live virus vaccines:** Use a weakened (attenuated) virus form. Examples are the vaccine for measles, mumps, and rubella (MMR) and varicella (chickenpox).
- ✓ **Killed (Inactivated) vaccines:** They are produced of protein or other small pieces of virus. A good example is the vaccine for whooping cough (pertussis).
- ✓ **Toxoid vaccines:** Contain a toxin or virus toxin or chemicals. They make you immune from the infection's harmful effects rather than from the infection itself. Examples are vaccines for diphtheria and tetanus.
- ✓ **Biosynthetic vaccine:** Contain substances manufactured that are very comparable to virus parts. The vaccine for hepatitis B is and specific example.

(All are discussed in detail in the next pages)



(1) Subunit, recombinant and conjugate vaccines:

• Subunit vaccines: Subunit vaccines involve only the antigens that stimulate the immune system. These vaccines use epitopes the very special parts of the antigen which antibodies recognize and bind to. They are surface protein and used for stimulating the immune system against the virus from which it is derived. Subunit vaccines do not contain live components of pathogen but contain only antigenic parts of the pathogen which is the main axis that gives the immune response. Subunit vaccines are very safe due to absence of live components. Subunit vaccines have less strong immune response than live vaccines. Example: plague immunization.

• Recombinant vaccines: they are similar to DNA vaccines. They introduce the microbial DNA to cells of the body through a bacterium which is called "victor" Example: DPT

• Conjugate vaccines: The immune response in this type is achieved by attaching the saccharide to the carrier protein, which can stimulate long-term immune response. Conjugate vaccines are used in the presence of an outer coating called polysaccharide found on the bacterium .The immature immune system sometimes fails to resist bacteria covered by the Polysaccharide coating as the polysaccharide coating protects the antigens of bacterium. Conjugated vaccines provide a protective response against diseases caused by encapsulated organisms such as meningococcal. Example: Haemophilus influenzae type B vaccines.

These vaccines stimulate the immune system and cause a very strong immune response that is resistant to the pathogenesis of the disease in the germ, because these vaccines do not use the entire organism, but use certain parts of the germ. These vaccines are used by a large number of patients and also with people with impaired immune systems.

These vaccines are used to protect against:

- Hib (Haemophilus influenza type b)
- Hepatitis B
- HPV (human papilloma virus)
- (part of the DTaP combined vaccine)
- Pneumococcal diseases
- Meningococcal diseases
- Shingles
- Pertussis (part of DTaP combined immunization)

(2) Inactivated vaccines:

Inactivated vaccines uses ‘wild’ viruses or bacteria that are grown in a culture medium and should be inactivated before being involved in a vaccine, or made using a protein, toxin or polysaccharide (sugar) from bacteria (subunit vaccines) or viruses. After immunization, nothing in an inactivated vaccine is alive the vaccine antigens cannot grow in the disease or vaccinated person.

So these types of vaccine is safe to give to a person with an double immune system response although a person with an impaired immune system response cannot develop the same amount of protection after immunization because of receiving the vaccine and this requires multiple doses. Some of this can require periodic supplemental doses to increase protection. Some examples for inactivated vaccines:

1- Hepatitis A,

2- Influenza

3- Polio vaccines.

(3) Toxoid vaccines:

Toxoid type can use a poison (harmful product) made by the microbe that causes an illness. They made immunity by using some parts of the microbe that cause illness in state the same microbe so the targeted response can achieve in parts which infected so that you need some shots to protect against diseases. We can protect human from some disease by using toxoid vaccine the example for this type: Diphtheria, and Tetanus

(4) Live-attenuated vaccines:

Theses vaccines consist of modified strains of a pathogen and use weakened form of the pathogen for causing the disease. These vaccines stimulate the immune system in a strong and long-term and achieve a very strong preventive response where two doses of these live vaccines are sufficient to protect against the diseases and germs caused by them.

However, there are some limitations for these live vaccines, including the care of patients before receiving these vaccines, because these vaccines contain the proportion of weakened live viruses.

Example: oral poliovirus vaccines (OPV), yellow fever virus vaccines, varicella zoster vaccines

Live vaccines are used to protect against:

- Measles, mumps, rubella (MMR combined vaccine)
- Rotavirus
- Smallpox
- Chickenpox
- Yellow fever

Importance of Immunization:

1. Promote health: They make healthy people remain healthy, removing a significant barrier to human development, unlike many other health interventions.
2. Expansive reach: They secure people, communities and entire populations, like the eradication of smallpox.
3. Rapid impact: Most vaccines have an almost instant effect on groups and populations. Vaccination reduced global deaths from measles by 78% between (2000 and 2008).
4. Save lives and costs.

Benefits of immunization:

- Immunization is a simple and effective way for you and your family to be protected. In addition, it helps others to control serious diseases in our community.
- In order to combat certain illnesses, immunization acts by triggering the immune system. If a vaccinated individual comes into touch with these illnesses, they can react more efficiently to their immune system. This either avoids or decreases the seriousness of the illness.

Routes of administration:

A vaccine may be administered orally, by injection (intramuscular, intradermal, subcutaneous), by puncture, transdermal or intranasal .Several latest clinical trials directed at delivering the vaccines via mucosal surfaces to be taken up by the common mucosal immunity system, thus removing the need for injections.

Table (3): Side effects

Mild side effects	Moderate side effects	Severe side effects
1. **Mild fever** 2. **Redness, soreness, swelling at the injection site** 3. **Fatigue, poor appetite** 4. **Vomiting**	1. Seizure 2. High fever	1. Serious allergic reaction (1 in 1,000,000) 2. Other severe problems including long-term seizure, coma, brain damage

Production of vaccination:

1-Generation of the antigen:

The first step to produce a vaccine is creating the antigen that will increase the immune response. The pathogen's proteins or DNA must be grown by using these mechanisms:

-Viruses are built on primary cells like cells from fertilized eggs or using chicken embryos (e.g. influenza).

-Bacteria is grown in bioreactors which are devices that use a part of medium that ideals the preparation of the antigens.

-Recombinant proteins in the pathogen can be generated either in cell culture, bacteria or yeast.

2. Isolation and release of the antigen:

The goal of this second step is to release bacteria or viruses as possible. The antigen will be disconnect from the cells and isolated from the other part of the growth medium and protein that are still present.

3. Purification:

In a third step the antigen will must be purified in order to create a high purity/quality product. This will be important for using different techniques from protein purification.

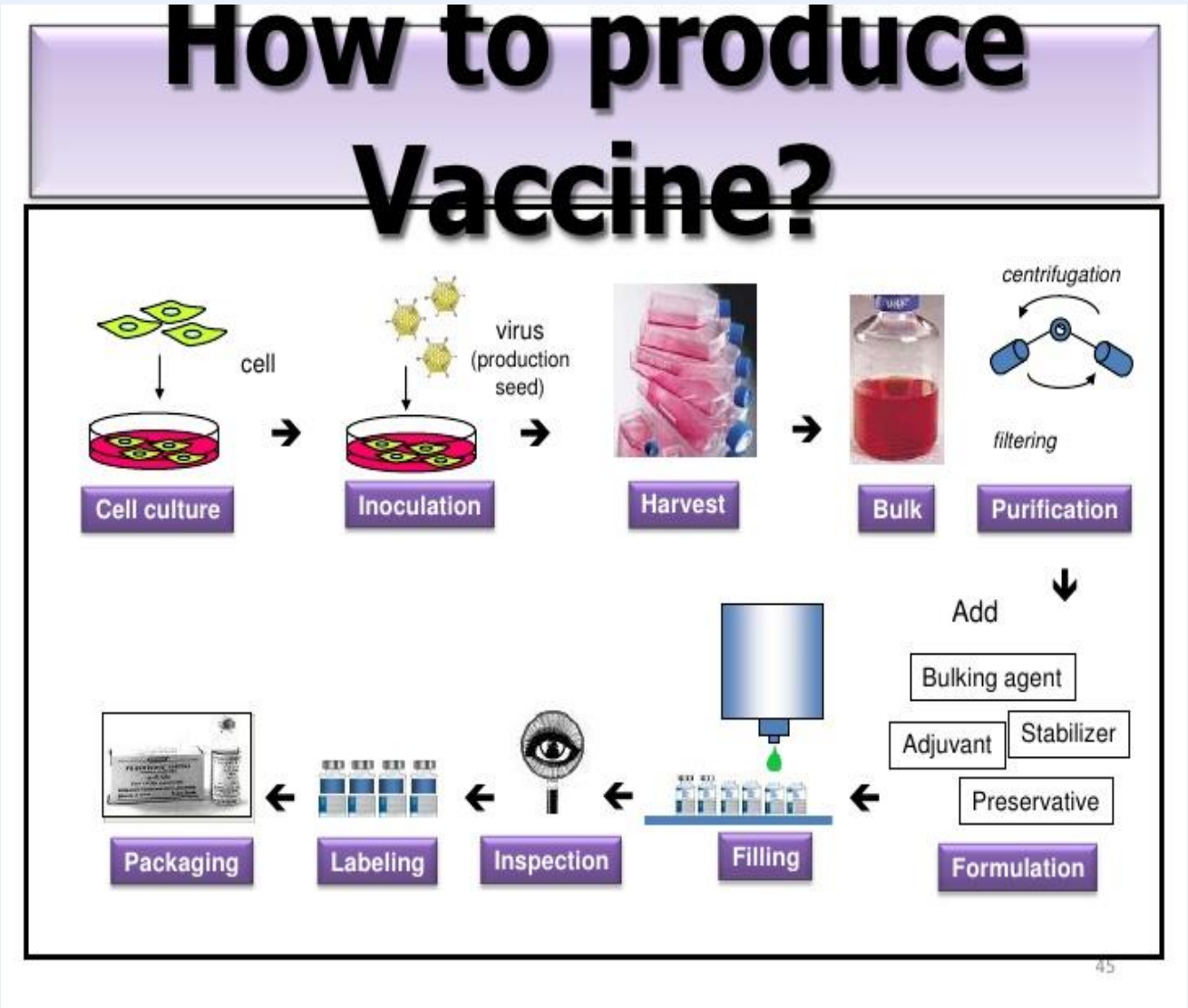
Because of different in protein size, we use many separation techniques like chemical, physical and biological activity.

4. Addition of other substance:

The fourth step may involve the addition of an adjuvant, which is a material that enhances the recipient's immune response to a supplied antigen. The vaccine is then formed by adding preservatives to prolong the storage life or stabilizer to allow multi dose vials to be used safely as needed. Interactions between other substance and antigen, all components that constitute the final vaccine are mixed and combined uniformly in a syringe or single vial.

5. Packaging:

Once the vaccine is put in recipient vessel (either a syringe or a Vail), it is sealed with sterile stoppers. It is shown in the diagram below. Finally, the vaccine is labeled and distributed in all over the world.


How to produce Vaccine?
cell
virus (production seed)
centrifugation
filtering
Cell culture
Inoculation
Harvest
Bulk
Purification
Add
Bulking agent
Adjuvant
Stabilizer
Preservative
Formulation
Filling
Inspection
Labeling
Packaging
45

Doses of vaccines:

1-Toxin vaccines:

Tetanus, pertussis, and diphtheria:

Any child need 5 doses ,first in 2 months, second in 4 months ,third in 6 months, fourth in 15-18 months and fifth in 4-6 years

In the adult need (.5) ml

2-Live attenuated vaccines:

Measles vaccination:

Any child needs 2 doses from MMR, first 12-15 months, and the second 4-6 years

In adult need (1) ml

Chickenpox:

Child needs 2 doses of chickenpox, first dose at 12-15 months and second 4-6 months

3-Cojugated vaccine:

Hepatitis B:

Any child needs 3-4 doses of hepatitis b

The first dose at birth, the second 1-2 months, the third 4 months if need, at least 6-18 months and in adults >20 need (1) ml

4-Inactivated vaccine:

Hepatitis A:

Any child needs 2 doses, first dose at 1years and second 6-12 months later

In adult <18 need (.5) ml/>19 need (1) ml

5-DNA vaccine:

Influenza:

Everyone have 6 months should vaccine every winter ,younger 9 years need 2 doses and may take more than 1 if he need it

For adult need (.5) ml

6-Recombinant vaccine:

Papilloma viruses:

In adult need (.5) ml

Immunization for infants and children:

Studies determined the dose for vaccination, first in animals and then in humans. To secure kids, small quantities of vaccine are used. These have been shown to be secure and efficient in children and young people with premature and low birth weight, who are obviously much bigger. While the patients are not the same, the response of the immune system is similar.

There are exceptions, of course. For instance, adults are given a greater dose of hepatitis B vaccine than children. The amount of doses sometimes varies based on the patient's age. Children under the age of 9 receive 2 doses of vaccine for influenza. People over the age of 13 receive 2 doses of vaccine for chickenpox.

Table (4): Immunization schedule for children less than 1 year:

Table showing Routine Immunization Schedule for Children less than 1 year

Vaccine	No of Doses	Age	Minimum interval between doses	Route of Administration	Dose	Vaccination site
BCG	1	At birth or as soon as possible after birth	-	Intradermal	0.05ml	Upper left arm
OPV	4	At birth and at 6,10,and 14 weeks of age	4weeks	Oral	2drops	Mouth
DPT	3	At 6,10,and 14 weeks of age	4weeks	Intramuscular	0.5ml	Outer part of thigh
Hepatitis B	3	At birth,6 and 14 weeks of age	4weeks	Intramuscular	0.5ml	Outer part of thigh
Measles	1	at 9months of age	-	Subcutaneous	0.5ml	Upper right arm
Yellow fever	1	at 9months of age	-	Subcutaneous	0.5ml	Upper right arm

Immunization for adults:

All adults need immunizations to assist avoid and spread severe illnesses that may lead to bad health, missed work, medical bills, and family care. Every year, every adult needs a seasonal flu (influenza) vaccine. Flu vaccine is particularly important for individuals with chronic health, pregnant females and older adults.

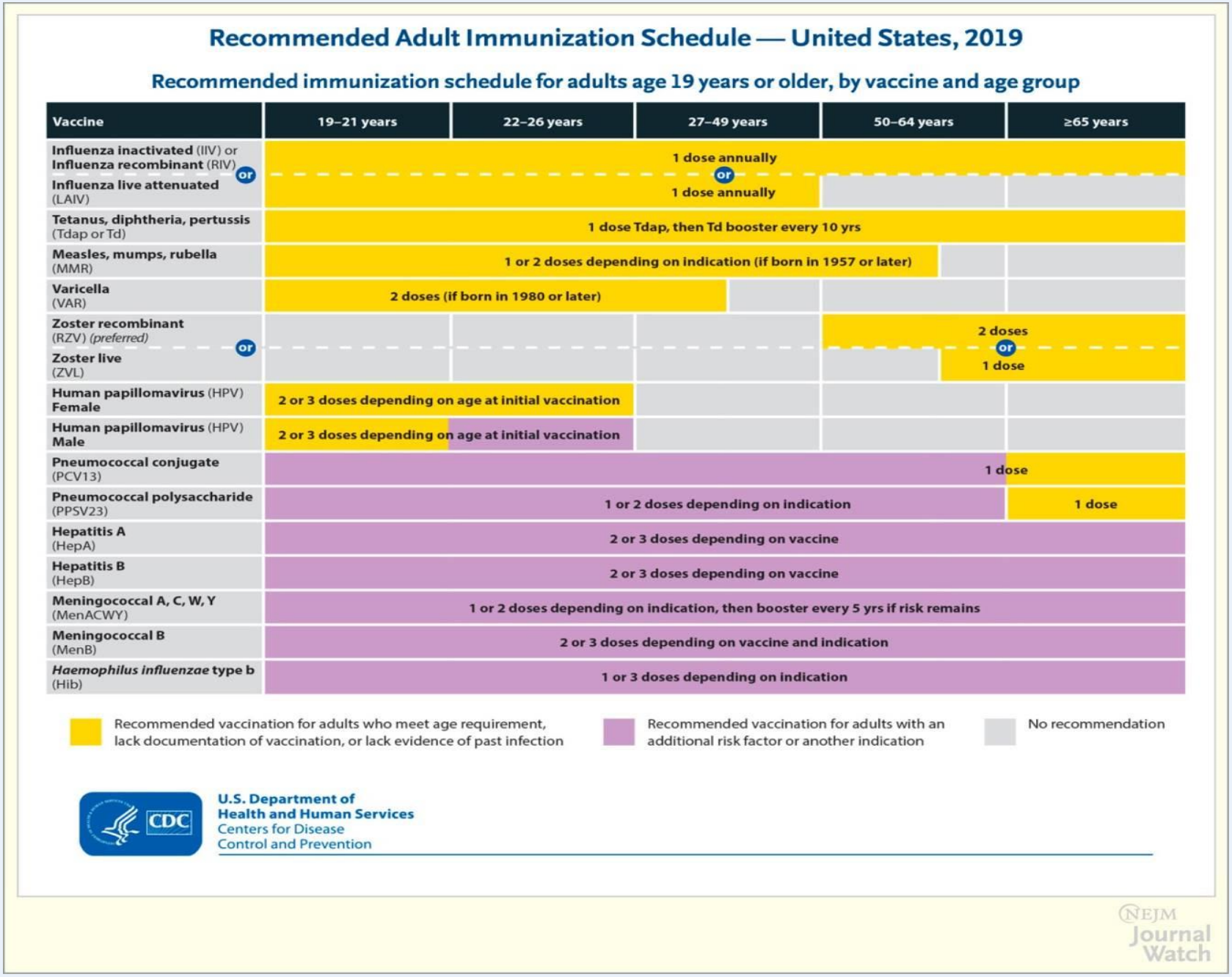
Every adult should get the Tdap vaccine once if they did not receive it as a teenager to defend against pertussis (whooping cough), and then a booster shot every 10 years with Td (tetanus, diphtheria). Furthermore, every time females are pregnant, females should receive the Tdap vaccine, preferably at 27 to 36 weeks.

(Shown on next page)

Who Should NOT be immunized

Some individuals should not get certain vaccines or should wait before they get them because of age, health situations or other factors. For example, Tdap vaccine (combined tetanus, Diphtheria and pertussis). An individual who has ever had a life-threatening allergic reaction following an earlier dose of any vaccine-containing diphtheria, tetanus or pertussis, OR has a serious allergy to any portion of this vaccine should not receive Tdap vaccine and Anyone with coma or long lasting seizures within 7 days of a childhood dose of DTP or DTaP or an earlier dose of Tdap.

Table (5): Immunization schedule for adults:



Recommended Adult Immunization Schedule — United States, 2019

Recommended immunization schedule for adults age 19 years or older, by vaccine and age group

Vaccine	19–21 years	22–26 years	27–49 years	50–64 years	≥65 years
Influenza inactivated (IIV) or **Influenza recombinant** (RIV)	1 dose annually				
or					
Influenza live attenuated (LAIV)	1 dose annually				
Tetanus, diphtheria, pertussis (Tdap or Td)	1 dose Tdap, then Td booster every 10 yrs				
Measles, mumps, rubella (MMR)	1 or 2 doses depending on indication (if born in 1957 or later)				
Varicella (VAR)	2 doses (if born in 1980 or later)				
Zoster recombinant (RZV) *(preferred)*				2 doses	
or				or	
Zoster live (ZVL)				1 dose	
Human papillomavirus (HPV) **Female**	2 or 3 doses depending on age at initial vaccination				
Human papillomavirus (HPV) **Male**	2 or 3 doses depending on age at initial vaccination				
Pneumococcal conjugate (PCV13)	1 dose				
Pneumococcal polysaccharide (PPSV23)	1 or 2 doses depending on indication				1 dose
Hepatitis A (HepA)	2 or 3 doses depending on vaccine				
Hepatitis B (HepB)	2 or 3 doses depending on vaccine				
Meningococcal A, C, W, Y (MenACWY)	1 or 2 doses depending on indication, then booster every 5 yrs if risk remains				
Meningococcal B (MenB)	2 or 3 doses depending on vaccine and indication				
***Haemophilus influenzae* type b** (Hib)	1 or 3 doses depending on indication				

Recommended vaccination for adults who meet age requirement, lack documentation of vaccination, or lack evidence of past infection

Recommended vaccination for adults with an additional risk factor or another indication

No recommendation

CDC

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

NEJM Journal Watch

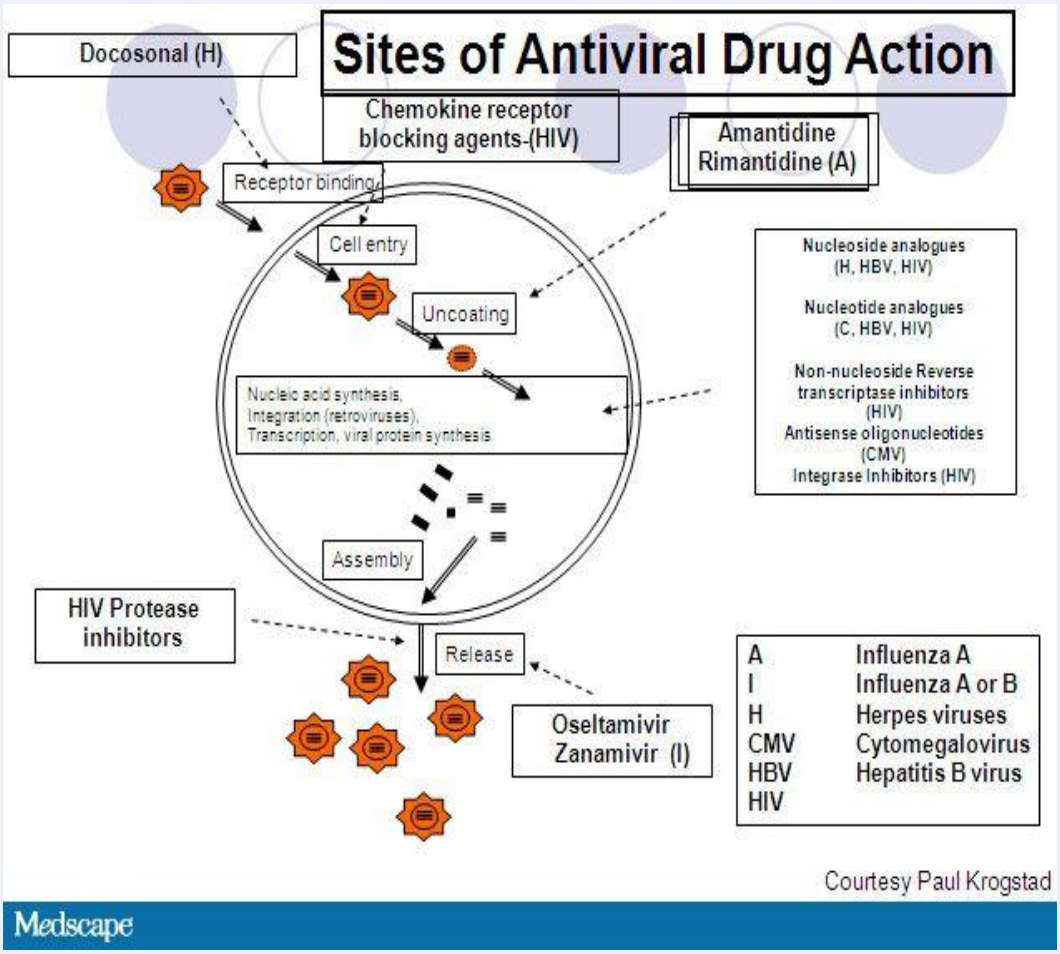
➔ **Antiviral drugs:** Antiviral drugs are used to treat particular viruses by type, and preventing viruses from beginning its development.

Classes	Drugs
1- Anti-herpes virus	Idouridine, Trifluridine Acyclovir, Valacyclovir Famiciclovir Ganciclovir, Valganciclovir Cidofovir, Foscamet, Fomivirsen
2- Anti-influenza virus	Amantadine, Rimantadine Oseltamivir, Zanamivir
3- Anti-hepatitis virus/nonselective antiviral drugs	
a- primarily for hepatitis B	Lamivudine, Adefovir dipivoxil, Tenofovir
b- primarily for hepatitis C	Ribavirin, Interferon alpha
4- Anti-retrovirus	Zalcitabine, Stavudine, Lamivudine, Abacavir Acyclovir (for treatment of herpes)

Table (6): Classification of antiviruses (types of Antivirals)

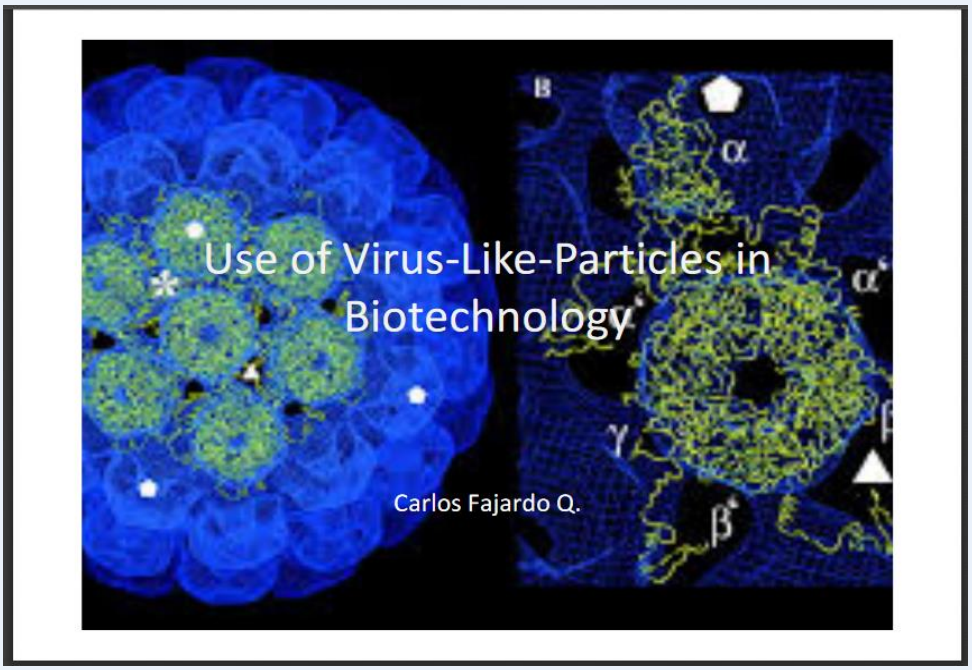
Mechanisms of action of antiviral drugs:

1. Nucleoside reverse transcriptase inhibitors
2. Integrase inhibitors
3. Protease inhibitors
4. Melting inhibitors
5. Non-nucleoside reverse transcriptase inhibitors



Anti-viral drug resistance

A virus **can resist Anti-viral drug** action through mutation procedure.. A virus will infect at the moment of infection and start to reproduce within a preliminary cell. Since subsequent cells were infected. Random mutations in viral genes can occur. The word has been used to manage HIV, the first virus that regularly uses genome sequencing to search for drug resistance.



A-Role of viruses in treatment

1-Development of vaccines.

Vaccines are substances that are introduced to the body which gives it resistance against a certain disease caused by pathogens, there are many types of viral vaccines that have been developed which are **live attenuated vaccines, toxoid vaccines, heterologous vaccines, inactivated vaccines, subunit vaccines, conjugate vaccines, DNA vaccines** and finally **recombinant vector vaccines**.

Vaccines work under the concept of active immunization which means that the body immune system have to develop antibodies for the certain antigens that are presented in the viral vaccine and this type of immunization is long lasting.

The first type is **inactivated vaccine.** They are the type that utilizes dead pathogens and introduce them to the body so it can gain immunization also this type of vaccine is much safer than any other type because the pathogens cant induce any pathogenic action to the body because it is killed, the typhoid inactivated vaccine was the leading developed inactivated vaccine back in 19th century but nowadays there are more inactivated vaccines like polio and hepatitis A vaccines.

Live attenuated vaccines are another widely used type of vaccine it was developed under the concept of using a virus with reduced virulence due to mutations, this type of virus depends on growing the virus in laboratory culture media so it can replicate and to give it a chance to mutate producing a virus with reduced virulence then these (Baxter, 2007 p.554) viruses are injected to humans either subcutaneously or intramuscularly which then activates the immune system to produce antibodies for the viral antigens introduced, the varicella-zoster vaccine and the oral poliovirus vaccine(OPV) are well known viral live attenuated vaccines.

Another important type of vaccine is **subunit vaccine,** which is a type of vaccine that uses the concept of dead pathogens likewise inactivated vaccines but it only uses specific parts of that dead pathogen to be introduced to humans, these specific parts can induce antigenic characters that the human immune system can react to and produce antibodies to neutralize it , a subunit vaccine can contain only a single type of antigen or it can be in combinations, as in Haemophilus influenzae b (Hib) and Hepatitis B.

These combinations of vaccines are types of subunit vaccines that use a singular antigen while influenza vaccine is a type of subunit vaccine that uses two antigens (neuraminidase and haemagglutinin).

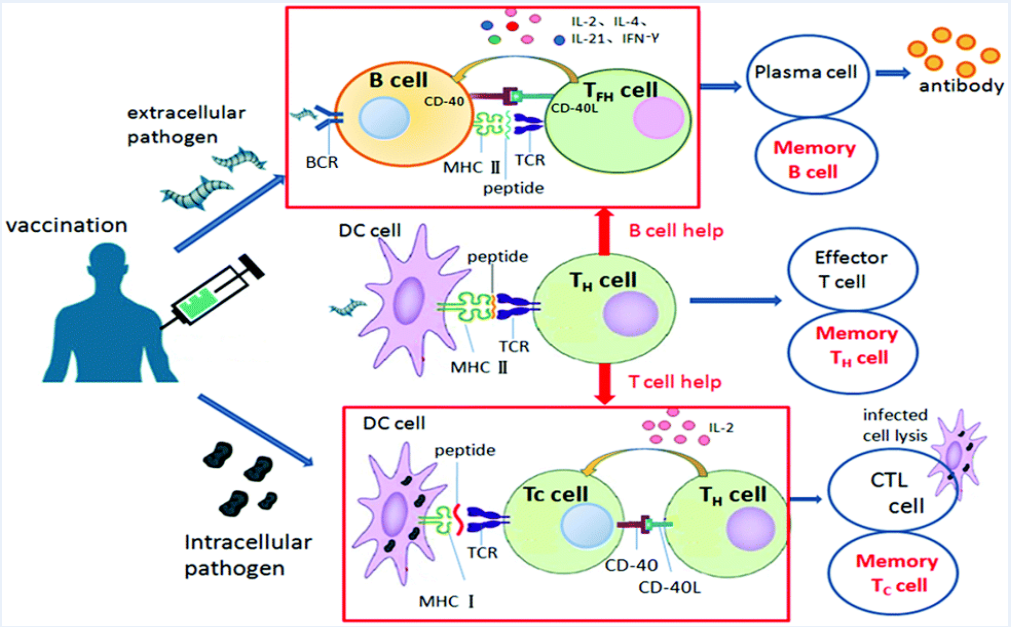
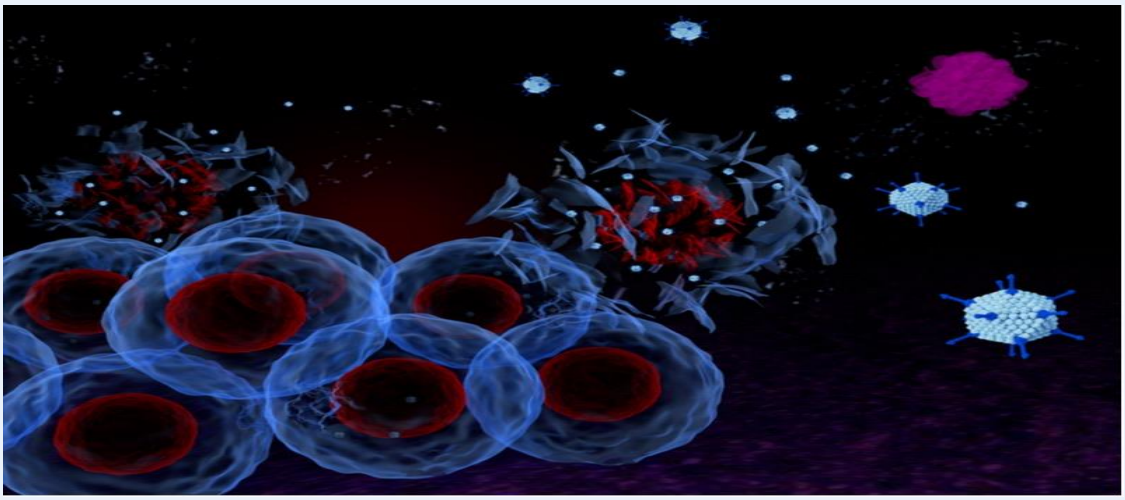


Fig (Va): diagram shows the standard mechanism for vaccines

2-Treatment of cancer:

One of the most innovative use of viruses in biotechnology is using it in the treatment of cancer as this field is growing fast day after day as the viruses has a higher selectivity in targeting the tumor cells and destroy it. Relying on the concept of that the viruses are naturally looking for specific cells to infect as this character make it an ideal option to be used in cancer treatment. The known used virus in this field is Amgen's T-VEC, its herpes virus that is genetically engineered and its used in treatment of melanoma.

However, many viruses are being developed by the scientists and giant pharmaceutical companies, like the studies that are concluded in Germany in order to develop the smallest oncolytic virus that is called ParvOryx this virus has a higher selectivity in killing cancer cell including pancreatic cancer and glioblastoma (Smith and Bundy, 2013).

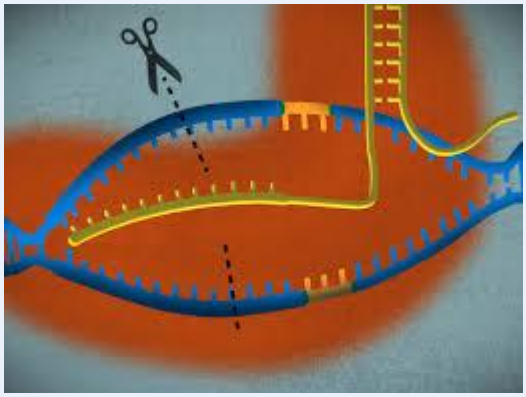


3-Virus-directed enzyme used in a prodrug therapy:

This kind of medication which exploits an inactive form for a cytotoxic drug that is given therefore, the active form of cytotoxic drug is only produced where this certain enzyme is found and active. There is an example like adenovirus stimulating the thymidine kinase enzyme of the herpes simplex virus that can polymerize with the system administration of Ganciclovir, then converted by the Thymidine Kinas to its active form only in cells where this enzyme is present so this is used in helping treatment.

B- Role of virus in biotechnology

How can Recombinant viruses have a role in gene therapy:



Gene therapy is made to introduce genetic material into cells that compensate for any non-functioning genes or a production of beneficial protein. A gene is inserted directly into a cell, and it usually doesn't function. Instead, the virus that is a carrier called a vector is genetically engineered or manufactured to deliver the gene to the cell. Not all viruses are often used as vectors (carriers), because it can deliver this new gene by infecting the cell directly.

These viruses are improvised so they can't cause any diseases when used on people. Some virus like retroviruses, improvise their genetic material means form the new genes into a chromosome in the human cells.

Another type of viruses like as adenoviruses, introduce their DNA into the nucleus of the cell directly, but they don't form new DNA is into a chromosome. The vector can be given IM or IV directly into a specific tissue in the body. Where absorbed up by individual cells. Another way, the sample of the patient's cells can be taken and exposed to the vector (viruses carrying new genes) in the laboratory for examination. The cells containing that virus's carrier are then inserted again to the patient. If this treatment is truly successful, these new genes delivered by the vector will form newly a functioning protein.

Scientists must overcome multiple technical challenges before the gene therapy will become truly a practical approach for treating cancer. For example: 1st scientists must find newly better ways to deliver genes, 2nd then target them to this particular type of cells. They must also ensure that these new genes are precisely controlled by the body through physiologically.

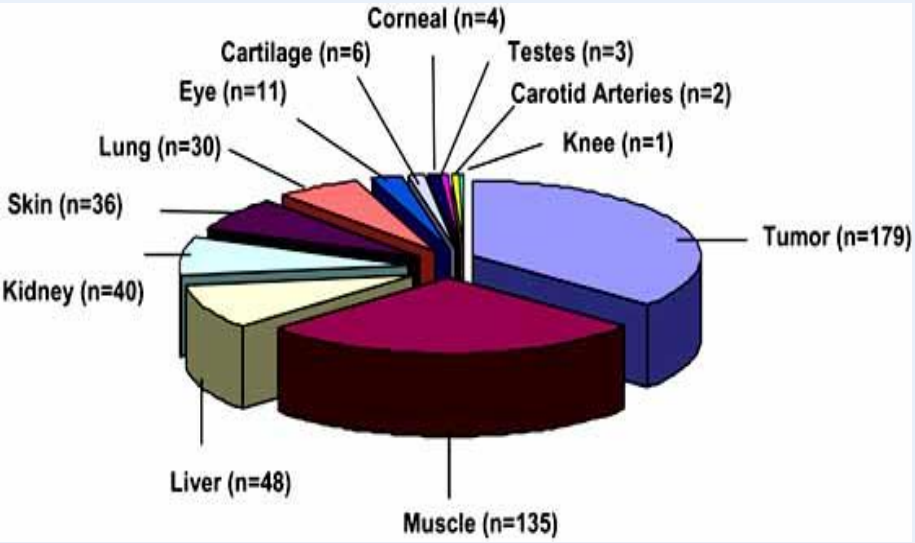


Fig (Vb) How electroporation effective against cancer using pub med as reference and

(n) represents number of cells needed to be exposed to let vectors can start to give its effect for each organ or for group of tissues.

References

1. A.Dusty Miller. Nature International journal of science. Human gene therapy comes of age. *Nature* volume 357, pages455–460 (1992)
2. Ahluwalia, G., D. A. Cooney, H. Mitsuya, A. Fridland, K. P. Flora, Z. Hao, M. Dalal, S. Broder, and D. G. Johns. (1987). Initial studies on the cellular pharmacology of 2',3'- dideoxyinosine, an inhibitor of HIV infectivity. Biochem. Pharmacol. 36:3797-3800

5- Anon., n.d. *Virus - PCR - PRRS diagnostics.* Available at: *https://www.prrs.com/en/prrs/diagnostics/visurs-pcr/*

6- Anon., n.d. *What is PCR (polymerase chain reaction)?* Available at: *https://www.yourgenome.org/facts/what-is-pcr-polymerase-chain-reaction*

7- Aryal, S. (2018). *Techniques of Virus Cultivation*. Microbiology Info.com. Available at: https://microbiologyinfo.com/techniques-of-virus-cultivation/

8- Baltimore D (1971). Expression of animal virus genomes. Bacteriological reviews, 35 (3), 235-41

9- Bauer, G., 2001. Simplicity through complexity: immunoblot with recombinant antigens as the new gold standard in Epstein-Barr virus serology. Clinical laboratory, 47(5-6), pp.223-230

10- Becker SA, Lee TH, Butel SJ, Slagle BL (1998) Hepatitis B virus X protein interferes with cellular DNA repair. J Virol 72:266–271

11- Block TM, Guo H, Guo JT (2007) Molecular virology of hepatitis B virus for clinicians. Clin Liver Dis 11:685–706

3. Blum, M. R., S. H. T. Liao, S. S. Good, and P. de Miranda. 1988. Pharmakokinetics and bioavailability of zidovudine in humans. Am. J. Med. 85(Suppl. 2A):189-194

12- *Bordenave G (May 2003). "Louis Pasteur (1822–1895)". Microbes and Infection / Institut Pasteur.* ***5*** *(6): 553–60. doi:10.1016/S1286-4579(03)00075-3. PMID 12758285*

13- Cattoir, L., Van Hoecke, F., Van Maerken, T., Nys, E., Ryckaert, I., De Boulle, M., Geerts, A., Verhelst, X., Colle, I., Hutse, V. and Suin, V., 2017. Hepatitis E virus serology and PCR: does the methodology matter?. Archives of virology, 162(9), pp.2625-2632

14- Contreras, M., Barbara, J.A.J., Anderson, C.A., Ranasinghe, E., Moore, C., Brennan, M.T., Howell, D.R., Aloysius, S. and Yardumian, A., 1991. Low incidence of non-A, non-B post-transfusion hepatitis in London confirmed by hepatitis C virus serology. The Lancet, 337(8744), pp.753-757

4. David Baxter. Active and passive immunity, vaccine types, excipients and licensing, Occupational Medicine 2007; 57:552–556

5. Delecluse, H. J., & Hammerschmidt, W. (2000). The genetic approach to the Epstein-Barr virus: from basic virology to gene therapy. *Molecular pathology*, *53*(5), 270.

15- Donnelly, J. J., Ulmer, J. B., Shiver, J. W., & Liu, M. A. (1997). DNA vaccines. Annual review of immunology, 15(1), 617-648

16- Erukhimovitch, V., Huleihil, M. and Huleihel, M. (2013). Identification of Contaminated Cells with Viruses, Bacteria, or Fungi by Fourier Transform Infrared Microspectroscopy. *Journal of Spectroscopy*, 2013, pp.1-6. Available at: https://www.hindawi.com/journals/jspec/2013/317458/cta/

17- Garibyan, Lilit; Avashia, Nidhi (2013). "Polymerase Chain Reaction". Journal of Investigative Dermatology

18- Genton, B., Al-Yaman, F., Beck, H. P., Hii, J., Mellor, S., Narara, A., & Alpers, M. P. (1995). The epidemiology of malaria in the Wosera area, East Sepik Province, Papua New Guinea, in preparation for vaccine trials. I. Malariometric indices and immunity. Annals of Tropical Medicine & Parasitology, 89(4), 359376

19- Gibbs, R. (1990); DNA Amplification by the Polymerase Chain Reaction. Analytical Chemistry, 62:1202- 1214

6. Gilbert, C., Bestman-Smith, J. and Boivin, G., 2002. Resistance of herpesviruses to antiviral drugs: clinical impacts and molecular mechanisms. Drug resistance updates, 5(2), pp.88-114

20- Gordon, M. H. Studies of the Viruses of Vaccinia and Variola. Med. Res. Council, Spec. Rep. No. 98

21- HADLER, S. C. (1988). Are booster doses of hepatitis B vaccine necessary? Annals of internal medicine, 108(3), 457-458

22- Hematian, A., Sadeghifard, N., Mohebi, R., Taherikalani, M., Nasrolahi, A., Amraei, M. and Ghafourian, S. (2016). Traditional and Modern Cell Culture in Virus Diagnosis. *Osong Public Health and Research Perspectives*, 7(2), pp.77-82. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4850366/

23- Hendrix, R.W., 2003. Bacteriophage genomics. *Current opinion in* microbiology, *6*(5), pp.506-511

24- Hsu, F. J., Benike, C., Fagnoni, F., Liles, T. M., Czerwinski, D., Taidi, B., ... & Levy, R. (1996). Vaccination of patients with B–cell lymphoma using autologous antigen–pulsed dendritic cells. Nature medicine, 2(1), 52.ISO 690

25- International Symposium on RNA Viruses and Host Genome in Oncogenesis. (1971). *JNCI: Journal of the National Cancer Institute*

26- John N. Barr1 J.N. and Fearns R.(2010). How RNA viruses maintain their genome integrity.Journal of General Virology, 91;1373–1387

27- Joshi, M. polymerase chain reaction: methods, principles, and applications. International journal of biomedical research

28- Joshua O (1974). Expression of Nucleotides genomes. Bacteriological adaption, 33 (4), 236-43

29- Kim, B.K., Han, K.H. and Ahn, S.H., 2011. Hepatitis B virus serology to predict antiviral response in chronic hepatitis B. Digestion, 84(Suppl. 1), pp.29-34

30- Lu, Z., Yokoyama, M., Chen, N., Oka, T., Jung, K., Chang, K. O., ... & Saif, L. J. (2016). Mechanism of cell culture adaptation of an enteric calicivirus, the porcine sapovirus Cowden Strain. Journal of virology, 90(3), 1345-1358

31- Luman, E.T., McCauley, M.M., Stokley, S., Chu, S.Y. and Pickering, L.K., 2002. Timeliness of childhood immunizations. Pediatrics, 110(5), pp.935-939

32- Marintcheva B. (2018). in Harnessing the Power of Viruses, Introduction to Viral Structure, Diversity and BiologyPages 1-26

33- Nestle, F. O., Alijagic, S., Gilliet, M., Sun, Y., Grabbe, S., Dummer, R., ... & Schadendorf, D. (1998). Vaccination of melanoma patients with peptide-or tumorlysate-pulsed dendritic cells. Nature medicine, 4(3), 328

34- Rashtchian, A. (1994). Amplification of RNA. *Genome Research*, 4(2), pp.S83-S91

35- Rerks-Ngarm, S., Pitisuttithum, P., Nitayaphan, S., Kaewkungwal, J., Chiu, J., Paris, R., ... & Benenson, M. (2009). Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. New England Journal of Medicine, 361(23), 2209-2220.ISO 690

36- Said, E.A., Diaz-Griffero, F., Bonte, D., Lamarre, D. and Al-Jabri, A.A., 2018. Immune Responses to RNA Viruses. Journal of immunology research, 2018

7. Salar, R. K., Gahlawat, S. K., Siwach, P., & Duhan, J. S. (Eds.). (2013). *Biotechnology: prospects and applications*. Springer

37- Seto, D. (2010). Viral Genomics and Bioinformatics. Viruses, 2(12), pp.2587- 2593

8. Smith, M.T., Hawes, A.K. and Bundy, B.C., 2013. Reengineering viruses and virus-like particles through chemical functionalization strategies. Current opinion in biotechnology, 24(4), pp.620-6206

38- Stanford Medicine: Clinical virology in the Department of Pathology (2019). *Viral Culture - Clinical Virology - Stanford University School of Medicine*. Clinicalvirology.stanford.edu. Available at: http://clinicalvirology.stanford.edu/culture.html

39- Tuaillon, E., Mondain, A.M., Meroueh, F., Ottomani, L., Picot, M.C., Nagot, N., Van de Perre, P. and Ducos, J., 2010. Dried blood spot for hepatitis C virus serology and molecular testing. Hepatology, 51(3), pp.752-758

40- Tyler CR, Krumwiede C. The danger of decolorizing vaccine virus. Am

J Public Health (N Y). 1925 Apr;15(4):303-4.PMID: 18011486

41- V**an Helvoort T. History of virus research in the twentieth century: the problem of conceptual continuity. Hist Sci. 1994 Jun;32(2):185-235. PMID: 11639267**

42- Van Regenmortel, M.H.V., 1967. Plant virus serology. In Advances in virus research (Vol. 12, pp. 207-271). Academic Press.

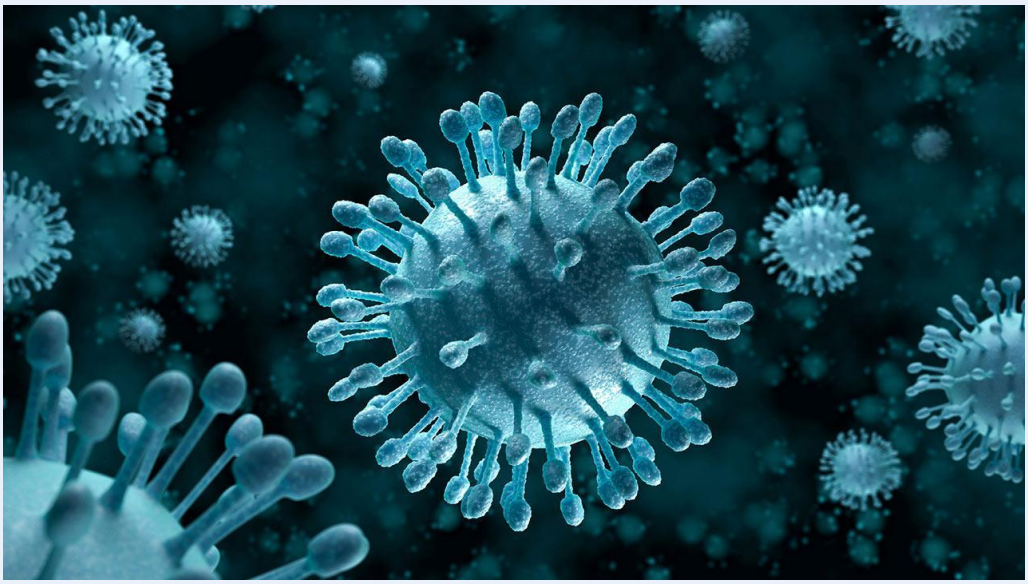
9. Verma, I.M., Naldini, L., Kafri, T., Miyoshi, H., Takahashi, M., Blömer, U., Somia, N., Wang, L. and Gage, F.H., 2000. Gene therapy: promises, problems and prospects. In Genes and Resistance to Disease (pp. 147-157). Springer, Berlin, Heidelberg

43- Walker, S., Wayne, T., Alexis, P. and Raul, R. (2011). DNA Replication process Journal of immunology, 84(5), pp.1406-1422

44- Waterson A.P. and Wilkinson L. An introduction to the history of virology. (Cambridge, 1978)

45- Wroe, A.L., Turner, N. and Salkovskis, P.M., 2004. Understanding and predicting parental decisions about early childhood immunizations. Health Psychology, 23(1), p.33

46- Yong Huang, N. X., Z. W. X. Z. X. Z. Q. D. L. C. D. T., 2015. Ultrasensitive Detection of RNA and DNA Viruses Simultaneously Using Duplex UNDP-PCR Assay.



“An inefficient virus kills its host. A clever virus stays with it.” – James Lovelock

6th October Campus

Mehwar Road intersection with
Wahat Road, 6th October City, Egypt.

Tel : 3837-1517
Tel : 3837-1518
Fax : (+202) 3837-1543
Hotline : 16672