Lec-1

Definition of viruses•

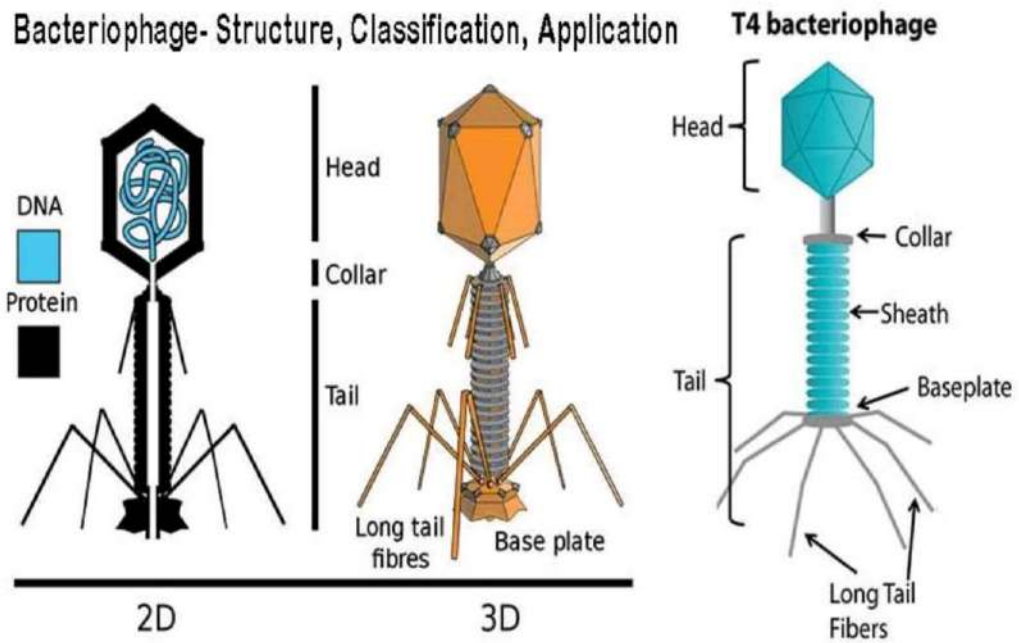
- Are infectious agents
- too small be seen with a light microscope
- Acellular (absence of nucleus, organelles, cytoplasm, plasma membrane).
- No ATP generating metabolism•
- Do not undergo binary fission• Sensitive to interferon

General Characteristics of Viruses

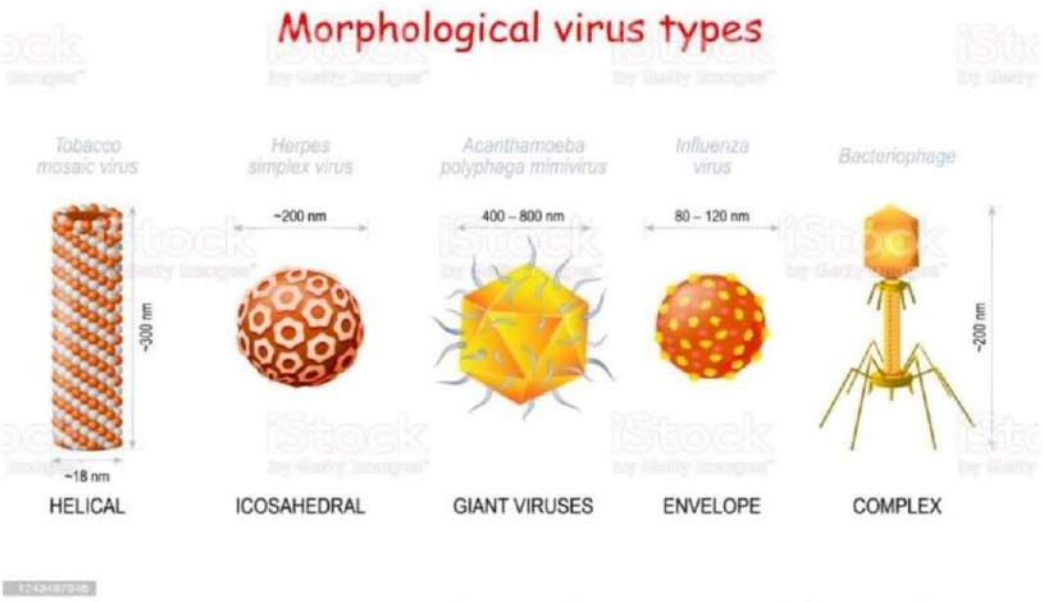
- Obligatory intracellular parasites
- Contain DNA or RNA
- Contain a protein coat
- Some are enclosed by an envelope
- Some viruses have spikes
- Viruses replicate through replication of their nucleic acid and synthesis of the viral protein.
- Viruses do not multiply in defined media.

Components and Function of viruses

- Nucleic Acid Core (DNA or RNA)–to replicate themselves in host cells
- Capsid: Surrounding protein coat –play a key role in the attachment of some viruses. Each capsid is composed of protein subunits called capsomeres
- Envelope: Some viruses have additional surrounding lipid bilayer membrane–outside their capsids Helps to protect from drying (enhances transmission), makes virus more susceptible to chemical agents that dissolve lipids and helps to attach to host cell membrane. Nucleocapsid comprises the viral genome together with the capsid
- Virion: A complete virus particle
- Naked: viruses with a nucleocapsid and no envelope Spikes: projections that extend from the viral envelope that may aid in attachment to the host cell Glycoprotein: these surface projections serve to attach virions to specific receptor sites on susceptible host cell surfaces



Viral morphology



Laboratory Diagnosis of Viral infection

- Specimens sending to the viruses Laboratory.

1- Anticoagulated blood.

2- Skin scraping in case infections caused by poxvirus and herpes virus.

3-Faeces: In case enterovirus infection caused by Rota virus.

4- Swab: Collected from (nose, eye, trachea, vaginal, Rectal).

5- Organs (heart, spleen, liver, kidney, lung.

- In case of died animals it's called (Autopsy).

Transporting of specimens to the viruses Laboratory

1- Specimens transport into strong a sterile screw cap tube or bottles which contains viral transport medium like 199 media add to mixture antibiotics to save the Specimens.

2- They should transported insulated box that contains ice packs or ice cubes in plastic bags.

3- Using some time persevered substance like glycerin 50%or phosphate Buffer saline.

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Lec-2

- **Key elements of a virology laboratory**

The key elements for the establishment of a virology laboratory and diagnostic services are:

(1) Physical infrastructure

(2) Human resources

(3) Equipment and supplies

- **Essential equipment**

 Biosafety cabinets Class II – Three (one for handling cell cultures, one for handling stock viruses and one for processing clinical specimens).

 One incubator and two CO2 incubators (one for uninfected cell cultures and the other for infected cell cultures).

 20 °C and –70 °C freezers..

 Fluorescent microscope with photography attachments.

 Filtration apparatus for preparation of tissue/cell culture media.

 Refrigerate centrifuge.

 water bath.

 pH meter

 Magnetic stirrer.

 Vortex mixer.

 Electronic balance for weighing chemicals.

 Elisa Reader and washer.

 Micropipttes (100ul, 200 ul, 20 ul).

 Multi-channel pipettes – 8 and 12 channel pipettes (20-200 ul and 50-300 ul).

 Autoclave – Two (one for decontamination and one for sterilization).

 Hot air oven for sterilizing glassware.

 Inverted light microscope.

 One personal computer with printer, photocopier, fax machine and telephone lines.

 PCR machine (conventional and real-time).

 Gel electrophoresis apparatus.

 UV transilluminator.

- Ice-making machine.
- Liquid nitrogen containers.
- Water purification

purification/distillation system, which provides high-grade water suitable for tissue culture work.

Glassware such as volumetric flasks, measuring cylinders, pipettes (1 ml, 2 ml, 5 ml and 10 ml), conical flasks, reagent storage bottles (50 ml, 100 ml, 250 ml, 500 ml and 1000 ml.(

Electric brushing machine and automatic pipette washer.

- Desirable equipment
- Shaker water bath.
- Rocking platform.
- Ultracentrifuge

.

*** Reagents and supplies**

A diagnostic virology laboratory requires a whole range of reagents and supplies. Most of the reagents and supplies listed below are generic and would suffice for the diagnosis of a variety of viral infections.

- Diagnostic kits as per requirements of the laboratory
- Tissue culture media
- Foetal bovine serum
- Fluorescent conjugates
- ELISA plates, antibodies and conjugates
- Analytical-grade fine chemicals for preparation of buffers
- Sterile tissue culture plastic ware (25 cm2 and 75cm2 flasks, 24 and 96 well plates, Petri

dishes, tissue culture tubes centrifuge tubes and pipettes(

- V-bottom polystyrene microtitre plates for haemagglutination
- Serum storage cryovials and boxes
- Micropipette tips
- PCR tubes
- PCR reagents (Taq polymerase, reverse transcriptase, primers, probes and agarose(

Electron microscope

Electron microscope constructed by Ernst Ruska

• Definition

- An electron microscope is a microscope that uses a beam of accelerated electrons as a source of illumination.
- It is a special type of microscope having a high resolution of images, able to magnify objects in nanometres, which are formed by controlled use of electrons in vacuum captured on a phosphorescent screen.

• Types of Electron Microscopes

There are several different types of electron microscopes, including the:

1- transmission electron microscope (TEM)
2- scanning electron microscope (SEM)
3- reflection electron microscope (REM)

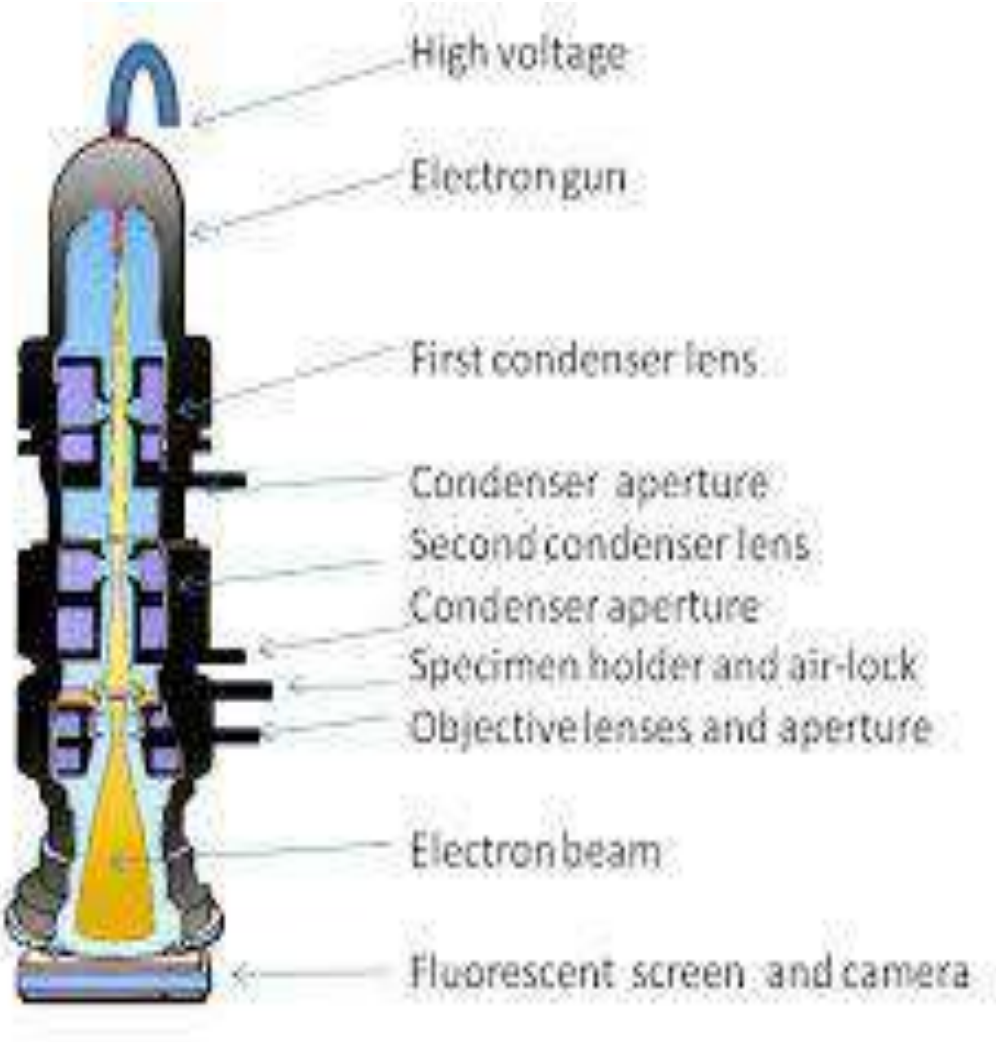
- TEM: magnifies **50 to ~50** million times; the specimen appears flat
- SEM: magnifies **5 to ~ 500,000** times; sharp images of surface features
- STEM: magnifies **5 to ~50** million times; the specimen appears flat

Parts of Electron microscope

EM is in the form of a tall vacuum column which is vertically mounted. It has the following components:

1. **Electron gun**
- The electron gun is a heated tungsten filament, which generates electrons.
2. **Electromagnetic lenses**
- **Condenser lens** focuses the electron beam on the specimen. A second condenser lens forms the electrons into a thin tight beam.
- The electron beam coming out of the specimen passes down the second of magnetic coils called the **objective lens**, which has high power and forms the intermediate magnified image.
- The third set of magnetic lenses called **projector (ocular) lenses** produce the final further magnified image.
- Each of these lenses acts as an image magnifier all the while maintaining an incredible level of detail and resolution.
3. **Specimen Holder**
- The specimen holder is an extremely thin film of carbon or collodion held by a metal grid.
4. **Image viewing and Recording System.**
- The final image is projected on a fluorescent screen.

- Below the fluorescent screen is a camera for recording the image.



- **Working Principle of Electron microscope**

Electron microscopes use signals arising from the interaction of an electron beam with the sample to obtain information about structure, morphology, and composition.

1. The electron gun generates electrons.
2. Two sets of condenser lenses focus the electron beam on the specimen and then into a thin tight beam.
3. The specimen to be examined is made extremely thin, at least 200 times thinner than those used in the optical microscope. Ultra-thin sections of 20-100 nm are cut which is already placed on the specimen holder.

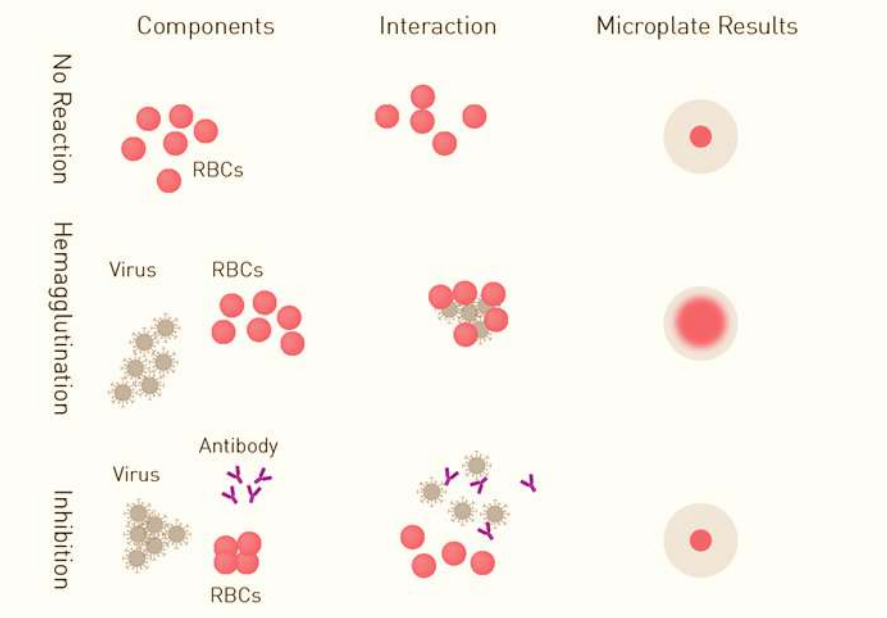
4. The electronic beam passes through the specimen and electrons are scattered depending upon the thickness or refractive index of different parts of the specimen.
5. The denser regions in the specimen scatter more electrons and therefore appear darker in the image since fewer electrons strike that area of the screen. In contrast, transparent regions are brighter.
6. The electron beam coming out of the specimen passes to the objective lens, which has high power and forms the intermediate magnified image.
7. The ocular lenses then produce the final further magnified image.

Lec-3

Enumeration of Virology

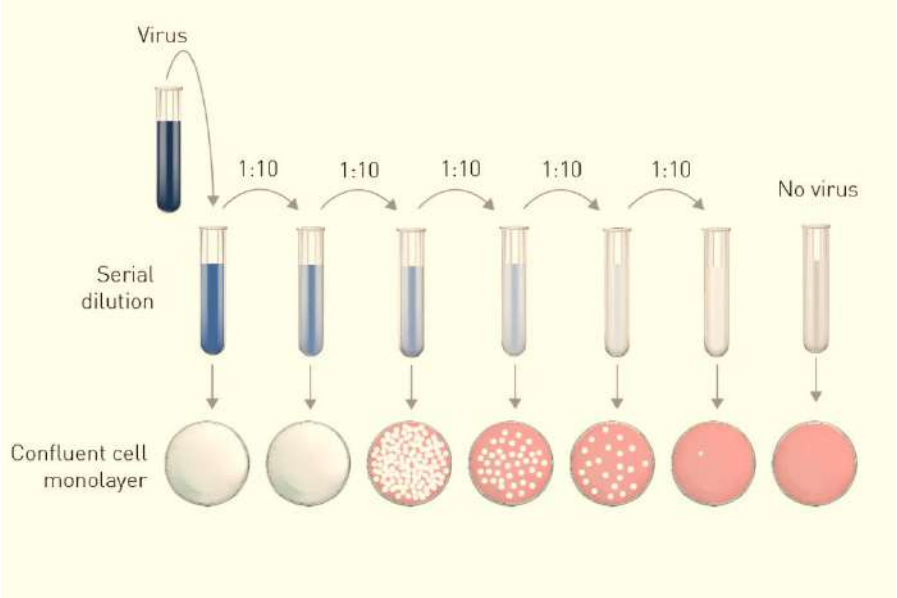
One direct method is to count virions using an electron microscope. However, this process required a long time and a high concentration of virus particles. Another direct counting method uses epi-fluorescence microscopy. This approach is often used to enumerate virus particles in natural water environment. This including stains the virions with fluorescent dyes before to microscopic examination. Finally, sometimes rather than counting virions, the nucleic acid of a virus can be quantified by quantitative-polymerase chain reaction (qPCR). This is possible, because PCR amplifies specific nucleic acids in a mixture of nucleic acids.

An indirect method of counting animal viruses is the hem-agglutination assay. Many animal virus particles bind to the surface of red blood cells. If the ratio of virions to cells is large enough, virions will join the red blood cells together; that is, they agglutinate, forming a network that keeps the red blood cells in suspension. In practice, red blood cells are mixed with diluted samples of the virus particles, and each mixture is examined. The hem-agglutination titer is the highest dilution of the virus preparation that still causes hem-agglutination.



Other indirect assays determine virion numbers based on their infectivity, and many of these are based on the same techniques used for virus cultivation. For example, the plaque assay is gold standard and most used quantitative virus assay. In plaque assays, a stacked monolayer of

cells is infected with unknown concentrations of a lytic virus at varying dilutions. Then infected monolayers are covered with an immobilizing medium to prevent viral spread. Zones of cell death (plaques) will begin to develop, as result of constrained infection and replication. Infected cells will propagate by the replication-lysis-infection cycle, resulting in increasingly distinct and discrete plaques. Visible plaque formation can take 2–14 days, depending on the virus and host cells used. Plaques are typically counterstained by neutral red or crystal violet to be counted manually. In combination with the dilution factor used, the viral titer is calculated in terms of plaque forming units (PFU) per milliliter (PFU/ml). The pfu/ml represents the number of infective particles within the sample and assumes that each plaque formed is representative of one infective virus particle.



The number of pocks on embryonic membranes or necrotic lesions on leaves is used to calculate the concentration of infectious units. When biological effects are not readily quantified by plaque and hem-agglutination assays, the amount of virions required to cause disease or death can be determined. Organisms or cell cultures are inoculated with serial dilutions of a preparation of virus particles. The lethal dose (LD50) is the dilution that contains a concentration (dose) of virions large enough to destroy 50% of the host cells or organisms. In a similar sense, the infectious dose (ID50) is the dose that causes 50% of the host organisms to become infected.

Lec-4

- # Cultivation of Viruses

Viruses can be grown **in vivo** (within a whole living organism, plant, or animal) or **in vitro** (outside a living organism in cells in an artificial environment, such as a test tube, cell culture flask, or agar plate). **Bacteriophages** can be grown in the presence of a dense layer of bacteria (also called a **bacterial lawn**) grown in a 0.7 % soft agar in a Petri dish or flat (horizontal) flask .The agar concentration is decreased from the 1.5% usually used in culturing bacteria. The soft 0.7% agar allows the bacteriophages to easily diffuse through the medium. For lytic bacteriophages, lysing of the bacterial hosts can then be readily observed when a clear zone called a **plaque** is detected. As the phage kills the bacteria, many plaques are observed among the cloudy bacterial lawn.

- **The Primary purposes of Viral cultivation are**:

1- To asolate and identify viruses in clinical spaecimen.
2- To prepare viruses for vaccines.
3- To do detalied research on viruses structure multiplication cycle, genetics and effects on host cells.

- **Method for cultivation of virus**

Generally three methods are employed for the viruses cultivation:

1. inoculation of virus into animals
2. inoculation of virus into embryonated eggs
3. Tissue culture

- **Inoculaton of viruses in animal**

_ Laboratiry animals play an essential role in studies of viral pathogensis

_ Live animals such as monkeys,mice,rabbits,guinea pigs,ferrets are widely used for cultivating viruses

_ Mice are most widely employed animals in virology

_ the selected animals should be healthy and free from any communicable diseases

_ Mice (less than 48 hours old) are most commonly used

_ Mice are susceptible to togavirus and coxsackie viruses,which are inoculated by intracerebral and intranasal route

_ After inoculation, virus multiply in host and develops disease. The animals are observed for symptoms of disease and death

_ then the virus is isolated and purified from the tissue of thes animals

_ Live inoculation was first used on human volunteers for the study of yellow fever virus.

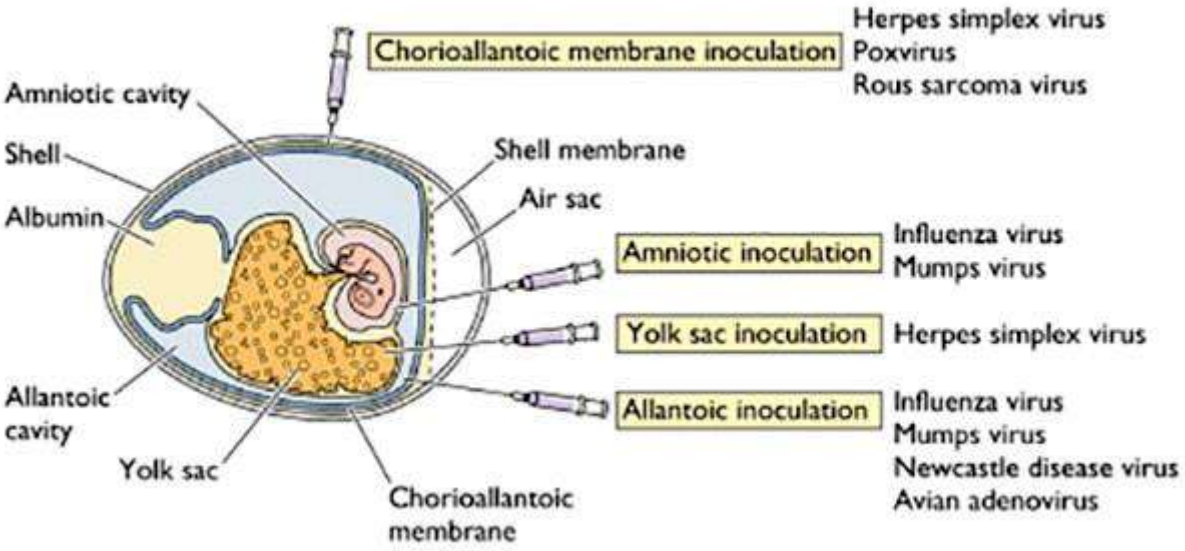
- **Advantages of Animal Inoculation**

1. Diagnosis, Pathogenesis and clinical symptoms are determined.
2. Production of antibodies can be identified.
3. Primary isolation of certain viruses.
4. Mice provide a reliable model for studying viral replication.
5. Used for the study of immune responses, epidemiology and oncogenesis.

- **Disadvantages of Animal Inoculation**

1. Expensive and difficulties in maintenance of animals.
2. Difficulty in choosing of animals for particular virus
3. Some human viruses cannot be grown in animals,or can be grown but do not cause disease.
4. Mice do not provide models for vaccine development.
5. Issues related to animal welfare systems.

2. Inoculation into embryonated egg



- Good pasture in 1931 first used the embryonated hen's egg for the cultivation of virus.
- The process of cultivation of viruses in embryonated eggs depends on the type of egg which is used.
- Viruses are inoculated into chick embryo of 7-12 days old.
- For inoculation, eggs are first prepared for cultivation, the shell surface is first disinfected with iodine and penetrated with a small sterile drill.
- After inoculation, the opening is sealed with gelatin or paraffin and incubated at 36°c for 2-3 days.
- After incubation, the egg is broken and virus is isolated from tissue of egg.
- Viral growth and multiplication in the egg embryo is indicated by the death of the embryo, by embryo cell damage, or by the formation of typical pocks or lesions on the egg membranes
- Viruses can be cultivated in various parts of egg like chorioallantoic membrane, allantoic cavity, amniotic sac and yolk sac.

Advantages of Inoculation into embryonated egg

1. Widely used method for the isolation of virus and growth.
2. Ideal substrate for the viral growth and replication.

3. Isolation and cultivation of many avian and few mammalian viruses.
4. Cost effective and maintenance is much easier.
5. Less labor is needed.
6. The embryonated eggs are readily available.
7. Sterile and wide range of tissues and fluids
8. They are free from contaminating bacteria and many latent viruses.
9. Specific and non specific factors of defense are not involved in embryonated eggs.
10. Widely used method to grow virus for some vaccine production.

Disadvantages of Inoculation into embryonated egg

1. The site of inoculation for varies with different virus. That is, each virus have different sites for their growth and replication.

3. Cell Culture (Tissue Culture)

There are three types of tissue culture; organ culture, explant culture and cell culture.

Organ cultures are mainly done for highly specialized parasites of certain organs e.g. tracheal ring culture is done for isolation of coronavirus.
Explant culture is rarely done.
Cell culture is mostly used for identification and cultivation of viruses.

- Cell culture is the process by which cells are grown under controlled conditions.
- Cells are grown in vitro on glass or a treated plastic surface in a suitable growth medium.
- At first growth medium, usually balanced salt solution containing 13 amino acids, sugar, proteins, salts, calf serum, buffer, antibiotics and phenol red are taken and the host tissue or cell is inoculated.
- On incubation the cell divide and spread out on the glass surface to form a confluent monolayer.

Types of cell culture

1. Primary cell culture:

- These are normal cells derived from animal or human cells.
- They are able to grow only for limited time and cannot be maintained in serial culture.
- They are used for the primary isolation of viruses and production of vaccine.
- Examples: Monkey kidney cell culture, Human amnion cell culture

2. Diploid cell culture (Semi-continuous cell lines):

- They are diploid and contain the same number of chromosomes as the parent cells.
- They can be sub-cultured up to 50 times by serial transfer following senescence and the cell strain is lost.
- They are used for the isolation of some fastidious viruses and production of viral vaccines.
- Examples: Human embryonic lung strain, Rhesus embryo cell strain

3. Heteroploid cultures (Continuous cell lines):

- They are derived from cancer cells.
- They can be serially cultured indefinitely so named as continuous cell lines
- They can be maintained either by serial subculture or by storing in deep freeze at -70°c.
- Due to derivation from cancer cells they are not useful for vaccine production.
- Examples: HeLa (Human Carcinoma of cervix cell line), HEP-2 (Humman Epithelioma of larynx cell line), Vero (Vervet monkey) kidney cell lines, BHK-21 (Baby Hamster Kidney cell line).

Susceptible Cell Lines

1. **Herpes Simplex** Vero Hep-2, human diploid (HEK and HEL),human amnion
2. **VZV** human diploid (HEL, HEK)
3. **CMV** human diploid fibroblasts
4. **Adenovirus** Hep2, HEK,
5. **Poliovirus** MK, BGM, LLC-MK2, human diploid, Vero, Hep-2,Rhadomyosarcoma

6. **Coxsackie B** MK, BGM, LLC-MK2, vero, hep-2
7. **Echo** MK, BGM, LLC-MK2, human diploid, Rd
8. **Influenza A** MK, LLC-MK2, MDCK
9. **Influenza B** MK, LLC-MK2, MDCK
10. **Parainfluenza** MK, LLC-MK2
11. **Mumps** MK, LLC-MK2, HEK, Vero
12. **RSV** Hep-2, Vero
13. **Rhinovirus** human diploid (HEK, HEL)
14. **Measles** MK, HEK
15. **Rubella** Vero, RK13

Advantages of cell culture

1. Relative ease, broad spectrum, cheaper and sensitivity

Disadvantage of cell culture

1. The process requires trained technicians with experience in working on a full time basis.
2. State health laboratories and hospital laboratories do not isolate and identify viruses in clinical work.
3. Tissue or serum for analysis is sent to central laboratories to identify virus.

Cultivation of plant viruses and bacteriophages

Cultivation of plant viruses

There are some methods of Cultivation of plant viruses such as plant tissue cultures, cultures of separated cells, or cultures of protoplasts, etc. viruses can be grown in whole plants.

Lec-5

Viral DNA Fingerprinting

A DNA fingerprint is a ladder of fragmented or newly synthesized DNA molecules that form a barcode-like pattern unique to an organism. The key to a DNA fingerprint, then, is having an identifiable pattern of bands on a gel.

- DNA is the chemical name for the molecule that carries genetic instructions in all living things. The DNA molecule consists of two strands that wind around one another to form a shape known as a double helix. Each strand has a backbone made of alternating sugar (deoxyribose) and phosphate groups. Attached to each sugar is one of four bases--adenine (A), cytosine (C), guanine (G), and thymine (T). The two strands are held together by bonds between the bases; adenine bonds with thymine, and cytosine bonds with guanine. The sequence of the bases along the backbones serves as instructions for assembling protein and RNA molecules.
- DNA fingerprinting was invented in 1984 by Professor Sir Alec Jeffreys after he realised you could detect variations in human DNA, in the form of these minisatellites.
- In the DNA fingerprint, the DNA of an organisms is cut up in to fragments using restriction enzyme producing a large number of fragments of DNA
- The methods that used to generate DNA fingerprints are pulsedfield gel electrophoresis (PFGE), the polymerase chain reaction(PCR),
- In this exercise, we will take advantage of the smallness of viral genomes, and generate DNA fingerprints simply through restriction enzyme digestion followed by agarose gel electrophoresis and staining. It will be possible to identify a simulated, unknown “clinical sample” by comparing its restriction pattern with those of known viral DNA samples.

steps to understanding DNA fingerprinting:

1. Extracting the DNA from cells.
2. Cutting up the DNA using an enzyme.
3. Separating the DNA fragments on a gel.
4. Transferring the DNA onto paper.
5. Adding the radioactive probe.
6. Setting up the X-ray film.

(a) Pulsed-field gel electrophoresis (PFGE):

1-Isolation of DNA from viruses: The DNA is mechanically sheared during this procedure, generating large fragments.

2- Restriction enzyme digestion: The large fragments of DNA are cut at specific sites with a restriction enzyme, generating restriction fragments characteristic of the organism.

3- Agarose gel electrophoresis: Very long fragments of DNA (from 40 kb to 5 Mb) are separated by size using alternating electric fields.

(b) The polymerase chain reaction (PCR):

polymerase chain reaction or PCR is a laboratory technique that widely used to rapidly make millions to billions of copies of a specific DNA sample, allowing scientists to take a very small sample of DNA and amplify it to a large enough amount to study in detail.

*** steps of PCR**

The key ingredients of a PCR reaction are *Taq* polymerase, primers, template DNA, and nucleotides (DNA building blocks). The ingredients are assembled in a tube, along with cofactors needed by the enzyme, and are put through repeated cycles of heating and cooling that allow DNA to be synthesized.

The basic steps are:

1. **Denaturation** (96 ° C): Heat the reaction strongly to separate, or denature, the DNA strands. This provides single-stranded template for the next step.

2. **Annealing** (55 - 65° C): Cool the reaction so the primers can bind to their complementary sequences on the single-stranded template DNA.

3. **Extension** (72 °C): Raise the reaction temperatures so *Taq* polymerase extends the primers, synthesizing new strands of DNA.

4. **Repeat denaturation, annealing, and DNA synthesis** about 30 times. 6-Agarose gel electrophoresis: The PCR products are separated by size, generating a fingerprint that can be used to identify of viruses

   This cycle repeats 25 - 35 times in a typical PCR reaction, which generally takes 2 - 4 hours, depending on the length of the DNA region being copied. If the reaction is efficient (works well), the target region can go from just one or a few copies to billions

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Lec-6

Types of cultures media that used to grow tissue cell culture.

1-Minimum Essential Medium (MEM). **2**-Media 199.

3-Hanks solution. **4**-Earls solution.

Culture medial contents

1- Inorganic salts: - Like Na, K, and Mg for growing cell, metabolic activity, maintains PH and given a suitable osmotic pressure.

2-Yeast extract:-as a source to amino acids for growth and diffusion the cell.

3- Glucose: as a source of energy and carbon.

4- Vitamins:-Folic acid, B6, B12, B, Thiamine and Colin.

5- Essential and non-essential amino acids.

6- Fetal calf serum (5-10%) treated at 56 c for 30 minutes and add to nutrient culture media in ratio 1-2 %.

7- Antibiotic solution (penicillin + streptomycin).

8- Phenol red. Given red color in optimum PH and red color in acidic media.

9- Naco3 (4.5-7.5) to give a suitable pH.

Preparation of tissue cell culture (fibroblast) from chicken egg embryo

1- Select the chicken egg embryo at age (10-11) days.

2- Killed the animal and put it in isotonic solution like hanks solution to remove the blood and remain embryo membranes.

3- Remove the capsule and cutting the embryo to small pieces and putting in the trypsin izing flask that contain magnetic rod.

4-add to pieces trypsin solution 0.25% and Leave for 10 minute in magnetic stirrer for digestion the cell.

5-filtrat the solution by using gauze.

6-Add 20 ml of nutrient culture media to stop of trypsin action or putting in cups ice.

7-Putting in cold centrifuge at 4c (1500 Revolutions per minute) for minutes.

8-Remove the supernatant and taken cell sediment. And add to 150 ml nutrient culture broth /1 ml cells

9-Distributed the mixture in falcon and incubator at 37c for 2-5 day to the formation of complete monolayer's cell (fibroblast) .inoculation the virus and observed cytopathic effect that virus happens.

Cytopathic effect virus in the cell tissue culture

1-gaint cells formation: - multi nuclear cell product from attached cell with other, can see observed when the cell infected with viruses contains fusion protein in external envelope e.g. Newcastle.

2-Inclusion bodies:-inside the cytoplasm e.g pox virus or inside nuclear e.g. Adeno virus.

3-Cell Trans-formation: - can see in case infected with oncogenic viruses e.g. Retro virus and paploma virus.

4-Increasing of cytoplasmic granule or rupture of the nuclear e.g. Myxo virus.

Haemagglutination Test (HA)

Haemagglutination Test:-biology phenomena are observed in some types of virus that contains in external envelope to the two types from peplomers or spikes are Heamagglutinin (HA) and Neuraminidase (NA).

Heamagglutinin (HA):-is responsible for attached the virus with cell (RBS) caused by agglutination. e.g. Newcastle and influenza virus,.

Neuraminidase (NA):-is responsible for destroy of the receptor present on the RBC wall and release the virus. This process called Elution.

Factors effected on the Heamagglutinaton test

1-Sex and type RBC. **2**-Temperature. **3**- pH.

Advantage of Heamagglutinaton test

1-Diagnostic and identification some types of (envelope virus).

2-measure titer of virus.

3- Measure Heamagglutinaton Inhibition Test (HI).

Positive: agglutination and clumping of RBC. **Negative**: precipitation of RBC.

Titer virus

Highest dilution of virus suspension producing agglutination.

Advantage of Heamagglutinaton Inhibition Test

1-Used for diagnosis of a number of viral diseases.
2-Antibody estimation.

Mode of action or mechanism of action

When treated the virus with serum contains specific Antibody to virus. Antibodies linking with peplomers virus and prevent happens agglutination.

Positive: no agglutination of RBC.

Negative: clumping and agglutination of RBC.

Titer of Abs: highest dilution of serum inhibiting haemagglutination ×8or 4 number HA units

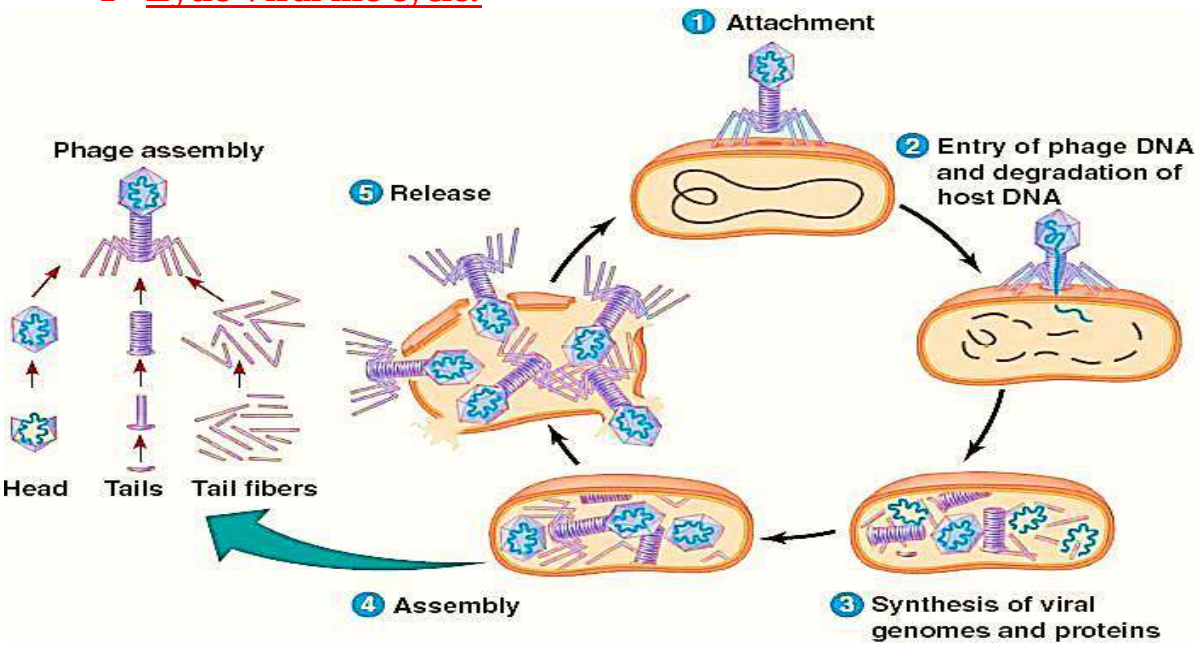
Lec-7

Diseases Affecting Specific Organs.

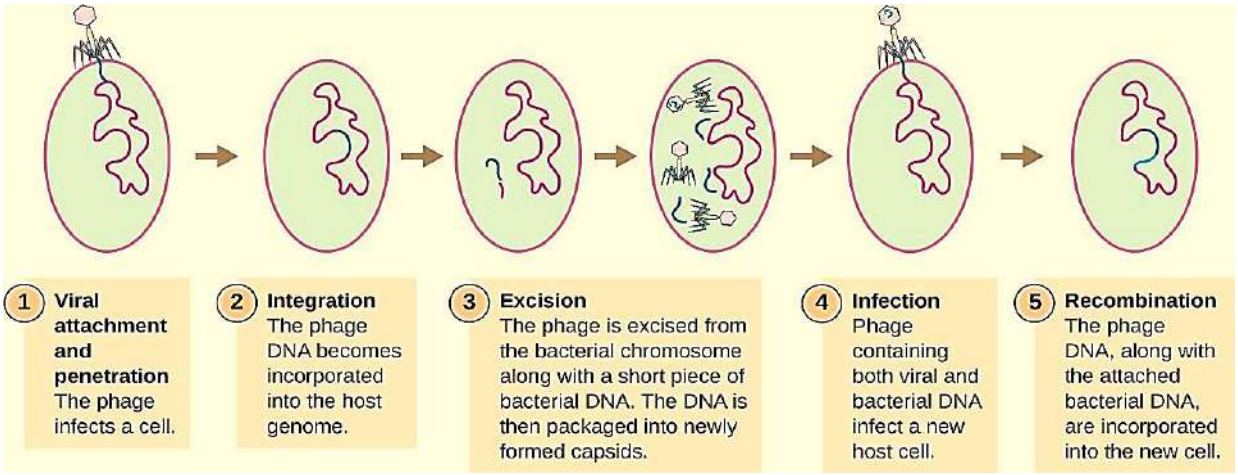
1. Diseases of the nervous system**:** polio myelitis
2. Diseases of the respiratory tract**:** Influenza, para influenza.
3. Localized diseases of the skin or mucous membranes**:** Herpes simplex type1.
4. Diseases of the eye**:** Adenovirus (conjunctivitis), herpes (krato-conjunctivitis).
5. Diseases of the liver**:** Hepatitis type A (infectious hepatitis)
6. Diseases of the salivary glands**:** Mumps and cytomegalovirus
7. Diseases of the gastrointestinal tract**:** Rotavirus.
8. Sexually transmitted diseases**:** Herpes simplex virus.

Growth cycle of virus.

1- **Lytic Viral life cycle:**



2- Lysogenic Viral life cycle:



- If host cell is a bacteria, Virus is a **bacteriophage**.

Differences between Lytic and Lysogenic cycles

Lytic Cycle	Lysogenic Cycle
Viral DNA destroys Cell DNA, takes over cell functions and destroys the cell.	Not destroys the cell.
The Virus replicates and produces progeny phages.	The Virus does not produce progeny.
There are symptoms of viral infection. Virtulant viral infection takes place.	There are no symptoms of viral infection. Temperate viral replication takes place.

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

Lec-8

Lab 8 : Vaccine

Whole virus vaccines. either live or killed, constitute the vast majority of vaccines in use at present. However, recent advances in molecular biology had provided alternative methods for producing vaccines. Listed below are the possibilities;-

1. Live whole virus vaccines
2. Killed whole virus vaccines
3. Subunit vaccines;- purified or recombinant viral antigen
4. Recombinant virus vaccines
5. Anti-idiotype antibodies
6. DNA vaccines

-Types of vaccine

1. Live Vaccines

Live virus vaccines are prepared from attenuated strains that are almost or completely devoid of pathogenicity but are capable of inducing a protective immune response. They multiply in the human host and provide continuous antigenic stimulation over a period of time, Primary vaccine failures are uncommon and are usually the result of inadequate storage or administration. Another possibility is interference by related viruses as is suspected in the case of oral polio vaccine in developing countries.

Several methods have been used to attenuate viruses for vaccine production.

1. **Use of a related virus from another animal** -
2. **Administration of pathogenic or partially attenuated virus by an unnaturalroute** -
3. **Passage of the virus in an "unnatural host" or host cell**
4. **Development of temperature sensitive mutants**

2. Inactivated whole virus vaccines

These were the easiest preparations to use. The preparation was simply inactivated. The outer virion coat should be left intact but the replicative function should be destroyed. To be effective, non-replicating virus vaccines must contain much more antigen than live vaccines that are able

to replicate in the host. Preparation of killed vaccines may take the route of heat or chemicals. The chemicals used include formaldehyde or beta-propiolactone. The traditional agent for inactivation of the virus is formalin. Excessive treatment can destroy immunogenicity whereas insufficient treatment can leave infectious virus capable of causing disease. Soon after the introduction of inactivated polio vaccine, there was an outbreak of paralytic poliomyelitis in the USA use to the distribution of inadequately inactivated polio vaccine. This incident led to a review of the formalin inactivation procedure and other inactivating agents are now available, such as Beta-propiolactone. Another problem was that SV40 was occasionally found as a contaminant and there were fears of the potential oncogenic nature of the virus.

Because live vaccines replicate inside host cells, bits of virus antigen are presented to the cell surface and recognized by cytotoxic cells

Potential safety problems

1-Live vaccines

1. Under attenuation
2. Mutation leading to reversion to virulence
3. Preparation instability
4. Contaminating viruses in cultured cells
5. Heat lability
6. Should not be given to immunocompromized or pregnant patients

2-Killed vaccines

1. Incomplete inactivation
2. Increased risk of allergic reactions due to large amounts of antigen involved

Present problems with vaccine development include

1. Failure to grow large amounts of organisms in laboratory
2. Crude antigen preparations often give poor protection. eg. Key antigen not identified, ignorance of the nature of the protective or the protective immune response.
3. Live vaccines of certain viru
4. ses can (1). induce reactivation, (2) be oncogenic in nature