South Valley University | Botany and microbiology Department | Faculty of Science

VIROLOGY

3-rd Microbiology and chemistry
3-rd special microbiology

Prepared by:

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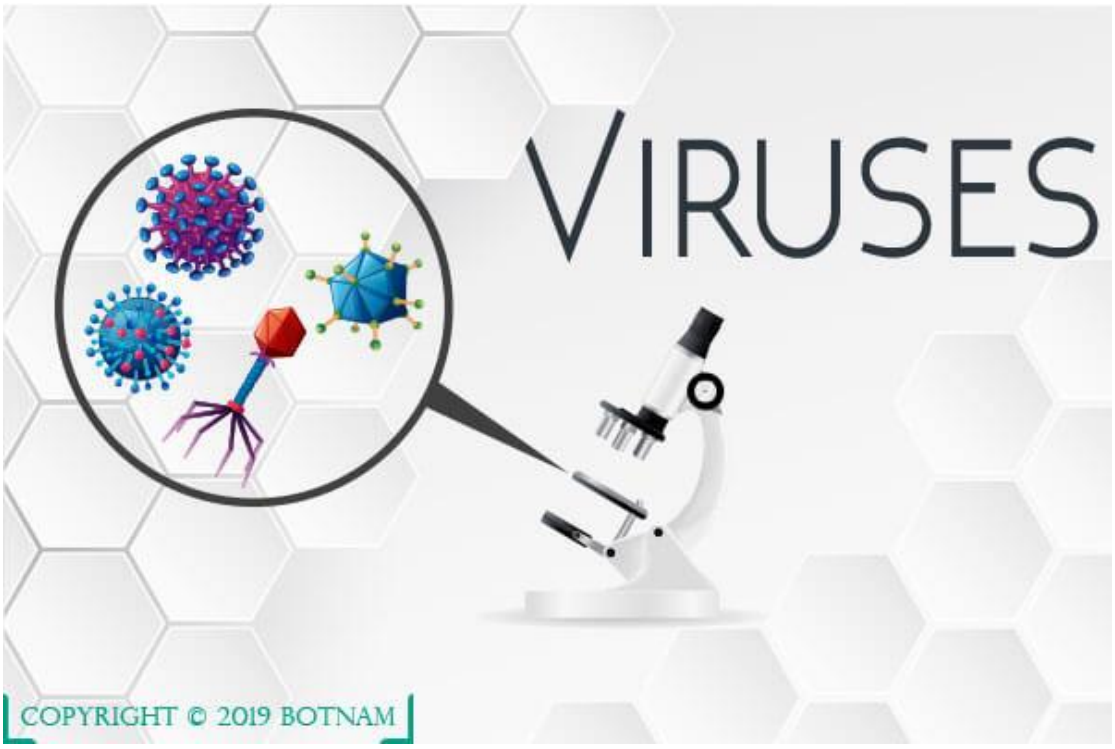
رؤية الكلية:

التميز في تعليم العلوم الاساسية والبحث العلمي للمساهمة في التنمية المستدامة.

رسالة الكلية:

تقديم تعليم مميز في مجالات العلوم الاساسية وانتاج بحوث علمية تطبيقية للمساهمة في التنمية المستدامة من خلال اعداد خريجين متميزين طبقا للمعايير الاكاديمية القومية وتوفير خدمات مجتمعية وبيئية تلبي طموحات جنوب الوادى وبناء الشراكات المجتمعية الفاعلة.

Contents:


VIRUSES
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Virology

Viruses:

Viruses are very small particles, ultramicroscopic, so they are not seen by ordinary microscope. They are obligate intracellular agents which can replicate only in the living susceptible cells because they depend on cell metabolites in their growth.

Example of viruses:-

Bacteriophage is a virus which infects bacteria.

Properties that distinguish viruses from other organisms

(1) They are very small in size (10-300 nm).

(2) They are metabolically inert as they don't possess ribosomes or protein synthesing apparatus. It uses host synthetic machinery.

(3) They require host cell for replication and survive i.e. adaptation.

(4) They cannot grow on artificial media but can grow in tissue culture and in living animals.

(5) Separation of viruses or isolation:

This requires ultra-centrifugation system which makes (10000-30000) cycle per minute.

As by using the centrifugation apparatus we can isolate viruses from sewage water as a suspension of liquid but by using the ultracentrifugation we can obtain virus in precipitate manner.

(6) Infection:

a) Some viruses are specific. If a virus infects a special organ like virus c.

b) Nonspecific viruses: if the virus infected more than organism different families in different plants.

c) Species specific viruses: if it infects a special plant of a family of a class.

Note: Different types of viruses can infect only a limited range of hosts and many are species-specific. Some, such as smallpox virus for example, can infect only one species—in this case humans, and are said to have a narrow host range. Other viruses, such as rabies virus, can infect different species of mammals and are said to have a broad range. The viruses that infect plants are harmless to animals, and most viruses that infect other animals are harmless to humans. The host range of some bacteriophages is limited to a single strain of bacteria and they can be used to trace the source of outbreaks of infections by a method called phage typing. The complete set of viruses in an organism or habitat is called the virome; for example, all human viruses constitute the human virome.

(7) Thermal death point:

It is the minimum temperature degree in which the virus loses its ability of reproduction and infection.

- At the beginning: the growth increase with increasing temperature.
- At a certain temperature the growth becomes constant. This point called stationary phase.
- Then the growth decrease with increasing the temperature due to the death of some viruses.
- Finally: the growth becomes zero at a certain temperature which known as thermal death point.

Living and non-living characters of viruses

Living characters of viruses:

(1) Incubation period:

It is the period that insides it occur all metabolic activities , life cycles and till symptoms appear; in case of bacteriophage it is the time that required by the virus to complete the lytic cycle.

In case of HIV, it is from 3 months to 7 years.

In case of influenza, 3 days.

(2) Host range :-

There are three types of host range

1) Wide host range: it is non-specific virus in which it can infect different species in different families.

2) Limited host range: it is specific virus in which virus can infect different species in one family.

3) Restricted host range: it is highly specific virus in which virus infects one species in one family.

(3) Enzyme production:

Viruses like any living organisms have the ability to produce enzymes for its biological effect (by using ribosomes' host cells).

(4) Obligate parasitism:

Viruses depend mainly and completely on the host.

(5) Mutation:

Viruses have the ability to produce mutations. If the viruses are exposed to rays.

(6) Thermal death point:

It is the lowest temperature in which the virus losses its ability for replication and growth.

Non-living characters of viruses:

(1) Viruses have no metabolic activity away from host cell.

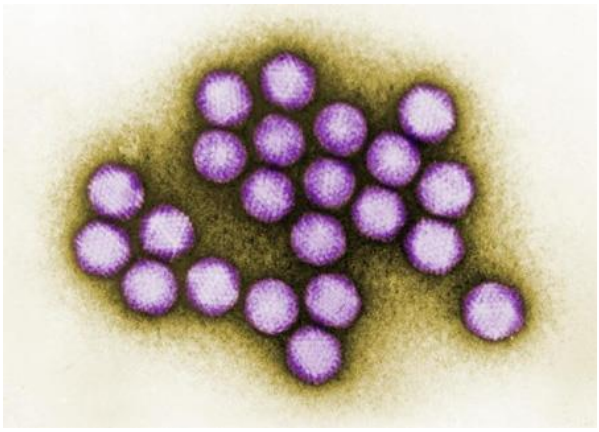
(2) Like any chemical substance they take crystalline form also we can obtain them in test tube and they have molecular weight.

Viral Morphology

There are three groups according to morphology:

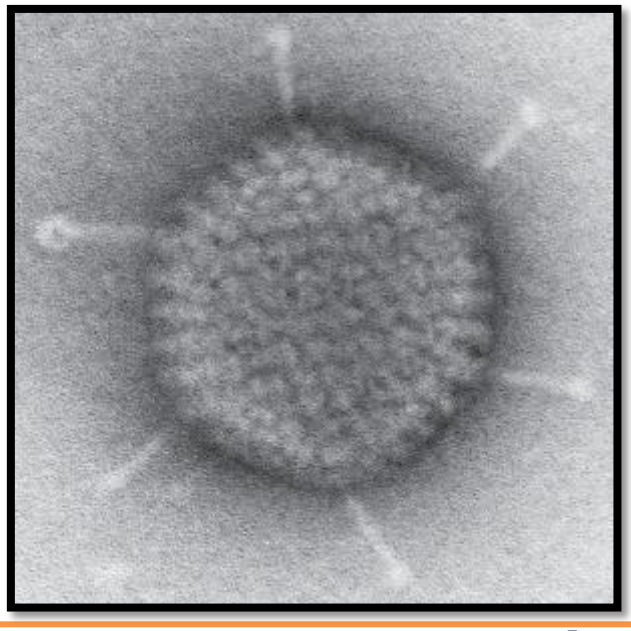
1- Polyhedral (Icosahedral) Viruses

Example *Adenovirus*: that infects glands



Adenoviruses **فايروس الغدد**

بالقوة الصغرى للمجهر الاليكتروني



Adenoviruses **فايروس الغدد**

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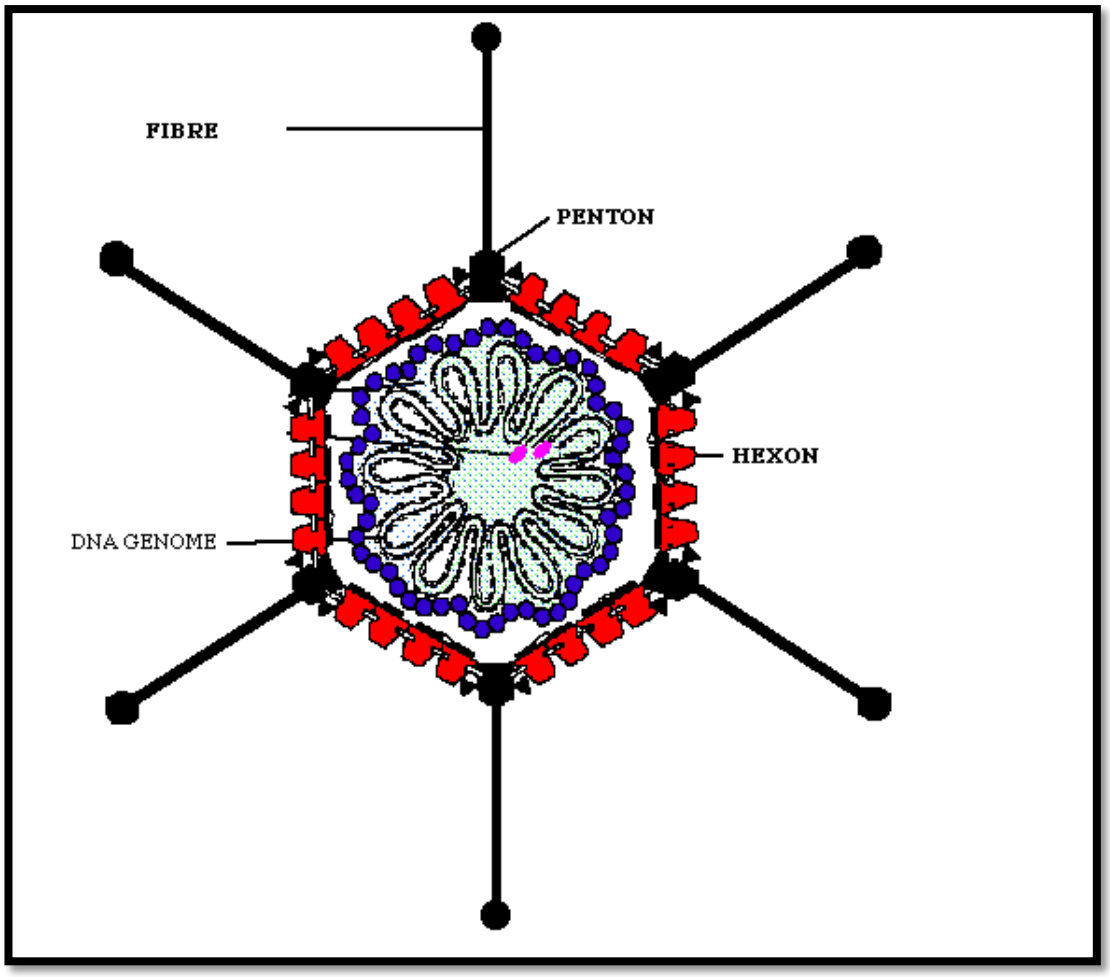


Adenoviruses

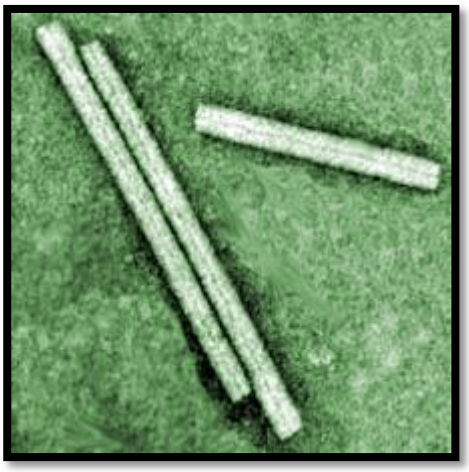
2- **Helical (Cylindrical) Viruses**

Examples:

A- Rod-shaped: Tobacco mosaic virus (TMV).

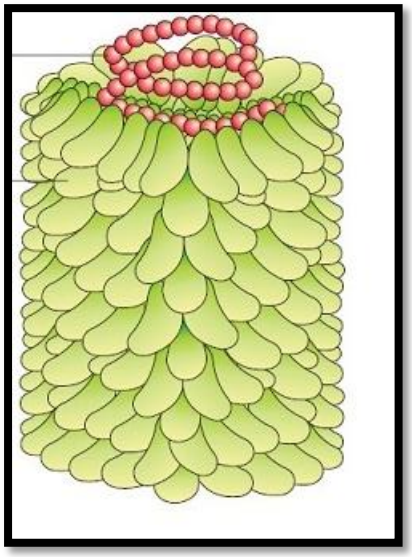
B- Enveloped Helical: Influenza Virus.

C- Filamentous Helical: Potato virus Y (PVY).



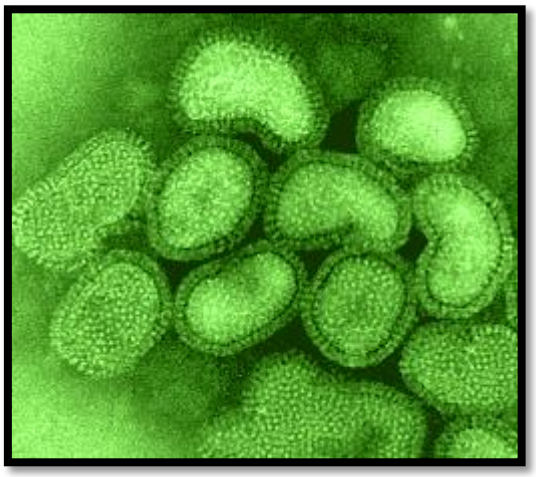
صورة بالمجهر الالكتروني الفايروس النباتي التبرقش الدخان

Tobacco mosaic (TMV) ***virus***



رسم توضيحي لفايروس النباتي التبرقش الدخان

Tobacco mosaic (TMV) ***virus***



صورة بالمجهر الالكتروني فايروس الانفلونزا Influenza Virus ***virus***.

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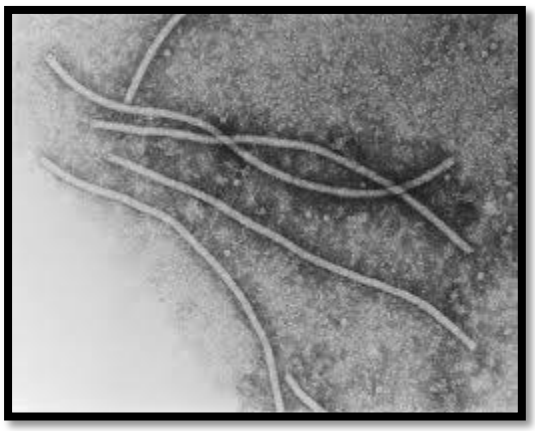

صورة بالمجهر الالكتروني فايروس Y للبطاطس

Potato virus Y (PVY)

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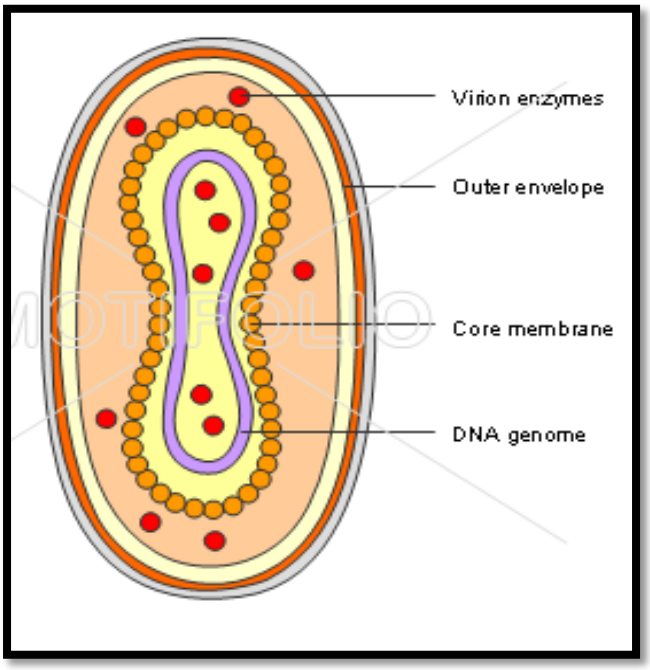
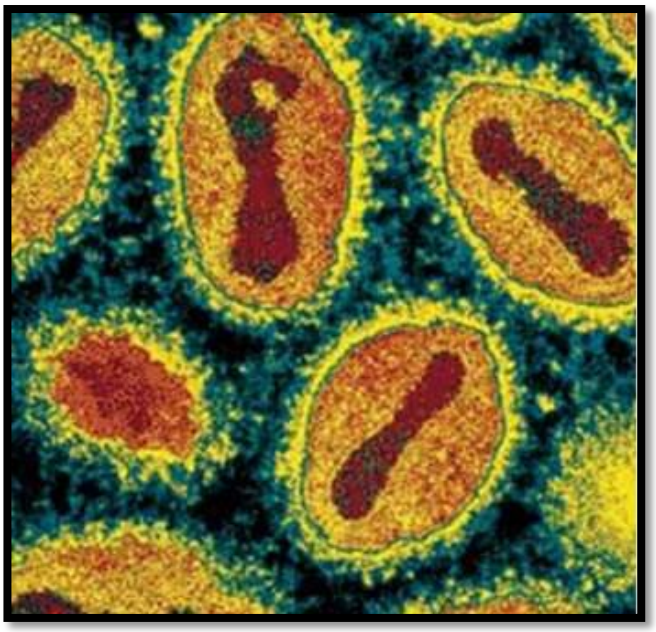
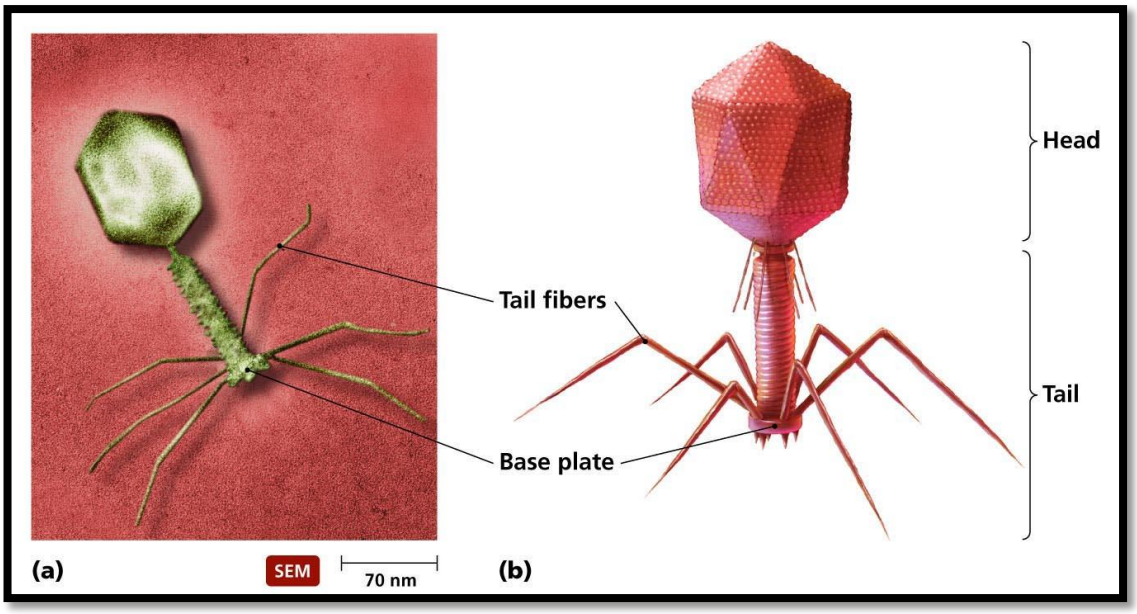
Complex Viruses



رسم توضيحي لفايروس الجدري variola virus.



صورة بالمجهر الاليكتروني لفايروس الجدري variola virus.



Morphological structure of Bacteriophage

Viruses' replication (Bacteriophage):

There are two methods for viral replication:-

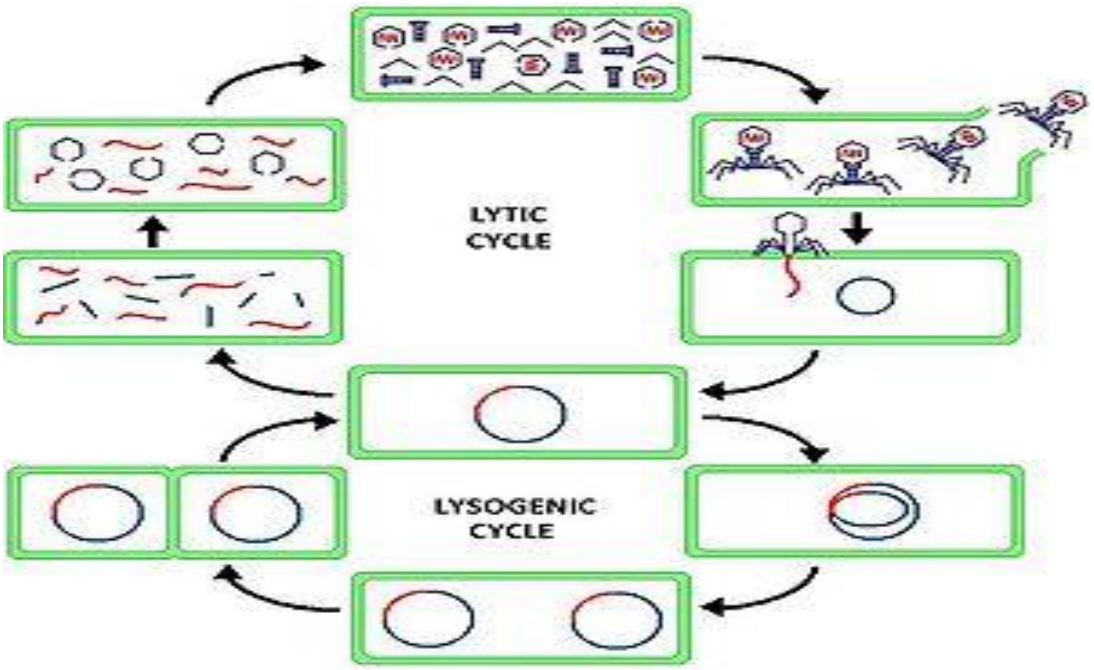
1. **lytic cycle.**
2. **Lysogenic cycle.**

Lytic cycle

- Bacteriophage is biologically extinct i.e. it can't activate singly but it must enter host cell.
- Lytic cycle called lysis cycle because bacteriophage lyse the cell wall of the bacterial cell and the bacterial cell die.

Phases of lytic cycle:-

- **Attachment phase:-**
  In this phase bacteriophage close from bacterial cell and by using plate core and fibers it begin to attach to bacterial cell wall. This called random collisions because if bacterial cell has a receptor. The virus will attack bacteria from it.
- **Penetration and injection of genome:-**
  In this phase bacteriophage begins to penetrate the wall of bacterial cell and injects its genome.
- **Multiplication phase:-**
  Also called copying phase. In this phase bacteriophage controls bacterial cell and dominates its activity and prevents it from division and use host machinery synthetic to make copies of its genome.
- **Maturation phase:-**
  In this phase each bacteriophage enveloped by a protein coat and fibers and plate core begins to form.
- **Releasing phase:-**
  In this phase the unmatured bacteriophage matures and begins to lyse the wall of the bacterial cell and releases and repeats the cycle.



Lysogenic cycle

- Also called lysogeny.
- It is one of the two methods of viral replication.
- It begins with the same phases of the lytic cycle as attachment phase, penetration and injection of genome.
- It is characterized by integration of the bacteriophage nucleic acid as a part of the linear structure of the host bacterium genome.
- In this cycle the bacteriophage doesn't dominate bacterial cell and doesn't prevent it from division but it copies itself with the usual bacterial cell division.
- The newly integrated genetic material which called prophage can be transmitted to each subsequent cell division and bacterial cell continue in division and bacteriophage division continue with it.
- Prophages called temperate phages because they have moderate effect on the cells i.e. they don't cause death of the cell and it allows bacterial cell to survive for some period.
- Under unsuitable conditions or if bacteriophage undergo any stress or mutation or if it exposed any radiation such as U.V. radiation that cause

releasing of prophages this occur through proliferation of phages via the lytic cycle.

- In case of lysogenic cycle the spread of viral DNA occurs through the usual prokaryotic reproduction.While in case of lytic cycle phages are spread through production of thousands of individual phages capable of surviving and infecting other bacterium.

Lysogenic conversion

In some interactions between lysogenic phages and bacteria, lysogenic conversion may occur; it is when temperate phages induce change in the phenotype of the bacterial cell.

Examples:-

1. **Corynebacterium diphtheria** produce the toxin of diphtheria only when it is infected with phage B.
2. **Vibrio cholera** is nontoxic strain also produce cholera toxin when infected with phage.

Viruses classification

The highest level of viral classification recognizes six major groups according to the nature of the genome:-

1. **Double stranded DNA viruses:**
   There are no plant viruses in this group. This group is defined to include only viruses that replicate without RNA intermediate, it is include those viruses with the largest known genomes about 400,000 base pairs and there is only one genome components, which may be linear or circular, well known viruses in this group includes herpes and pox viruses.
2. **Single strand DNA viruses:**
   There are two families of plant viruses in this group and both of these small circular genome components often with two or more segments.
3. **Reverse-transcribing viruses:**

These have dsDNA or ssRNA genomes and their replication includes the synthesis of DNA from RNA by the enzyme reverse transcriptase; many integrate into their host genomes. The group includes the retroviruses, of which Human immunodeficiency virus (HIV), the cause of AIDS, is a member. There is a single family of plant viruses in this group and this is characterized by a single component of circular dsDNA, the replication of which is *via* an RNA intermediate.

4. **Double-stranded RNA (dsRNA):**

   Some plant viruses and many of the mycoviruses are included in this group.

5. **Negative sense single-stranded RNA (-ssRNA):**

   In this group, some or all of the genes are translated into protein from an RNA strand complementary to that of the genome (as packaged in the virus particle). There are some plant viruses in this group and it also includes the viruses that cause measles, influenza and rabies.

6. **Positive sense single-stranded RNA (+ssRNA):**

   The majority of plant viruses are included in this group. It also includes the SARS coronavirus and many other viruses that cause respiratory diseases (including the "common cold"), and the causal agents of polio and foot-and-mouth disease.

   Within each of these groups, many different characteristics are used to classify the viruses into families, genera and species. Typically, combinations of characters are used and some of the most important are:-

- **Particle morphology:** the shape and size of particles as seen under the electron microscope.
- **Genome properties:** this includes the number of genome components and the translation strategy. Where genome sequences have been determined, the relatedness of different sequences is often an important factor in discriminating between species.
- **Biological properties:** this may include the type of host and also the mode of transmission.
- **Serological properties:** the relatedness (or otherwise) of the virion protein(s).

Vaccines

It is a biological preparation that improves the immunity to a particular disease.

Vaccine contains an agent resembles to a disease that are caused by microorganisms.

Vaccine made from weakened or killed forms of the microbes or its toxins.

The agent stimulate the body's immune system to recognize the agent as a foreign destroy it and remember it so that the immune system can more easily recognize and destroy any of these microorganisms that is later encounters.

There are several types of vaccines:

- **killed:**
  Some vaccines contained killed but previously virulent microorganisms that have been destroyed with chemicals, heat, radioactivity or antibiotics.
  Examples: influenza vaccine, cholera vaccine, hepatitis A vaccine
- **Attenuated**
  Some vaccine contain live attenuated microorganisms many of these are live viruses that have been cultivated under conditions that disable their virulent properties or which use closely related but less dangerous organisms to produce a broad immune response.
  Examples:
  Viral diseases like yellow fever, measles, rubella, bacterial disease like typhoid.
- **Toxoid:**
  They are made from inactivated toxic compound that cause illness rather than the microorganisms.
  Examples of toxoid based vaccines include tetanus and diphtheria.
- **Subunit:**
  Protein subunit rather than introducing inactivated or attenuated microorganisms to an immune system a fragment of it can create an immune response
  Examples:

The subunit vaccine agent hepatitis B virus, which is composed only from the surface protein.

- **Conjugate:**

  Certain bacteria have polysaccharides outer coats that are poorly immunogenic.

  By linking these outer coats to proteins e.g. toxins that immune system can be lead to recognize the polysaccharides as it if were a protein antigen.

  Example:

  Haemophilus influenza type B vaccine.

Isolation of animal viruses

(1) Enrichment process: which occur by prepare nutrient broth which consists of 3 gm beef extract. 5 gm pepton.1000 ml distilled water.
(2) Take 90 ml of nutrient broth and add 10 ml of sewage water.
(3) Incubation process for 24 at 37◦c.

Observation

The virus attached to *E. coli* and released by high numbers.

The isolation occurs by filter unit: gives bacterial cell and viral suspension.

Determination the activity and availability of viruses:

Procedures:

- Prepare treptocase say agar with sterilization and keeps it at 45°c to still liquid.
- Prepare bacterial suspension.
- Add 1ml of bacterial suspension to sterilized Petri dish.
- Add 15 ml of treptocase say agar to every Petri dish under sterilized conditions.
- Let the media to become solid i.e. solidify.
- Take sterilized filter paper discs and immerse it in viral suspension using sterilized forcipes.
- Put filter discs with virus to the surface of media.
- Incubate at 37°c for 24h in incubator.

Observation:

An appearance of clear zone that means virus is active and make lytic cycle of bacteria.

Absence of clear zone this means that virus is not active and don't make lytic cycle for bacteria.

Your observation and comments:

Titration of virus activity:

The main target is to determine the activity of viruses.

There are two methods to determine the activity of viruses.

(1) In case of liquid media:
The test is called broth clearing assay.

(2) In case of solid media:
The test used is called plaque clearing assay.

In case of liquid media

Procedures:

- Make serial dilutions of viral suspension.
- Put 9 ml of n.b. in sterilized test tubes and make sterilization process.
- By using sterilized pipit add 1 ml of the bacterial suspension in all tubes.
- Incubate for 24h at 37°c in incubator.

Observation

We must detect the titre of virus activity which is the highest dilution and the lowest concentration at which clearing is occurring and turbidity is disappeared i.e. absence of turbidity.

The titre

It is defined as the highest dilution or the lowest conc. of viral suspension which causes complete inhibition of bacteria.

Or the disporical of the highest dilution which cause complete inhibition of bacterial growth.

Your observation and comments:

In case of solid media

Procedure:

- Prepare nutrient agar media and sterilize it.
- Keep it at 45◦c to make it semisolid.
- Add in every sterilize Petri dish 9 ml of nutrient broth agar.
- Make viral suspension and add 1 ml of it to every Petri dish.
- Add 1 ml of bacterial suspension to every dish.
- Incubate for 24 h at 37◦c in incubator.

Observation

Formation of plaque ranged from 30-300.

Lower than 30 plaque doesn't consider titre as it is non active.

Higher than 300 doesn't titre as it is highly active.

Titre=no. of plaque×1÷the highest dilutions.

Your observation and comments:

Antigen antibody reactions

- **Antigen:**

Any foreign substance which when introduced into an animal produces a specific immune response (antibodies, cell mediated immunity).

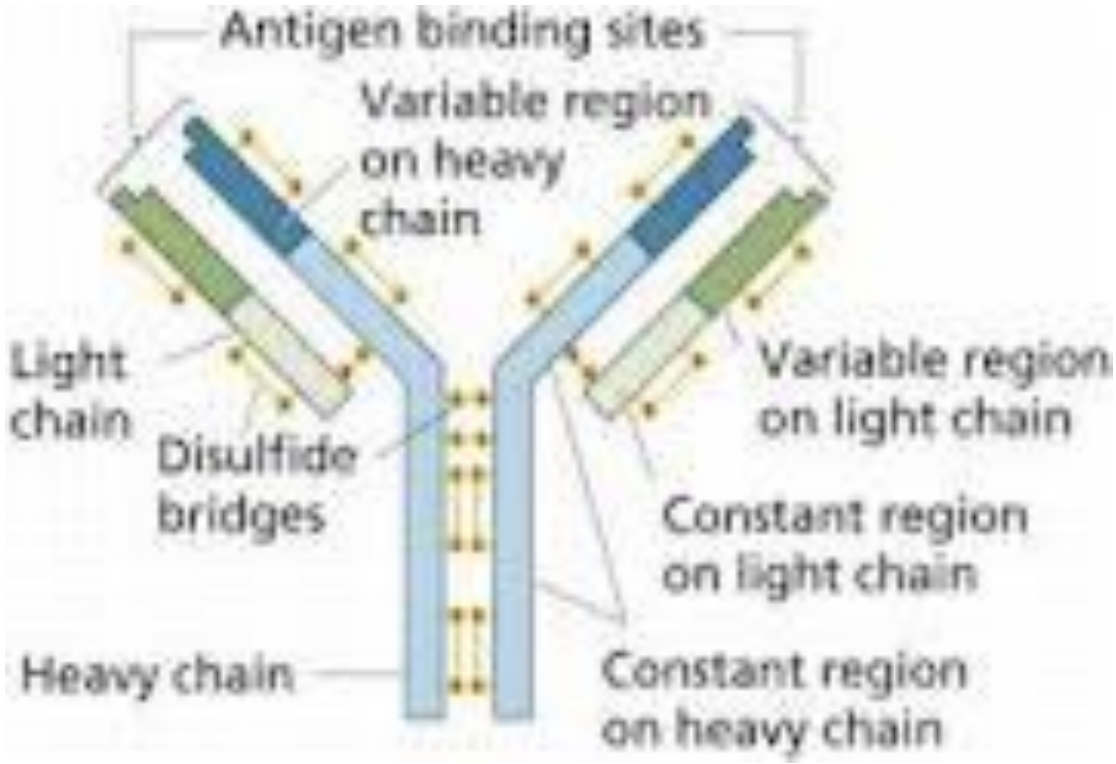
- **Antibody:**

It is a gamma globulin that appears in the serum, tissue fluids of animal after exposure to an antigen.

Types of antibodies:

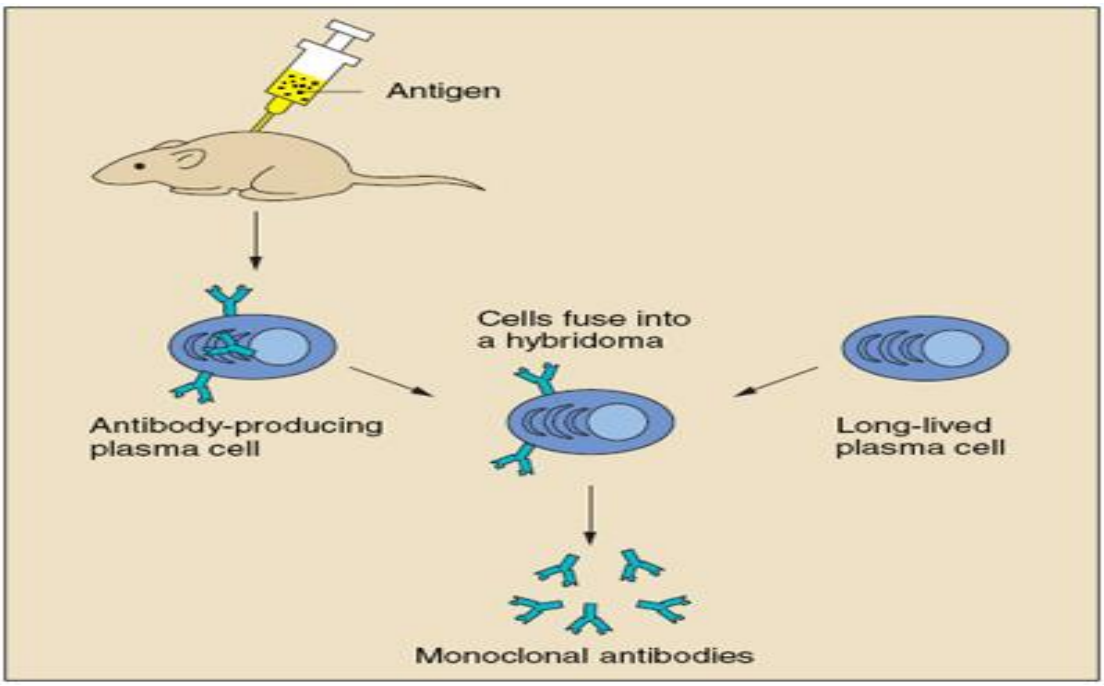
IgG, IgA, IgM, IgD, IgE

Structure of antibody



Monoclonal antibodies

They are made by aclone of cells that arouse from single cell.



Characters of antigen antibody reaction

1-Specific:

Sense antibody can combine only with the antigen which induced its formation.

2- The physical state of the antigen determines the observable result:

If antigen in the form of particles the resulting reaction is clumping of the particles or agglutination.

If in solution, the resulting reaction is precipitation.

3-The presence of electrolytes is essential for the reaction to occur as it decreases the repulsion forces between particles and facilitating anigen antibody reaction.

1- Zone phenomenon:

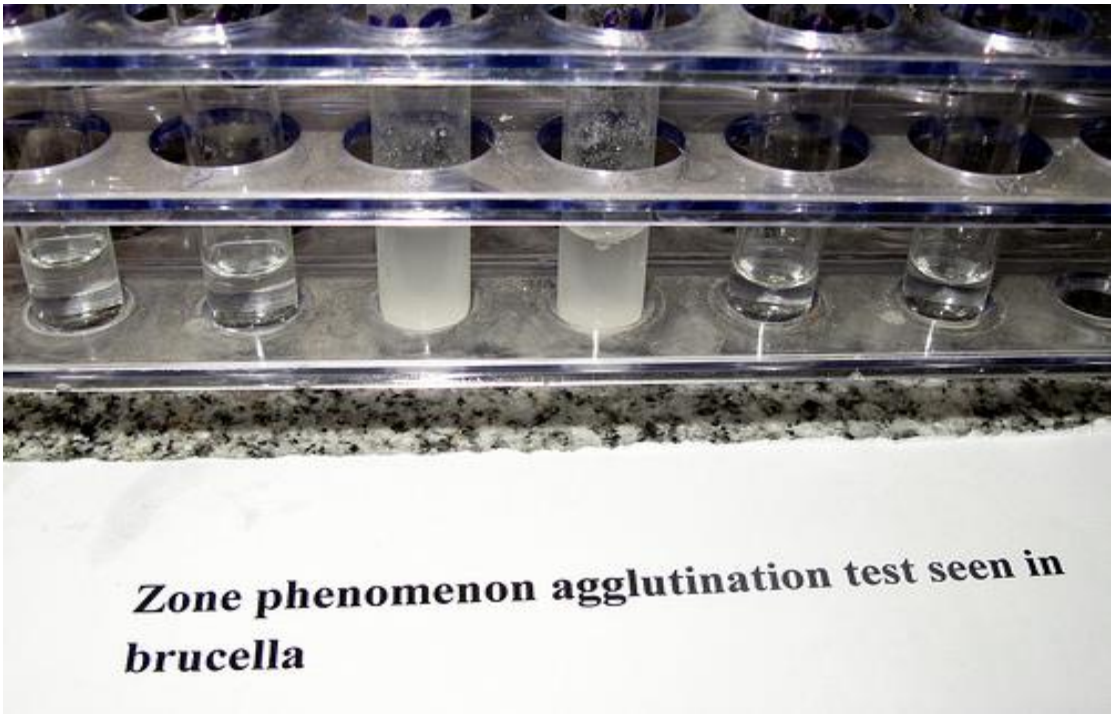
- In serological tests serum is prepared in a series of tubes to which constant amount of antigen is added for quantitative estimation of antibody and avoid zone phenomenon.

- In the first tubes antibody is present in excess→soluble complexes with antigen→no visible reaction.

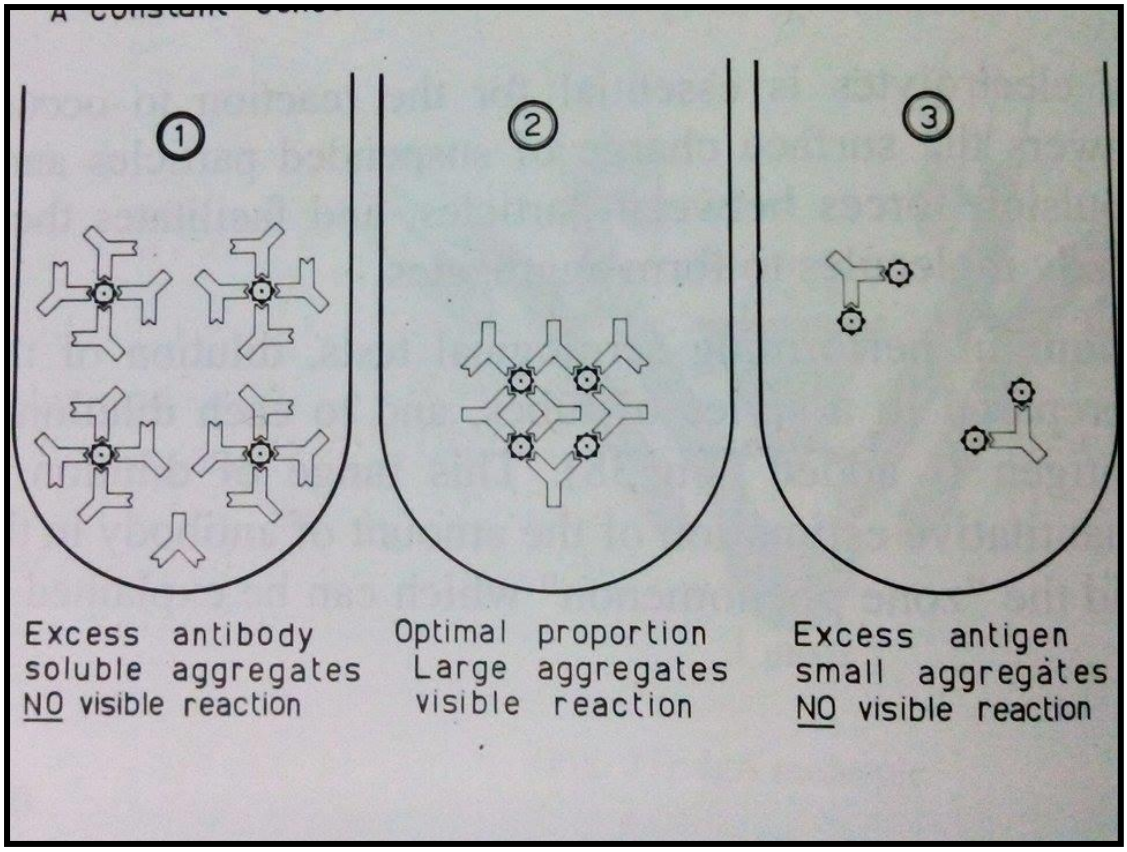
- In the far right tubes the antibody has been diluted beyond its capacity to bind free antigen→no large aggregates→no visible reaction.

- In between these two extremes antigen and antibody presnt at optimal concentrayion to each other→large aggregate is formed→visible reaction

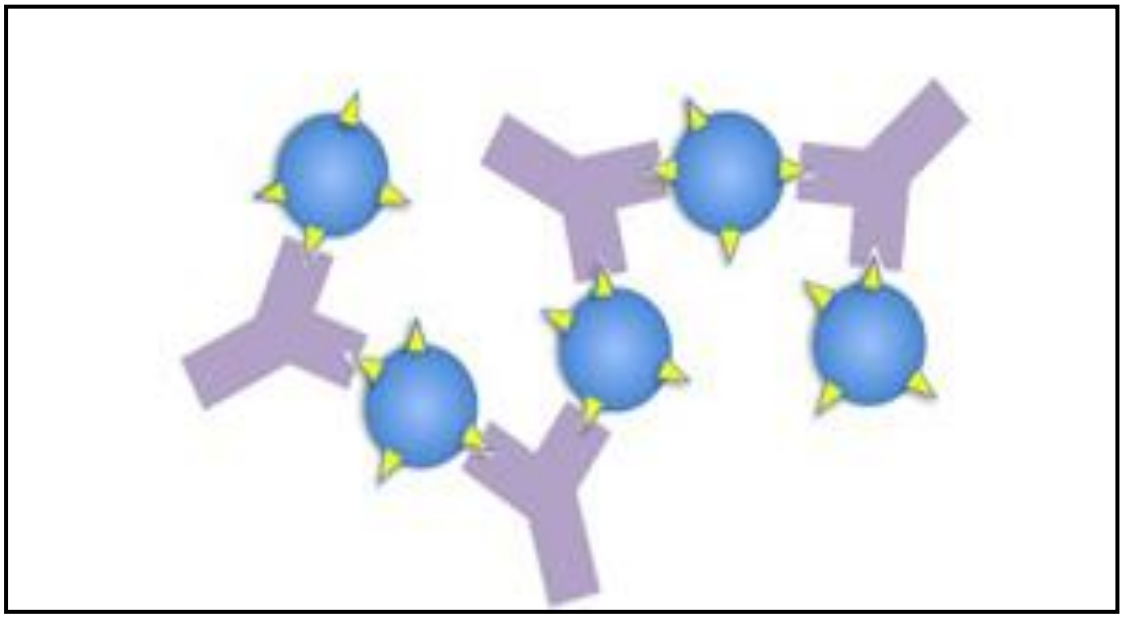
Zone phenomenon (cont.)



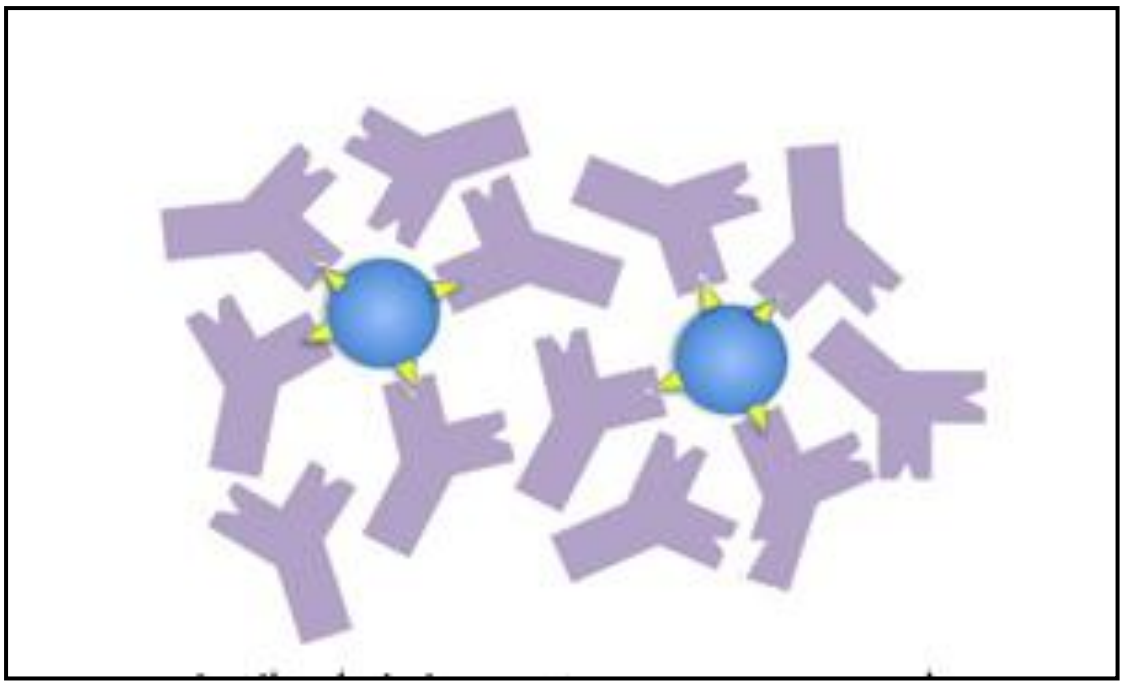
Zone phenomenon agglutination test seen in brucella



Diagramtic explanation of the zone phenomenon



Optimal proportion of antigen & antibody-large aggregates- visible reaction



Excess antibody- soluble aggregates – No visible reaction

Your observation and comments:

2- Agglutination

- Antigen in the form of particles e.g. microorganism, RBCs, latex particle

- When mixed with specific antisera particles become clumped.

Types of Agglutination:

- Direct agglutination.

- Antiglobulin agglutination test.

- Latex agglutination.

- Coagglutination (COA).

- Virus heamagglutination inhibition.

- Heterophile antibodies agglutination tests.

A- direct agglutination: has 2 forms

1- Slide agglutination:

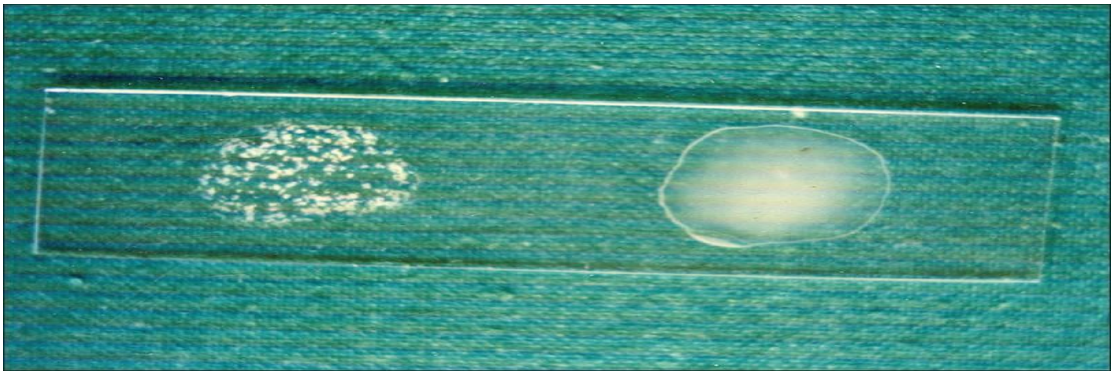
- 2drops of saline containing the unknown microorganism are placed on clean slide.

- known serum is added mixed well

- clumping occurs if serum is specific to the organism.

Application:

Blood grouping



Blood Group Testing

Usually restricted to the ABO and the Rhesus (D)

A) **ABO Blood Group**

- To determine the ABO type, red cells must be tested with anti-A and Anti-B and the serum/plasma tested with A and B red cells

- **Forward grouping** - identifies the **antigens** on the red cells
  - tests the recipient or donor red cells with anti-A and anti-B sera

eg Cells agglutinated only with anti-A serum are group A
Cells that do not agglutinate with anti-A or anti-B are group O

- **Reverse grouping** – identifies the presence of **antibodies** in the serum/plasma
  - confirms the reaction obtained by the forward grouping test.
  - tests the serum/plasma from the recipient or donor with group A red cells and group B red cells
    eg Agglutination with group B cells indicates the presence of
    anti-B in the plasma – Group A individual

B) **Rhesus Blood Group (Rh)**

Rhesus typing of red cells is determined by examining their reaction with anti-D serum.

There are no 'naturally- occurring' Rhesus antibodies, therefore reverse grouping is not performed.

Routine testing for other Rh antigens is not required.

Your observation and comments:

2- Tube agglutination

Quantitative test to determine amount of antibodies in the serum.

Steps:

- Serial dilution of the patient serum (1/10,1/20,1/40,1/80…..etc.) is done to which constant amount of known bacteria is added.

- Tubes incubated for 2-4hr. At 37^0c

- Examine for visible clumping

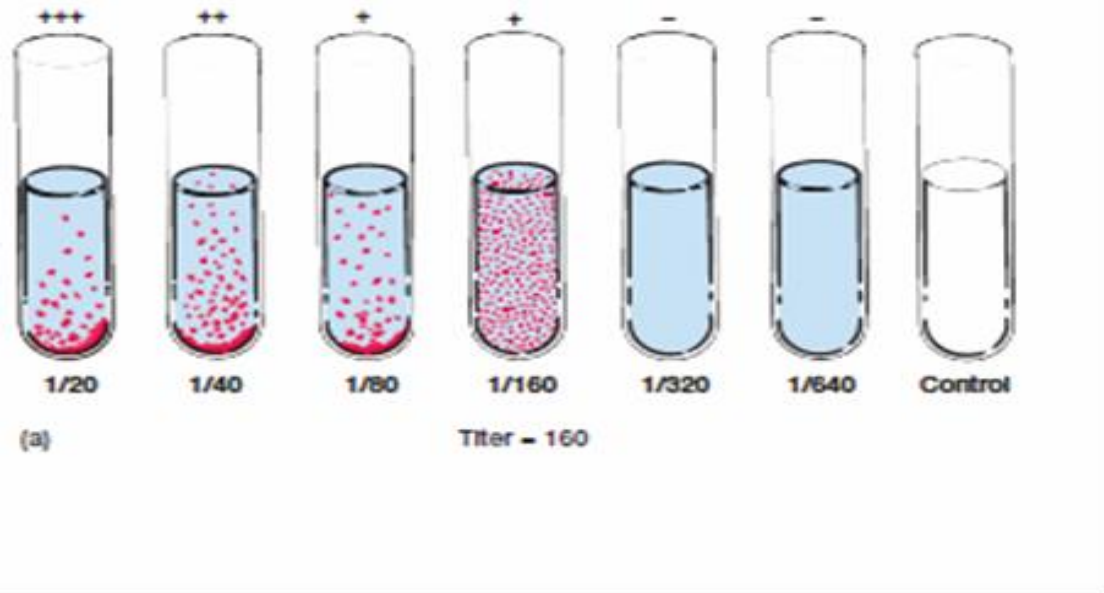
- Titre:

The hightest dilution that showing visible aggregate

- Titre measures the number of antibody units per unit volume e.g.1/320 means 320 units of antibody/ml of serum.

Application:

Widal test for diagnosis of Salmonella



Your observation and comments:

B-antiglobulin agglutination test=coomb's test.

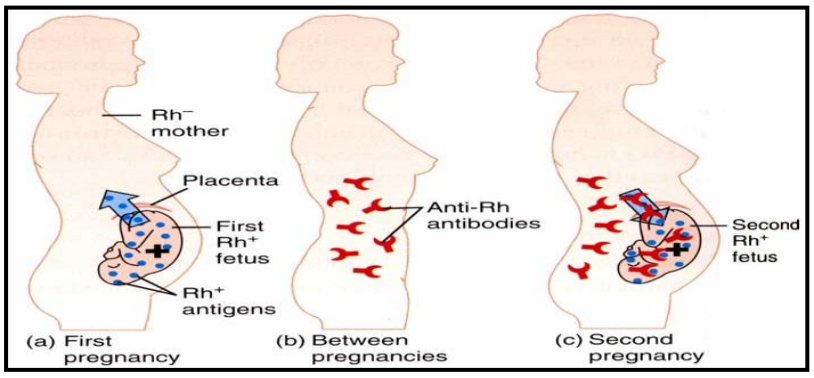
Application:

Used to determine the presence of Rh incompatibility which causes" *erythroblastosis foetalis*".

- red cells of Rh +ve foetus can induce Rh antibodies in his Rh –ve mother,these antibodies will pass through the placenta to the foetus of the next pregnancy causing heamolysis of his RBC's.

-anti-Rh antibodies are IgG incomplete antibodies(cannot bridge between 2 RBC's)so they can be detected by antiglobulin.

-there are 2 types of coomb's test:



Rh incompatibility which causes" *erythroblastosis foetalis*

1- Indirect coomb's test:

- Mother's serum, containing anti-Rh antibodies is mixed with Rh +ve RBC's(group O)

- Incubation at 37^0c for 30min-1hr.

- The mixture is centrifuged, deposit washed, antihuman globulin is added, tubes incubated

- The antihuman globulin causes agglutination by linking the incomplete antibodies together.

2-Direct coomb's test:

- It detects incomplete Rh antibodies coating the RBC's of newborn in *erythroblastosis foetalis.*

- *The antihuman* globulin is added directly to a washed suspension of the newborn RBC's, agglutination occurs.

- Both direct, indirect coomb's test are also used to detect autoimmune heamolytic anaemias.

3- Latex agglutination:

- Agglutination reaction in which inert particles e.g.latex are coated with various antigens or antibodies.

- These particles are aggregated in the presence of specific antibody or antigen.

Examples:

- Pregnancy test:

- The antigen is human chorionic gonadotrophic hormone (HCG) in the urine of pregnant female.

- The test is done by adding latex particles coated with anti-HCG to adrop of urine.

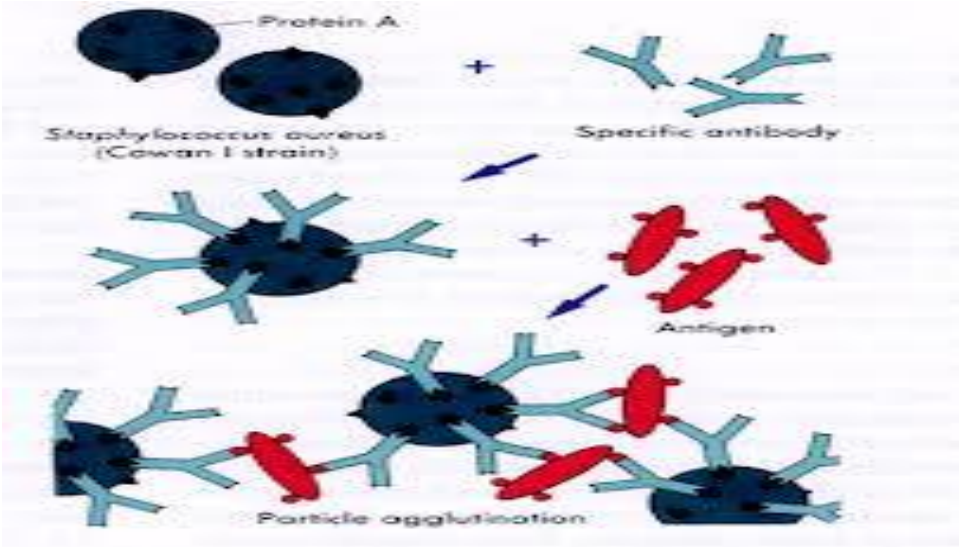
- Agglutination occurs if HCG is present.

Your observation and comments:

4 – Coagglutination (COA):

- *Staph. aureus* rich in protein A on there surface, can bind IgG non specifically through Fc region leaving Fab sites free.

- If staph. coated with antibody reacts with specific antigen →agglutination occurs.



Your observation and comments:

What is ELISA (enzyme-linked immunosorbent assay)?

ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. Other names, such as enzyme immunoassay (EIA), are also used to describe the same technology. In an ELISA, an antigen must be immobilized on a solid surface and then complexed with an antibody that is linked to an enzyme. Detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate to produce a measureable product. The most crucial element of the detection strategy is a highly specific antibody-antigen interaction.

ELISAs are typically performed in 96-well (or 384-well) polystyrene plates, which passively bind antibodies and proteins. It is this binding and immobilization of reagents that makes ELISAs so easy to design and perform. Having the reactants of the ELISA immobilized to the microplate surface makes it easy to separate bound from non-bound material during the assay. This ability to wash away nonspecifically bound materials makes the ELISA a powerful tool for measuring specific analytes within a crude preparation.

ELISA formats

ELISAs can be performed with a number of modifications to the basic procedure. The key step, immobilization of the antigen of interest, can be accomplished by direct adsorption to the assay plate or indirectly via a capture antibody that has been attached to the plate. The antigen is then detected either directly (labeled primary antibody) or indirectly (labeled secondary antibody). The most powerful ELISA assay format is the sandwich assay. This type of capture assay is called a “sandwich” assay because the analyte to be measured is bound between two primary antibodies – the

capture antibody and the detection antibody. The sandwich format is used because it is sensitive and robust.

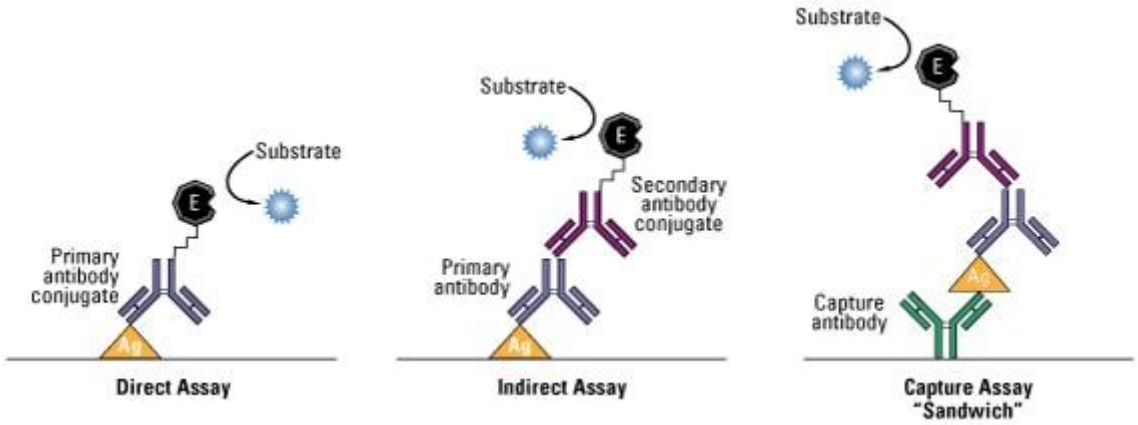


Diagram of common ELISA formats (direct vs. sandwich assays). In the assay, the antigen of interest is immobilized by direct adsorption to the assay plate or by first attaching a capture antibody to the plate surface. Detection of the antigen can then be performed using an enzyme-conjugated primary antibody (direct detection) or a matched set of unlabeled primary and conjugated secondary antibodies (indirect detection).

The direct detection method uses a labeled primary antibody that reacts directly with the antigen. Direct detection can be performed with an antigen that is directly immobilized on the assay plate or with the capture assay format. Direct detection while not widely used in ELISA is quite common for immunohistochemical staining of tissues and cells.

The indirect detection method uses a labeled secondary antibody for detection and is the most popular format for ELISA. The secondary antibody has specificity for the primary antibody. In a sandwich ELISA, it is critical that the secondary antibody be specific for the detection primary antibody only (and not the capture antibody) or the assay will not be specific for the antigen. Generally, this is achieved by using capture

and primary antibodies from different host species (e.g., mouse IgG and rabbit IgG, respectively). For sandwich assays, it is beneficial to use secondary antibodies that have been cross-adsorbed to remove any secondary antibodies that might have affinity for the capture antibody.

Comparison of direct and indirect ELISA detection methods

Direct ELISA detection	
Advantages	• Quick because only one antibody and fewer steps are used. • Cross-reactivity of secondary antibody is eliminated.
Disadvantages	• Immunoreactivity of the primary antibody might be adversely affected by labeling with enzymes or tags. • Labeling primary antibodies for each specific ELISA system is time-consuming and expensive. • No flexibility in choice of primary antibody label from one experiment to another. • Minimal signal amplification.
Indirect ELISA detection	
Advantages	• A wide variety of labeled secondary antibodies are available commercially. • Versatile because many primary antibodies can be made in one species and the same labeled secondary antibody can be used for detection. • Maximum immunoreactivity of the primary antibody is retained because it is not labeled. • Sensitivity is increased because each primary antibody contains several epitopes that can be bound by the labeled secondary antibody, allowing for signal amplification. • Different visualization markers can be used with the same primary antibody.
Disadvantages	• Cross-reactivity might occur with the secondary antibody, resulting in nonspecific signal. • An extra incubation step is required in the procedure.

Fluorescent tags and other alternatives to enzyme-based detection can be used for plate-based assays. Despite not involving reporter-enzymes, these methods are also generally referred to as a type of ELISA. Likewise, wherever detectable probes and specific protein binding interactions can be used in a plate-based method, these assays are often called ELISAs despite not involving antibodies.

Your observation and comments:

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