



South Valley University



Botany and microbiology Department



Faculty of Science

PRACTICAL VIROLOGY

4th Special Botany



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Virology

Viruses:

They are entities' its genome is DNA or RNA single or double strand enveloped by a protein coat called capsid consists of structural units called capsomers.

Virus's 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 include 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. 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.

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

The majority of plant viruses are included in this group.

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

Within each of these groups, many different characteristics are used to classify the viruses into families, genera and species. Typically, a combination of characters is 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).

Isolation and purification of plant viruses:

Isolation or purification of plant viruses is necessary to know about their structure and properties. By employing the method of purification, a virus is finally obtained in its pure form as a colourless pellet in a test tube and may be used for various purposes following are steps involved in virus purification (isolation) :-

1. Infected leaves are homogenized in water or in phosphate, borate or citrate buffer in an electric grinder or in a mortar with pestle.
2. Tissue homogenate is strained through a piece of muslin cloth or cheese cloth. Crude sap which comes out and contains virus is collected and then poured into centrifuge tube. The tube is spun at low speed (3000-17000g). As a result, the crude sap differentiates into supernatant and a pellet. The pellet is discarded and the supernatant with virus is collected.
3. The supernatant with virus is poured into centrifuge tube. The tube is placed in fixed angle rotor of ultracentrifuge and spun at high speed (40000-150000) after the tube settles, the virus sediments and forms tiny pellet at the bottom of the tube and a supernatant over it. Supernatant is discarded and the pellet of virus is mixed with buffer and stirred with rod so that it resuspends in buffer.
4. Low and high speed centrifugation steps are repeated 2-3 times and the virus is purified by density gradient centrifugation, the most frequently used technique. A tier of layer of sucrose solutions of different concentrations (e.g., 10-40%), and hence densities, is formed in the centrifuge tube, layer at the

bottom being the most dense and one at the top of the least dense with layer of intermediate concentration .Virus suspensions is placed at top of the top most layer and centrifuged in swimming –bucket rotors at high speed ultracentrifuge .

5. When settled, virus particles move together as a band in gradient solution of sucrose. The virus band is collected as separate fraction through puncture at the bottom of the centrifuge tube .The virus fraction is placed in cellulose dialysis tubing and sucrose is removed by dialysis in buffer solution or water . Thus the virus is obtained in pure form.

External symptoms of plant viral diseases:

1- Chlorosis:-

These symptoms represent the area which becomes weakened in green colour due to either destruction or inhibition of chlorophyll formation subsequent to infection and the cells with chlorotic symptoms contain less chlorophyll thus appearing pale green or yellowish in colour.

2- Mosaic:-

These symptoms represent irregular intermingling of light green, yellow or white areas with the normal green colour of the plants or fruits forming a mosaic like pattern.

3- Mottling:-

It is an irregular pattern of indistinct light and dark areas. Mottling is considered equivalent to the mosaic by some virologists.

4- Vein chlorosis and vein clearing:-

The former represents the chlorosis restricted to the veins while the latter represents the translucence of the veins rather than being chlorotic or yellow. These two symptoms make the veins appear lighter in colour against dark background of normal green tissue.

5- Vein mosaic:-

These represent the chlorosis along the main vein with the lighter areas irregular in shape and distribution. Sometimes vein mosaic extends to adjacent areas in the form of irregular patterns.

6- Vein banding:-

In these symptoms the chlorotic area occur along the veins in a regular manner resulting in the presence of chlorotic and regular green coloured bands.

7- Necrosis:-

These are the common viral symptoms occurring in plants representing the death of the cells in localized areas .These areas of dead tissues are generally differentiated by the presence of dark brown border around them . Necrosis may remain localized but sometimes it spreads as long streaks producing systemic necrosis resulting in the death of the whole plant. One strain of TMV develops streaks on the stems and causes complete necrosis of leaves and fruits resulting in the death of the infected tomato plants.

8- Ring spots:-

These symptoms represent the localized circular spots formed by concentric rings of chlorotic and normal green tissue .ring spots are generally accompanied with necrosis. They may be presents singly or in groups developing concentrically on infected parts.

9- Enations:-

Enations are the out growths generally occurring on vein or midrib on the lower surface of the leaves.they may vary in number and shape .they may be small, large, papillate or spin like in shape .in some cases like pea enation mosaic virus the outgrowths develop between or adjacent to the veins on the lower surface of the leaves and look like leaves , funnels,wings,cups etc.

10- Leaf narrowing:-

In these symptoms the infected leaves generally become narrow due to reduced growth of laminar tissue. The veins and the midrib remain normal in growth, in the case where leaf narrowing is at extreme as represented by tmv infected tomato only the midriband veins are present and the laminar tissue is almost absent.

11- Leaf curling :-

These symptoms represent irregular and extensive wrinkling and furrowing of leaves due to reduced growth of veins in comparison to the growth of laminar tissue resulting in shrunken veins and raised up laminar tissue leading to the curling of the leaves.

Example: leaf curl of papaya, leaf of tomato etc.

12- Leaf rolling:-

In these symptoms the downward and upward rolling of leaves takes place and this covers their entire length example leaf roll of potato.

13- Stunting or dwarfing:-

In this case the infected plants show general retardation in all their organs resulting in stunting of plants. The morphology of the stunted plants remains normal. Example: chrysanthemum stunt virus.

14- Rosetting:-

Shortening of internodes due to reduced growth brings the leaves together at the tip of the branches giving rosette like appearance.

15- Tumors:-

These are large and irregular outgrowths developed due to abnormal increase in size and number of the cells .tumors generally occur on roots of some leguminous plants.tumors develop on roots of remix sp.infected with wound tumor virus.

16- Pollen abortion and pollen sterility:-

In some infected plants either the pollen may not be produced (tomato ring spot) or may remain sterile (quirking virus on datura sp.)

17- Premature abscission of leaves:-

This is a common occurrence in many plant viral diseases.

18- Colour deviation:

* Chlorosis:

Appearance of light areas on the plant surface due to lack of plastid .chlorosis has two shapes either spotting (regular light areas) or mottling (irregular light areas).

* Mosaic:

Areas with different colours ,larger than chlorosis colours ranged from white to deep green.

* Yellowish:

All the leaf becomes yellow due to destruction of plastid.

* Colour breaking: changes of flower colour.

19- Death of tissue:

*Necrosis: destroying of group of cells or tissues or whole of plant body.

*Local lesions: Death of certain tissues or cells.

*Streaks:

The dead part of the leaves appears as longitudinal lines ranged from (7-10) lines with brown colour.

20- Deformations or malformations:

*Leaf curl or rolling of leaves.

*Fly form shape of leaf blade.

*Enation: abnormal growth on the leaf blade.

*Blister: pits formed due to group of cells the pits are more green spots than other parts of leaf blade.

21- Vein clearing:

The region around the vein appears colourless or white.

Internal symptoms of plant viral diseases:

Instead of the external symptoms described earlier there are many symptoms developed endogenously with the virus infected plants they are called internal symptoms. Some important ones are being given in brief:

1- Change in parenchymatous cell:

Mainly mosaic viruses alter the structural and functional characteristics of various parenchymatous cells e.g.

- a) Palisade parenchyma formation is poor. They also look like spongy parenchyma i.e. spherical
- b) Cells of chlorotic areas remain smaller in size.
- c) Intercellular spaces are reduced in size.
- d) Hyperplasia may result in the infected cells.
- e) Chlorophyll destruction may occur affecting the development of chloroplast and thus bringing various abnormalities in them.

2- Changes in xylem:

Viruses promote the formation of styluses and gummosis, tyloses the bladder like outgrowth. Block the xylem lumen. Gummosis represents the formation of gum like substances as a result of carbohydrate decomposition. These gums like substance sandy loses block the lumen of the xylem vessels stopping ascent of water and other inorganic substances. This results in finally wilting and even death of the host.

3- Change in phloem:

Necrosis is the ultimate fate of those phloem cells which are infected with viruses. These infections are however localized and mainly occur in the vicinity

of sieve elements. Sometimes hyperplasia has been observed before necrosis in infected phloem elements. Since phloem elements represent the food channel of the plant, any disturbance in it affects the food supply of the host or formation of callose in the sieve tube and companion cells.

4- Changes in cell and cell organelles

Following are some important changes that have been observed either in the cell organelles due to viral infections:

- (a) Infected cell increase vacuole formation in their cytoplasm.
- (b) The concentration of the cytoplasmic matrix becomes low.
- (c) Ribosomes are reduced in number.
- (d) There occur change in shape and size of nuclei of the infected cells.
- (e) Abnormality may operate in number and morphology of mitochondria of infected cells. Mitochondria is the unit of energy decreasing the no. of it lead to decrease energy of plant.
- (f) Meiosis and mitosis may also be affected in viral infected cells instead of above described internal symptoms, many amorphous and crystalline inclusions such as x-bodies, spindle or needle like crystals etc, have been reported within the cells infected by viruses. Such inclusions have never been observed in cells which are free from the concerned viral infection. On the lower surface of the leaves.
- (g) Formation of vesicles of vacuoles inside plastid.

5- Destroying of cell membrane which is permeable membrane.

Examples for plant viral diseases:

Potato virus y (PVY)

Pvy is a member of the *Potyviridae* family of viruses.

Common symptoms:

Once infected, a potato plant can express symptoms in as few as 10 days, depending on the variety. Symptoms of PVY infection are variable and range from mild (foliar mottling, streaking and mosaic) to severe (leaf necrosis, leaf drop and stunting). The severity of the symptoms depends on the potato cultivar, environmental conditions and the strain of PVY infecting the plant.



An example of how different PVY strains cause different levels of symptom severity. On the Pike variety, PVY^{N-WI} symptoms (top) are mild. PVY^O symptoms are more severe (bottom).

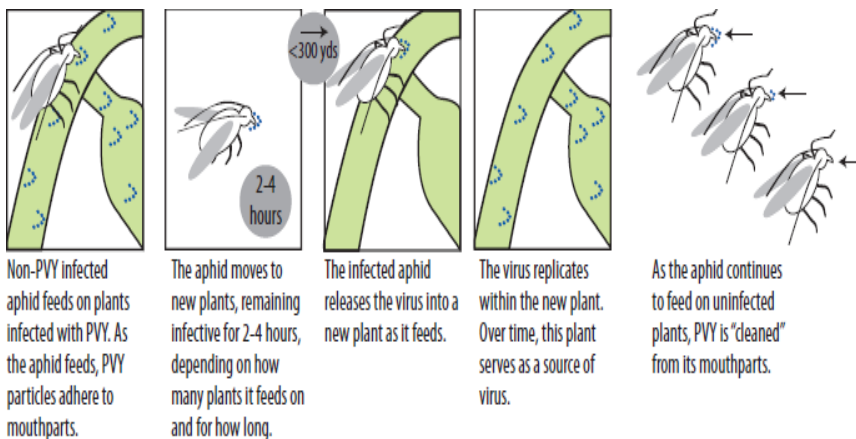


▲ **Figure 3.** A Yukon Gold tuber (top) when infected with certain strains of PVY is prone to develop potato tuber necrotic ringspot disease (bottom).

Usual means of spread:

PVY is spread from plant to plant by mechanical means or by aphids. Mechanical transmission generally occurs when an infected plant and an adjacent healthy plant are wounded by wind or human activity. The wounds of an infected plant leak sap that contains the virus and the wound of a nearby

healthy plant may take in some of that virus when the two plants touch. The more efficient and rapid form of transmission in fields involves aphid vectors. When an aphid feeds on a Pvy infected plant, virus particles adhere to the tips of its mouthparts. If the aphid then moves to a healthy plant and begins to feed, the virus particles are released and transferred to the healthy plant, leading to Pvy infection. After an aphid acquired the virus, it generally transmits it to uninfected plants for only a short period of time, usually less than two to four hours.



Principal hosts:

Solanaceous crops including tomato, pepper, tobacco and potato.

Control:

Excluding sources of the virus from the planted crop (by using seed certified to be free of Pvy is one strategy to exclude the virus from the farm), reducing the attractiveness of the growing crop to migrating aphids and reducing the likelihood that infectious aphids will feed on the crop.



Using seed certified to be free of PVY is one strategy to exclude the virus from the farm.

Banana bunchy top disease (rosetting)

What is banana bunchy top disease?

- The responsible for this disease is banana bunchy top virus.
- It is one of the most important and dangerous virus that infect banana.
- The virus becomes widely spread in presence of aphids.

Common symptoms:

- The initial symptoms are appearance of dark green lines on the down surface of midrib.



- The edges of the leaves become yellow then brown and become easy to break.
- As progress of infection the small leaves become crowded at the false tip of the branches.
- Forming the rosette shape (rosetting).
- The leaves emerges difficulty from infected adult plants and the edges become wavy shape and narrow.
- Usually the infected plants become unproductive and if the plants product fruit the fruit will be disfigured.
- The next generation of the infected plants will be stunting and the leaves will be hard and erected and not flat and shorter than the leaves of normal plant.



Usual means of spread:

- It doesn't transmit mechanically by cellular sap.
- It transmit with banana aphid (*Pentalonia nigronervosa*).

Control:

- Plant uninfected seeds.
- Put a cup of kerosene inside the plant to avoid aphids and cut the plant from the middle.
- Take off the infected plants with roots (the full plant) and burn the location of the removed infected plants.
- Pour kerosene on the soil (the location of the removed infected plants) and leave the soil exposed to air for two weeks and put (CaO) inside the plant.

Banana bract mosaic virus

Banana bract mosaic virus (BBrMV) is also called banana bract mosaic and Kokkan disease. This disease was first found in the Philippines in 1979.

Description

Initial symptoms of banana bract mosaic virus include green or red-brown streaks or spindle shaped lesions on the leaf stalks and sometimes on the midribs of the new banana leaves. Chlorotic (pale yellow or yellow-white) streaks may also appear on the bunch stems and fingers. In severe cases the affected fruit can be rejected. If the outer leaves are removed from the banana plant stem; red-brown, spindle-shaped streaks can be seen on the exposed stem.

Life cycle

Banana bract mosaic virus infects the vegetative, flowering and fruiting stages of banana plants. The disease is transmitted by aphids which acquire the virus while feeding on infected plants. The virus only lives inside the aphids for a short period of time and is transmitted in a nonpersistent manner. Banana bract mosaic virus can be spread by four aphids that are present in Australia: banana aphid (*Pentalonia nigronervosa*), corn aphid (*Rhopaloshiaphum maidis*) cotton or melon aphid (*Aphis gossypii*), cowpea aphid (*Aphis craccivora*). Double infections of banana bract mosaic virus and banana bunchy top virus (also transmitted by the banana aphid) can occur in banana plants.

Hosts

Banana bract mosaic virus infects cultivated and wild bananas (Musaceae plant family).

Spread

Banana bract mosaic virus is spread longer distances by infected banana planting material, including suckers, bits, corms and unindexed tissue culture plantlets.

Distribution

Banana bract mosaic virus is found in India, the Philippines, Sri Lanka, Thailand, Vietnam and Western Samoa.

Actions to minimise risks

Put in place biosecurity best practice actions to prevent entry, establishment and spread of pests and diseases: practise “Come clean, Go clean” ensure all staff and visitors are instructed in and adhere to your business management hygiene requirements source propagation material of a known high health status from reputable suppliers monitor your crop regularly keep records isolate banana plants or areas with suspect symptoms to prevent further spread



Figure 1 A dark, red-brown mosaic pattern on the banana flower bract is the main symptom of banana bract mosaic virus



Figure 2 Red-brown, spindle-shaped streaks on the exposed banana stem (when the outer leaves are removed) indicating banana bract mosaic virus

Tomato spotted wilt virus (TSWV):

Taxonomic position: Viruses: Bunyaviridae: *Tospovirus*

Symptoms

On tomatoes, plants show bronzing, curling, necrotic streaks and spots on the leaves. Dark-brown streaks also appear on leaf petioles, stems and growing tips. The plants are small and stunted as compared with healthy plants. The ripe fruit shows paler red or yellow areas on the skin. Sometimes, affected plants are killed by severe necrosis.



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Usual means of spread:

- TSWV is spread by tiny insects called thrips, which pick up the virus by feeding on infected plants.
- If the virus and the thrips are present, the severity of the disease depends on the weather.

- TSWV is not seed-transmitted.

Principal host:

TSWV is polyphagous on a great number of mostly herbaceous hosts. *Capsicum annuum*, lettuces, tobacco, tomatoes and various ornamental crops are the main hosts.

Control

ž The presence of thrips in the crops should be monitored using yellow sticky cards. If the disease appears in a crop, infected plants should be rogued and destroyed immediately and the house treated with insecticide against thrips.

Sugarcane mosaic virus

List of symptoms/signs

- Leaves: abnormal colours, abnormal forms, abnormal patterns and necrotic areas
- Stems: discoloration of bark and stunting or rosetting
- Whole plant: dwarfing

The particular symptoms depend on the virus strain, the host cultivar and the environmental conditions, particularly temperature.

Prevention and control

Control of SCMV:

- Applying herbicides may be useful in maintaining mosaic-free seed plots of cane if the level of infection is lower than 5%.

- Use of mosaic-free seed cane is an effective control measure where inoculum pressures are not intense. Thermotherapy of planting material can result in some plants that are free of SCMV.

Control of Aphid Vectors

- Altering the times of planting and harvesting so that they do not coincide with high aphid vector populations and can reduce losses.
- Should not be grown near infected sugarcane crops.
- The use of insecticides failed to prevent aphid vectors from spreading SCMV.
- Aphids which transmit SCMV come from outside as well as inside the sugarcane crop, care should be given to reduce the build up of the vector species in the vicinity.





Cucumber mosaic virus {CMV }

- Cucumber mosaic virus (CMV) is one of the most common plant viruses and causes a wide range of symptoms, especially yellow mottling, distortion and stunting. Expect damage whenever susceptible plants are growing well in spring and summer.

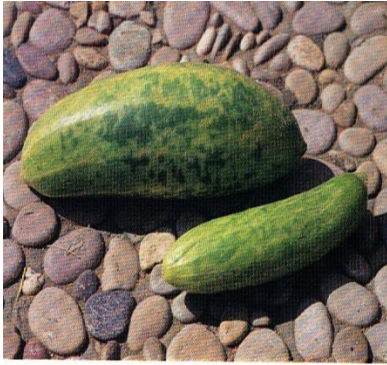


Principle Hosts

Apart from cucumbers and other cucurbits, it also attacks spinach ,lettuce and celery and many flowers, especially lilies.

Usually means of spread

- CMV is vectored by several aphid species which feed on a broad range of plants and this contributes to the very wide host range of this virus
- CMV is occasionally transmitted through seed in around 20 of plant species
- It can spread mechanically by a sap.
- It can also be transmitted in seeds and by the parasitic weeds, *Cuscuta* sp.
- It naturally spread in soil by zoospores of fungus.
- CMV is easily transmitted on garden tools and gardeners' fingers.



Cucumber: mosaic by cucumber mosaic virus (fruit symptoms) (Middle East)



Control

- **Chemical control:** There are no chemical controls available to control viruses. The use of insecticides to reduce aphid transmission is not practical.
- **Non chemical:** Avoid handling healthy plants after working with suspected infected ones until tools or hands have been washed with soapy water. Destroy suspect plants promptly to reduce the risk of transmission. Keep the garden weed free.

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.

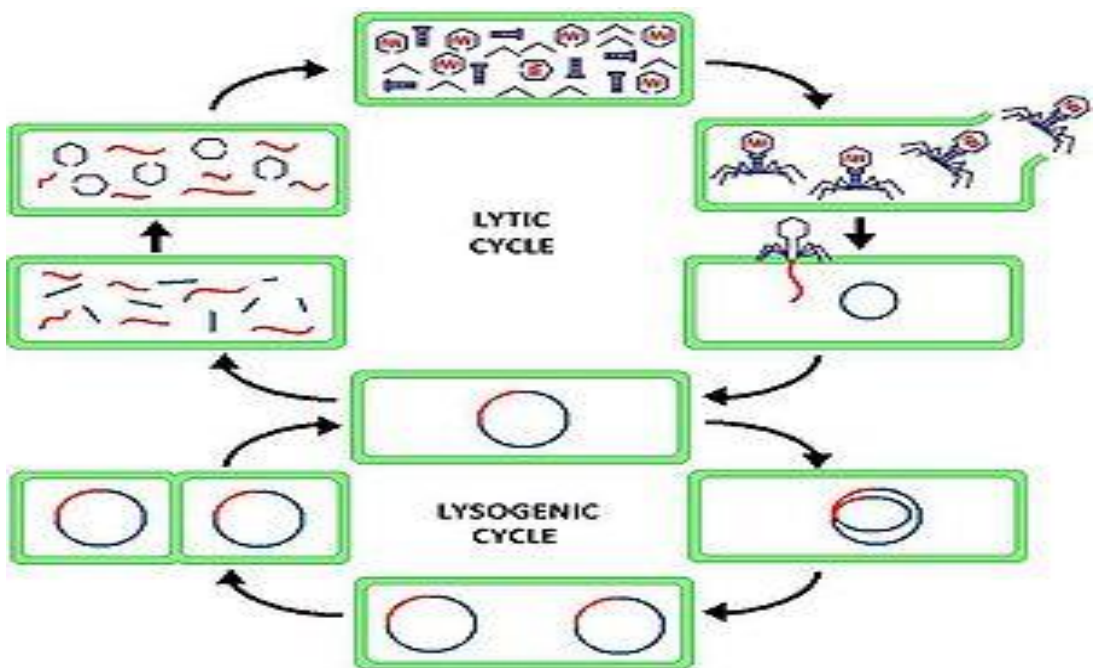
Phases of lytic cycle:-

- **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 unmaturing 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.

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).

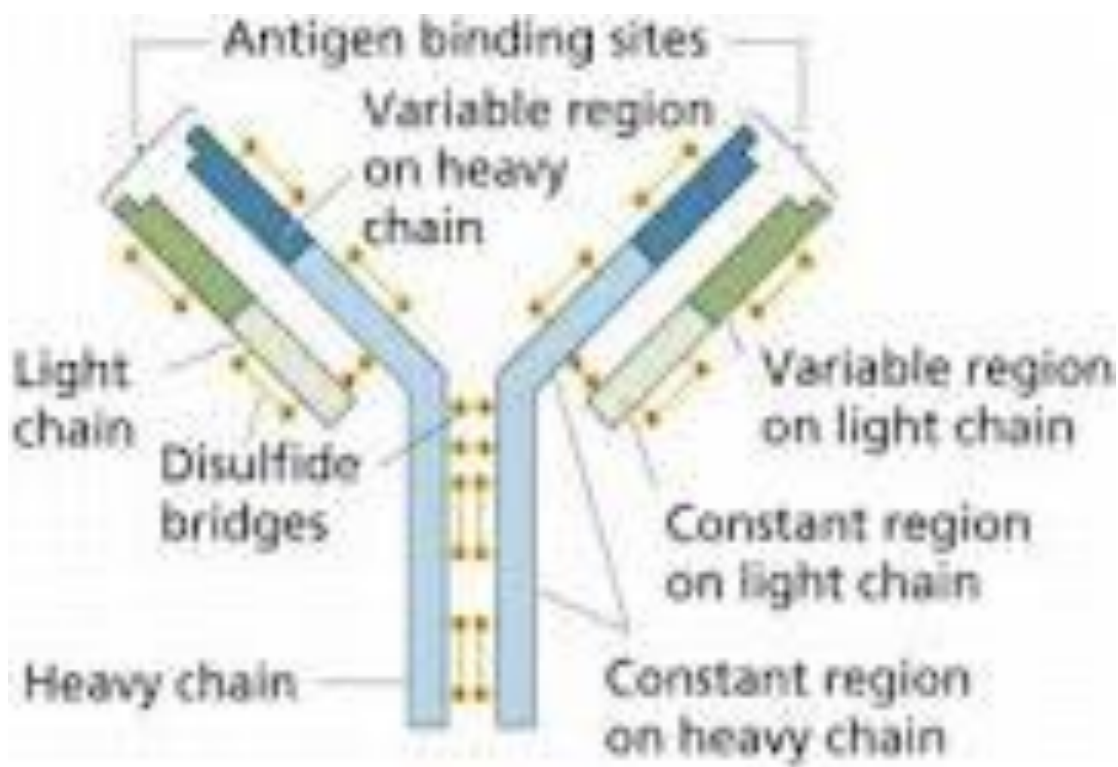
- **Antibody:**

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

Types of antibodies:

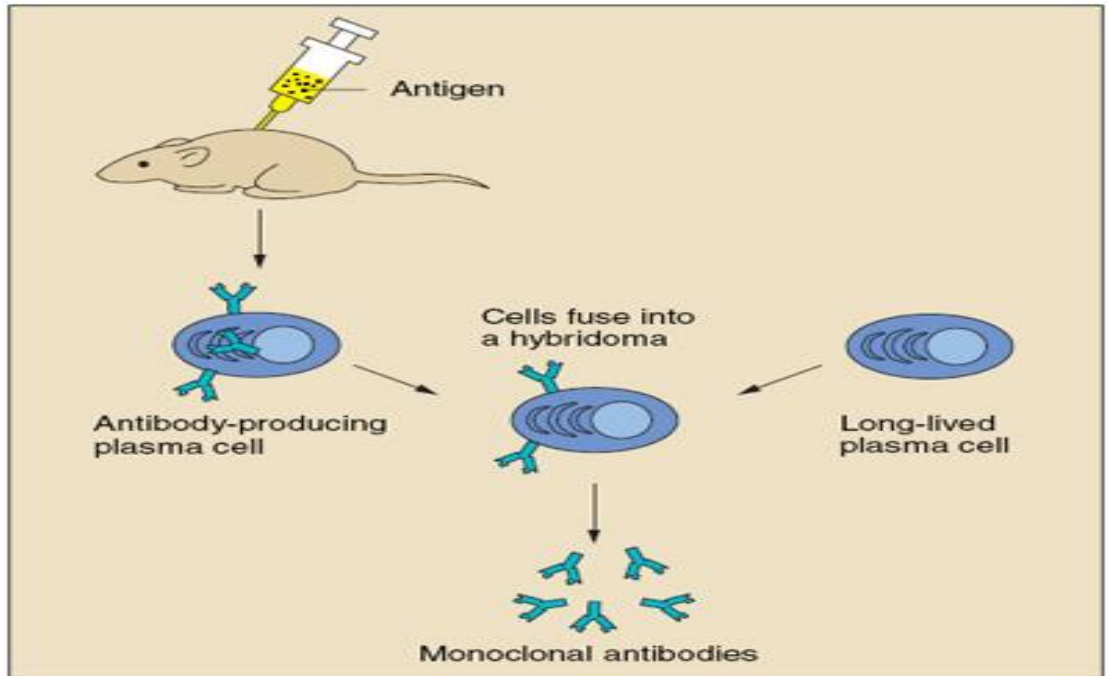
IgG, IgA, IgM, IgD, IgE

Structure of antibody



Monoclonal antibodies

They are made by a clone of cells that arise from a 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.

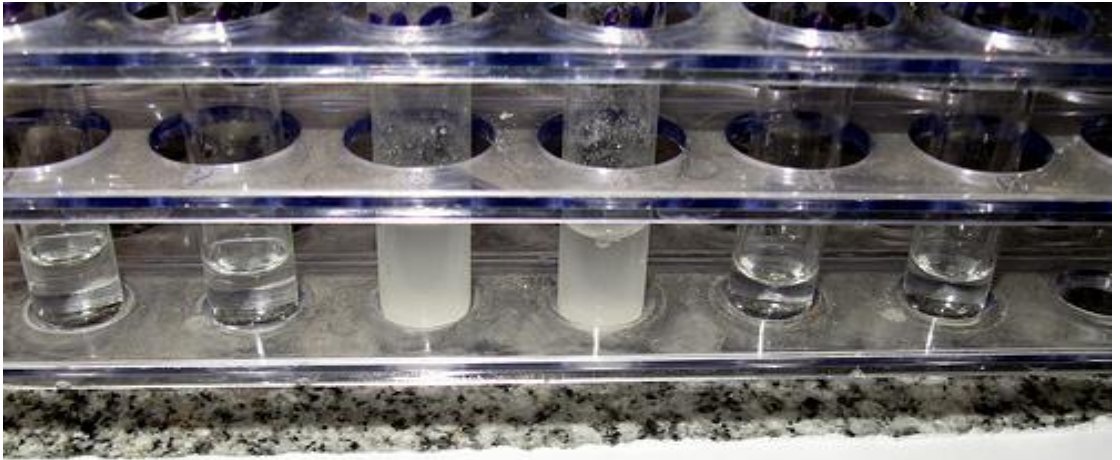
If in solution, the resulting reaction is precipitation.

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

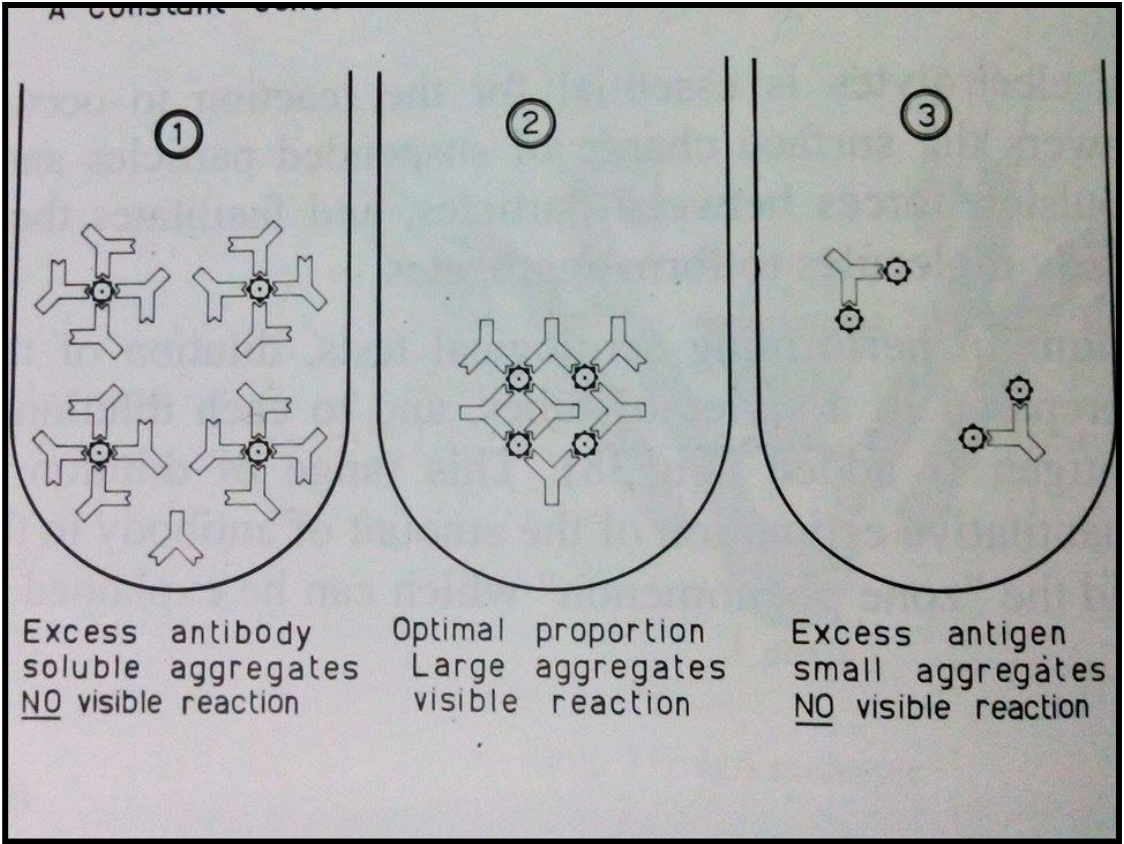
1- Zone phenomenon:

- 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 present at optimal concentration to each other→large aggregate is formed→visible reaction

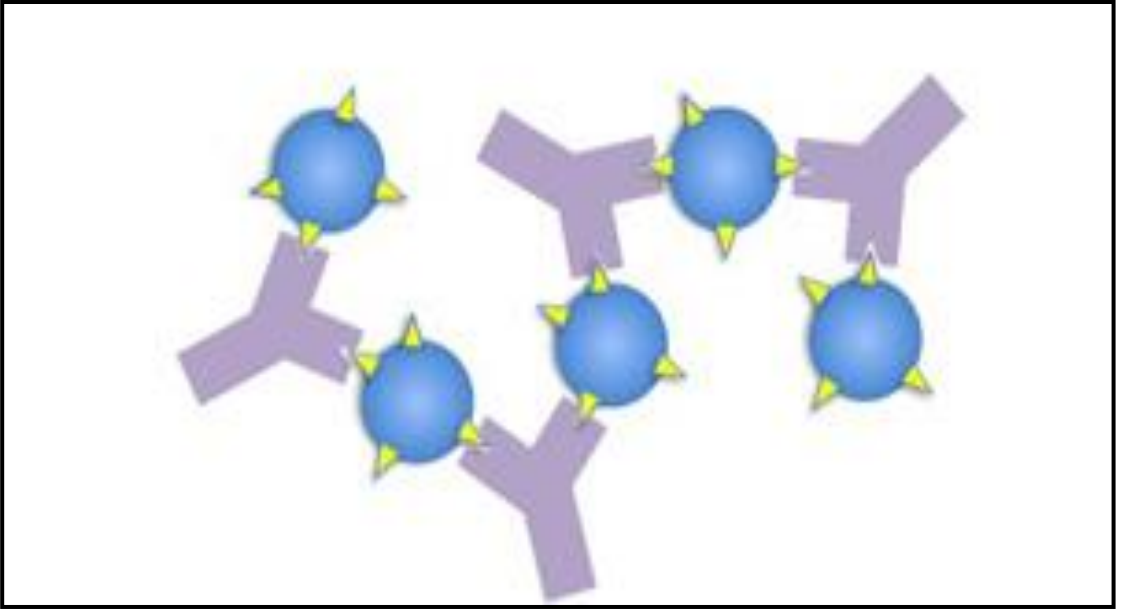
Zone phenomenon (cont.)



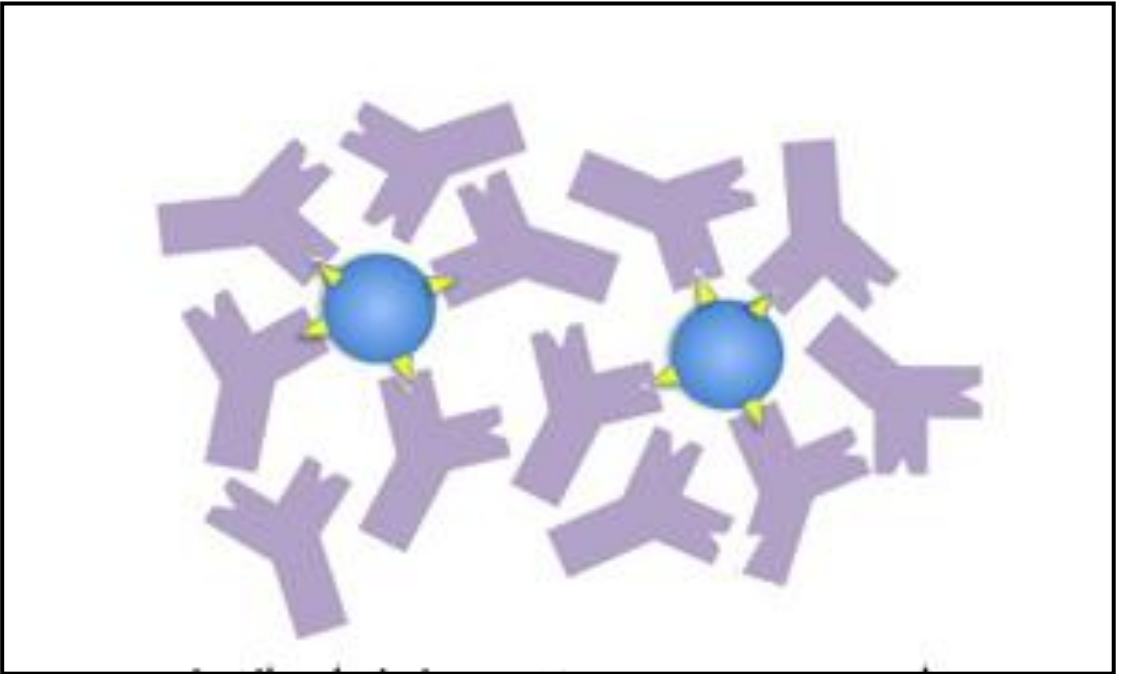
Zone phenomenon agglutination test seen in brucella



Diagrammatic 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 hemagglutination inhibition.
- Heterophile antibodies agglutination tests.

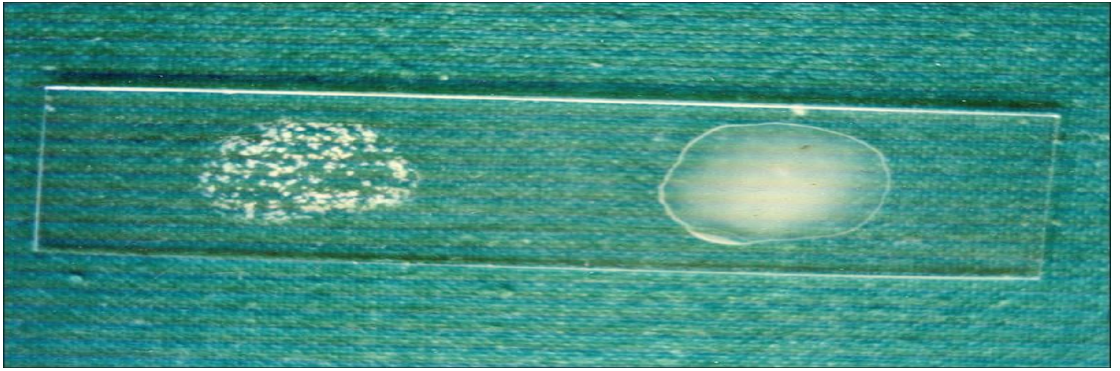
A- direct agglutination: has 2 forms

1- Slide agglutination:

- 2 drops 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 37⁰c

- Examine for visible clumping

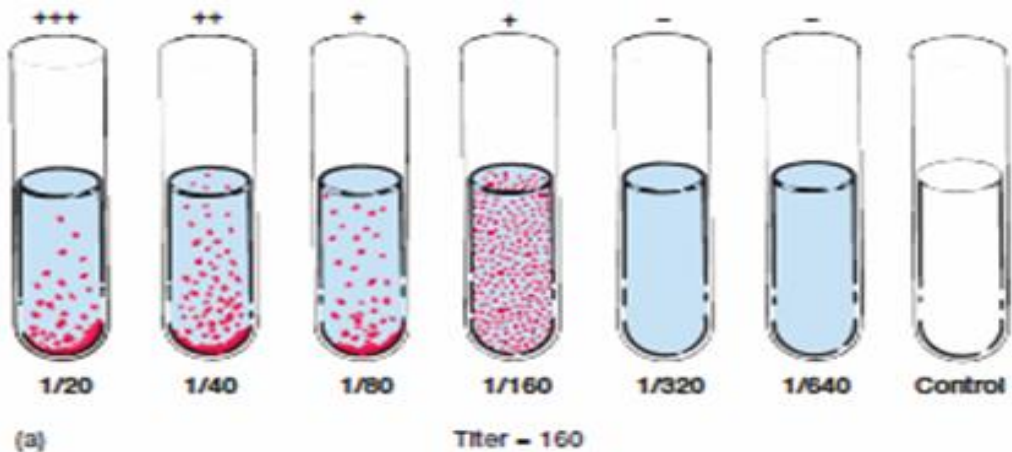
- Titre:

The highest 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.

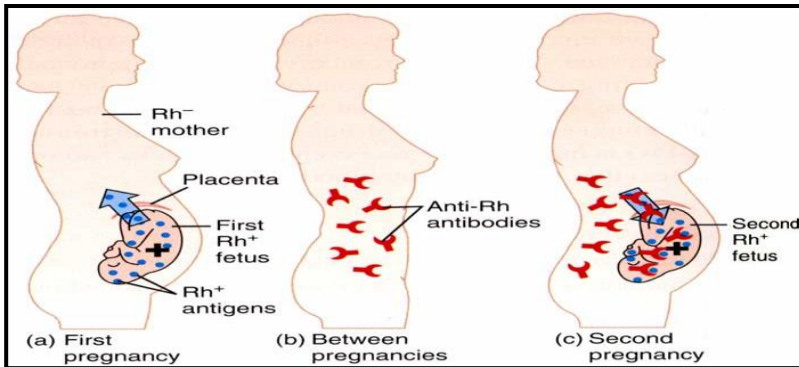
Application:

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 37⁰c 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 hemolytic 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:

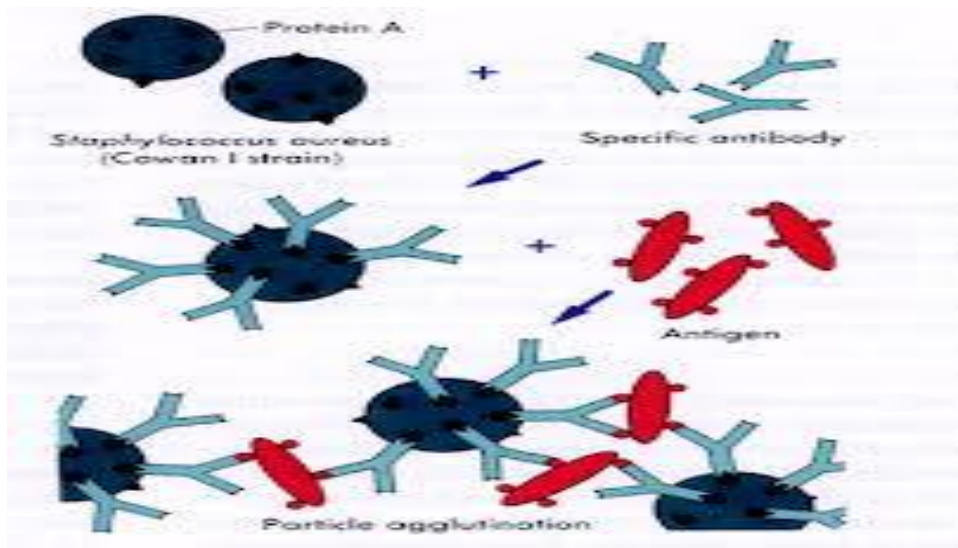
- Pregnancy test:
 - The antigen is human chorionic gonadotrophic hormone (HCG) in the urine of pregnant female.
 - The test is done by adding latex particles coated with anti-HCG to a drop of urine.
 - Agglutination occurs if HCG is present.

Your observation and comments:



4 – Coagglutination (COA):

- *Staph. aureus* rich in protein A on their 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:

