Antigen-Antibody Reactions: Salient features

Microbiology V

2.2.21

Introduction

- Ag and Ab combine with each other specifically & in an observable manner.
- Reaction b/w Ag-Ab serve several purposes.

➢ In the body:

  – Form the basis of AB mediated immunity in infectious diseases
  – Tissue injury – some types of hypersensitivity
  – Autoimmune diseases

- Laboratory: help in diagnosis of infections
- Epidemiological surveys:
  - Identification of infectious agents &
  - Noninfectious Ags such as enzymes

- General: reactions used for detection & quantification of either Ags or Abs

- Ag-Ab reactions *in vitro* are known as **serological reactions**

Antigen – Antibody reactions

Ag-Ab reaction occur in 3 stages:

1. Primary stage:

- initial reaction b/w the two, **without any visible effects**.
- Reaction is rapid
- Occurs even at low temperatures
- Obeys laws of physical chemistry & thermodynamics

- Reaction is reversible:
  - Ag-Ab combine by weaker intermolecular forces
  - Van der Waal's forces
  - Ionic bonds
  - Hydrogen bonding

- **Primary reaction detection:**
- Estimation of free & bound Ag or Ab separately – reaction mixture by several methods
- Physical & chemical methods
- Includes use of markers:
  - Radioactive isotopes
  - Fluroscent dyes
  - Ferritin

2. Secondary stage:

- Most cases primary stage is followed by secondary stage
- Leads to demonstrable events:
  - Precipitation
  - Agglutination
  - Lysis of cells
  - Killing of live Ag
  - Complement fixation
  - Immobilization of motile organisms
  - Enhancement of phagocytosis

- Based on the nature of the reaction the Ag & Ab are termed respectively
- Ex. Agglutination reaction
- Ab – agglutinin, Ag – agglutinogen
- Precipitation reaction
- Ab – precipitin, Ag – precipitinogen

- Ag and Ab property:
- Antibody: Single Ab can cause different reaction – precipitation, agglutination & other serological reactions
- Based on the nature of Ag and reaction conditions.
- Antigen: stimulate production of different immunoglobulins classes (IgG, IgM etc)
- Differ in their reaction capacities & other properties

3. Tertiary reactions:

- Some Ag-Ab reactions that occur in vivo initates chain reactions that leads to:
  - Neutralization
  - Destruction of injurious Ag
  - Tissue damage

- Tertiary reactions include:

➢ **Humoral immunity** against infectious diseases

➢ Clinical allergy

➢ Immunological diseases

Comparative efficiency of Ig classes in different serological reactions

Reaction	IgG	IgM	IgA
Precipitation	Strong	Weak	Variable
Agglutination	Weak	Strong	Moderate
Complement fixation	Strong	Weak	Negative
Lysis	Weak	Strong	Negative

General features of Ag-Ab reactions:

1. Reaction is specific.

- Ag combine only with its homologous Ab and vice versa.
- Specificity is not absolute and cross reactions may occur
- due to antigenic similarity or relatedness

2. Entire molecule react and not in fragments.

- Antigenic determinant present in a large molecule or on a carrier particle – reacts with its Ab
- Whole molecules or particles are agglutinated

3. No denaturation of Ag or Ab during the reaction

4. Combination occurs on the surface.

- Surface Ag that are immunologically relevant
- Ab to the surface Ag of infectious agents are generally protective.

5. Combination is firm but reversible.

- Firmness of union is influenced by the affinity and avidity of the reaction

- **Affinity** – intensity of attractionb/w Ag-Abmolecules
- Function of closeness of fir b/w epitope – Ag combining region of it Ab

- **Avidity** – strength of the bond after the formation of Ag-Ab complexes

- Reflects the overall combining property of the various Ab molecules in an antiserum

- Possess different affinity constants with multiple epitopes of the Ag

6. Both Ag and Ab participate in the formation of agglutinates or precipitates

7. Ag and Ab combine in varying proportions, unlike chemicals with fixed valencies.

- Both Ag and Ab are multivalents.

- **Ab** are generally **bivalent** (2)
- IgM has 5 or 10 combining sites

- **Ag** have valencies up to 100

Measurement of Ag and Ab

- Measurement methods are available for Ag-Ab reactions – primary, secondary & tertiary reactions
- Measurement:
- Mass – mg of nitrogen
- Units or **titre** – commonly used

- **Ab titre - serum**:
- highest dilution of the serum that shows an observable reaction with the Ag in the particular test.

- Serum titre influenced by:
  - nature & quantity of Ag
  - type & test conditions

- Ag may also be titrated against sera

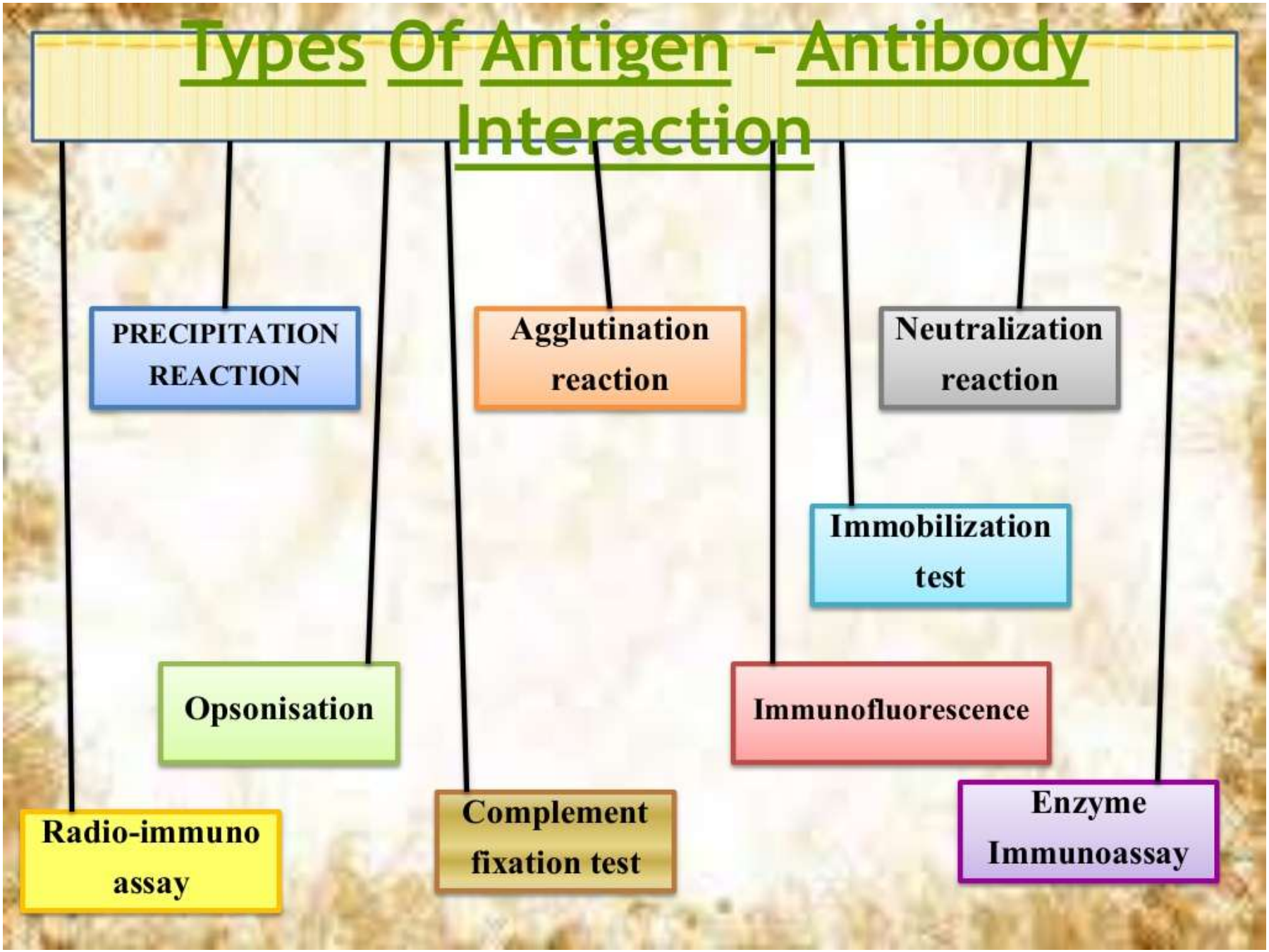
Parameter in serological tests:

- **Sensitivity:** ability of the test to detect even very minute quantities of Ag or Ab.
- Highly sensitive test – false negative results are absent or minimal

- **Specificity:** ability of the test to detect reactions b/w homologous Ag–Ab only, and with no other.
- Highly specific test – false positive reactions are absent.
- Sensitivity and specificity of a test are inversely proportional.

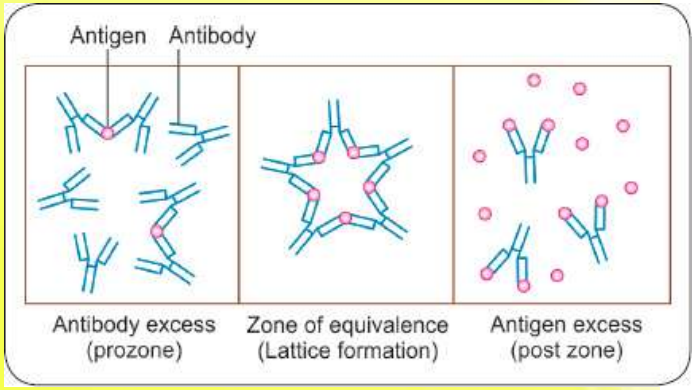
- Serologicals test are performed using **standard reagents** for reliable results, reproducibility & comparability.
- Thus prevents variability in results.

Types Of Antigen - Antibody Interaction



PRECIPITATION REACTION

- Precipitation refers to an antigen-antibody reaction between a soluble antigen & its antibody resulting in the formation of insoluble precipitate. The antibody causing precipitation is called PRECIPITIN.



- Precipitation occurs in two media:

a) Liquid or solution,

b) Gel - agar, agarose or polyacrylamide.

- Formation of an Ag-Ab lattice depends on the valency of both the antibody and antigen.

- The antibody must be bivalent; a precipitate will not form with monovalent Fab fragments. The antigen must be either bivalent or polyvalent; that is, it must have at least two copies of the same epitope, or have different epitopes that react with different antibodies present in polyclonal antisera.

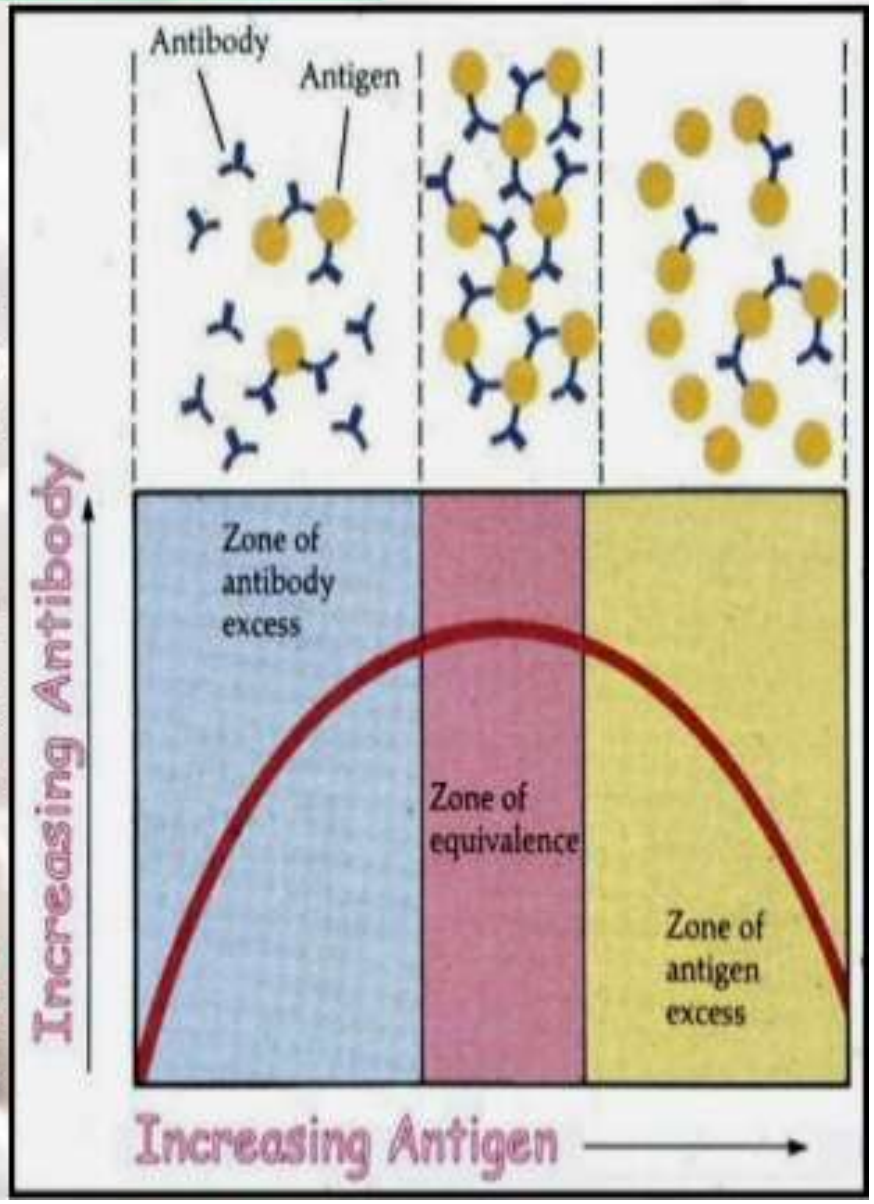
Precipitation curve

- *Plots of the amount Ag/Ab complexes precipitated when increasing Ag concentrations are added to constant concentration of Ab. It reveals 3 zones:*

1. ***Zone Of Antibody Excess (PROZONE)*** - **precipitation is inhibited and antibody not bound to antigen can be detected in the supernatant.**

2. ***Zone Of Equivalence*** - **maximal precipitation in which antibody and antigen form large insoluble complexes and neither antibody nor antigen can be detected in the supernatant.**

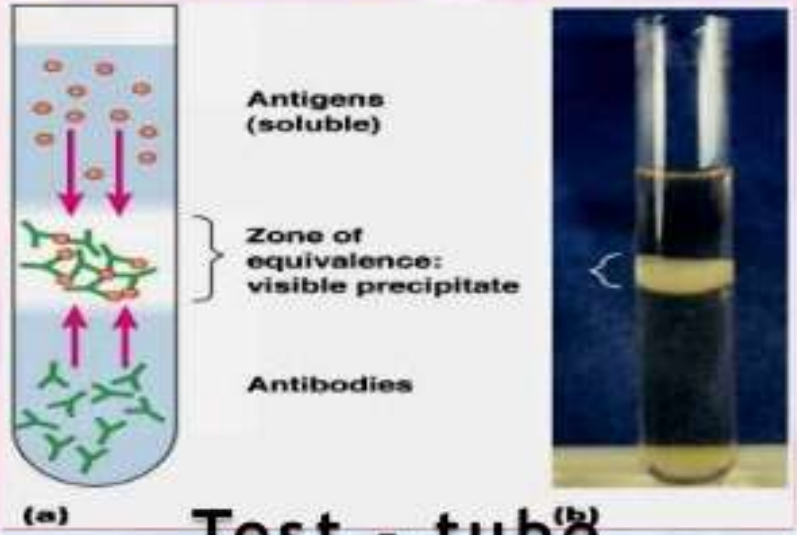
3. ***Zone Of Antigen Excess (POSTZONE)*** - **precipitation is inhibited & Ag. not bound to Ab. can be detected in the supernatant.**



Precipitation In Liquid Or Solution

- Soluble antigen + antibody (in proper proportions) → visible precipitate
- Ring test and flocculation test are examples of precipitation in solution.

a) *Ring test* :- In this test, antigen solution is layered over antiserum in a test tube or capillary tube. Precipitation between antigen & antibodies in antiserum solution is marked by the appearance of a ring of precipitation at the junction of two liquid layers. C–reactive protein (CRP) & streptococcal grouping by the Lancefield methods are the examples of the ring test.



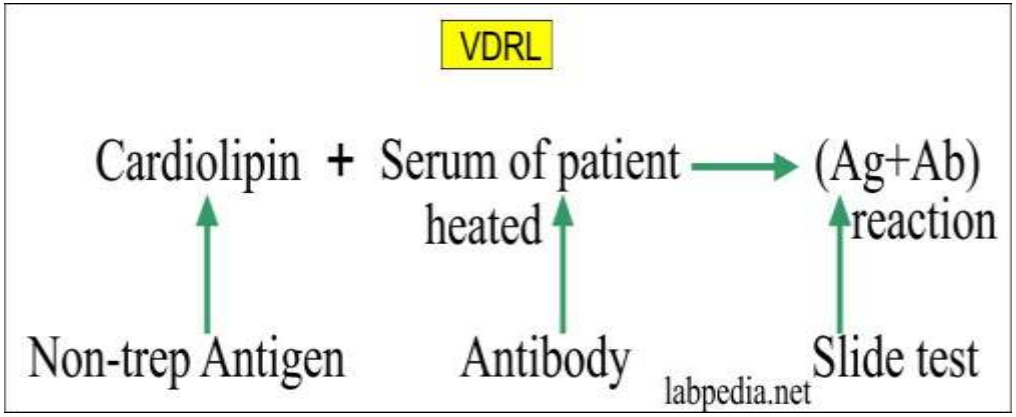
Test - tube Reaction

Capillary - tube Reaction

VDRL test

Slide precipitation/flocculation test

- **VDRL** stands for **Venereal Disease Research Laboratory** test.
- It is used for serological diagnosis of **syphilis** and it is an example of Slide flocculation test.
- In this test **cardiolipin** antigen is used as reagent to detect auto-antibody in serum of patients.



- Cardiolipin antigen is an alcoholic extract of bovine heart muscle to which lecithin and cholesterol are added.
- Cardiolipin reacts non-specifically to cardiolipin auto-antibodies (IgM and IgG) to form flocculation (floccular precipitation)

- The autoantibodies are not only produced in case of syphilis but also in other treponemal infection
- So this VDRL test is non –specific test.
- For the test, at first a drop of antigen is placed on a slide and then a drop of serum is added to it.
- The slide is rotated to mix the content. In case of positive test, flocculation occurs.

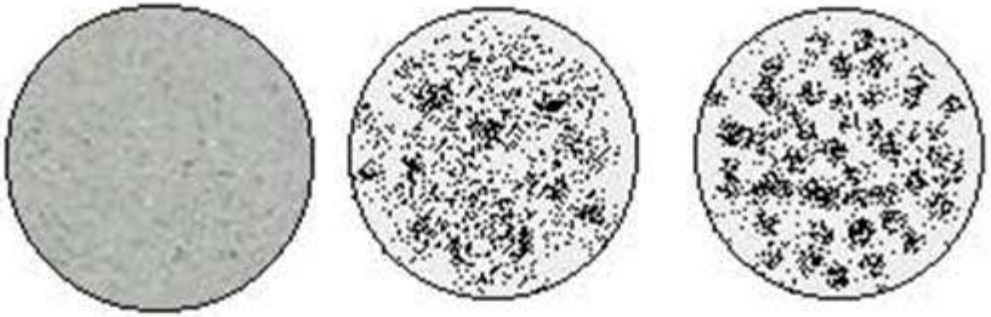
Procedure:

- Take patient's serum, heat is for 30 minutes at 56°C and then allow to cool to room temperature
- Take a measured volume of diluted antigen suspension (a colloidal suspension of tissue cardiolipid or chemically synthesized cardiolipin) and add to a measured volume of serum in a glass slide.
- Rotate the slide for about 4 minutes,
- Examine the slide microscopically for clumping of Ag-Ab complex using 10X objective and eye piece.

Result interpretation

- Reactive (Positive test): If Ag-Ab clump of large or medium size then it is reported as reactive (Positive VDRL test)
- Weakly reactive: If Ag-Ab complex is small sized, it is weakly reactive
- Non-reactive: If no Ag-Ab clump is formed, it is non-reactive
- All reactive and weakly reactive serum require serial dilution to estimate antibody titer.

VDRL test result



Non reactive Weakly reactive Strongly reactive

Precipitation in Gels

- The precipitation test in agar gel is termed as *immunodiffusion* test. In this test, reactants are added to the gel and antigen – antibody combination occurs by means of diffusion. The rate of diffusion is affected by the size of the particles, temperature, gel viscosity, amount of hydration, and interactions between the matrix and reactants.

- Immunodiffusion reactions have the following advantages:

- In this test, the line of precipitation is visible as a band, which can also be stained for preservation.

- The test can be used to detect identity, cross-reaction, and nonidentity between different antigens in a reacting mixture.

- Immunodiffusion reactions are classified based on the -

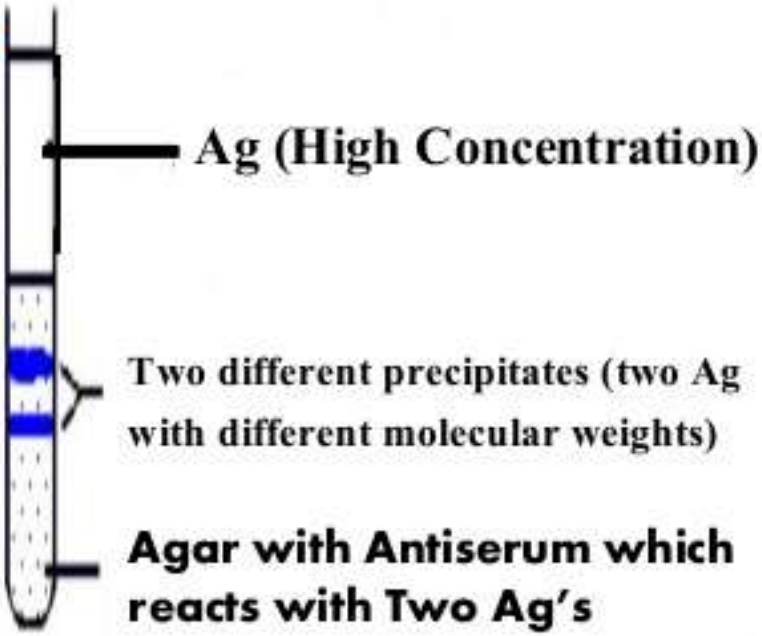
(*a*) *number of reactants diffusing and* Single diffusion/ double diffusion

(*b*) *direction of diffusion,* One dimension/ two dimension

as follows:

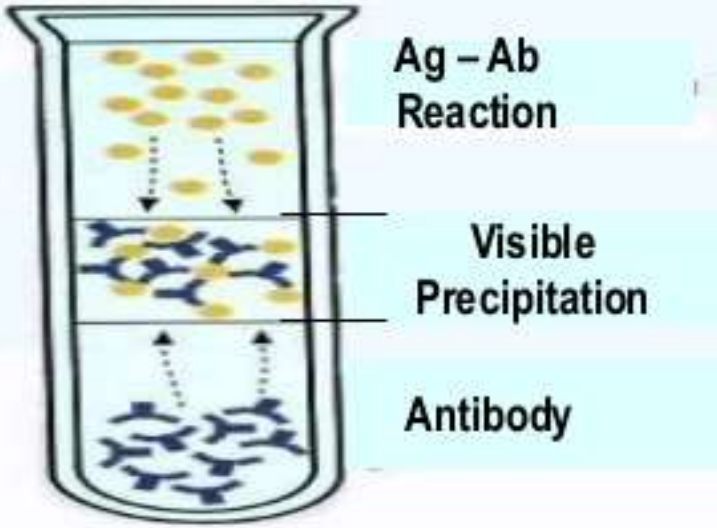
❖ ***Single Diffusion In One Dimension (Oudin Procedure) :-***

- Ab is incorporated in agar gel in a test tube & Ag solution is layered over it.
- Ag diffuses downward through the agar gel – forming a line of precipitation.
- The number of precipitate bands shows the number of different antigens present in the antigen solution.



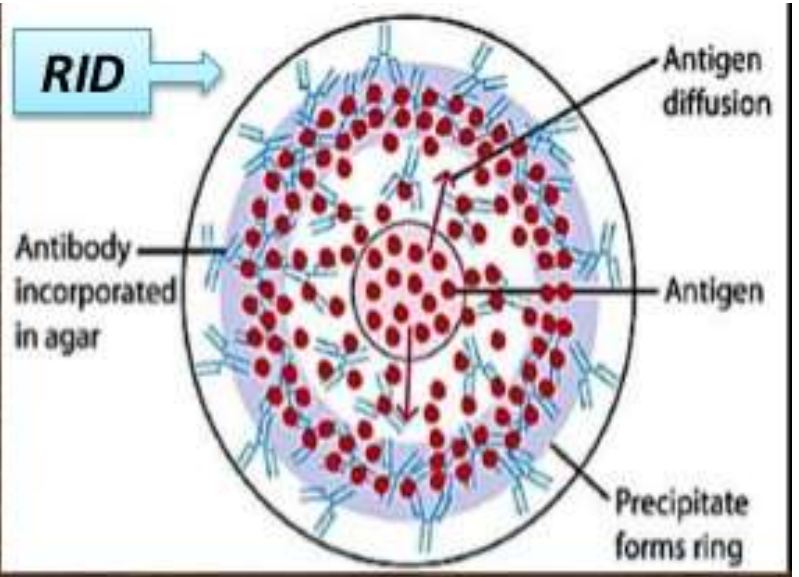
❖ ***Double diffusion in one dimension (Oakley-Fulthorpe procedure) :-***

- Ab is incorporated in agar gel. Above which is placed a column of plain agar.
- The Ag is layered over it. The Ag & Ab move towards each other through the intervening column of plain agar & form the precipitate.



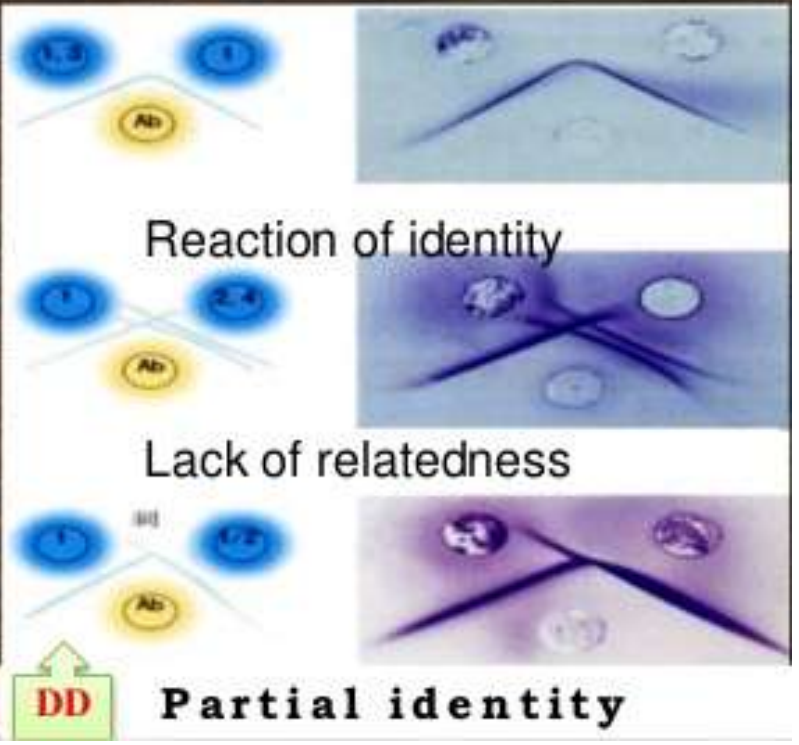
❖ Single Diffusion In Two Dimension (Radial Immunodiffusion) :-

- In this method antiserum solution containing antibody is incorporated in a agar gel on a RID slide or petri plate.
- Wells are cut and antigen is applied in the gel. Then Ab present in the gel reacts with the Ag which diffuses out of the well. Precipitation rings are formed around the wells.



❖ Double Diffusion In Two Dimensions (Ouchterlony Procedure) :-

- Ab is incorporated in agar gel.Above which is placed a column of plain agar. The Ag is layered over it. The Ag & Ab move towards each other through the intervening column of plain agar & form the precipitate.
- Antiserum – central well. Different Ags in the surrounding wells.



DOUBLE DIFFUSION IN TWO DIMENSION

Three types of reactions can be demonstrated as follows:

- **Fusion of lines at their junction to form an arc.**

-Serologic identity / presence of common epitope.(two identical Ag's are present)

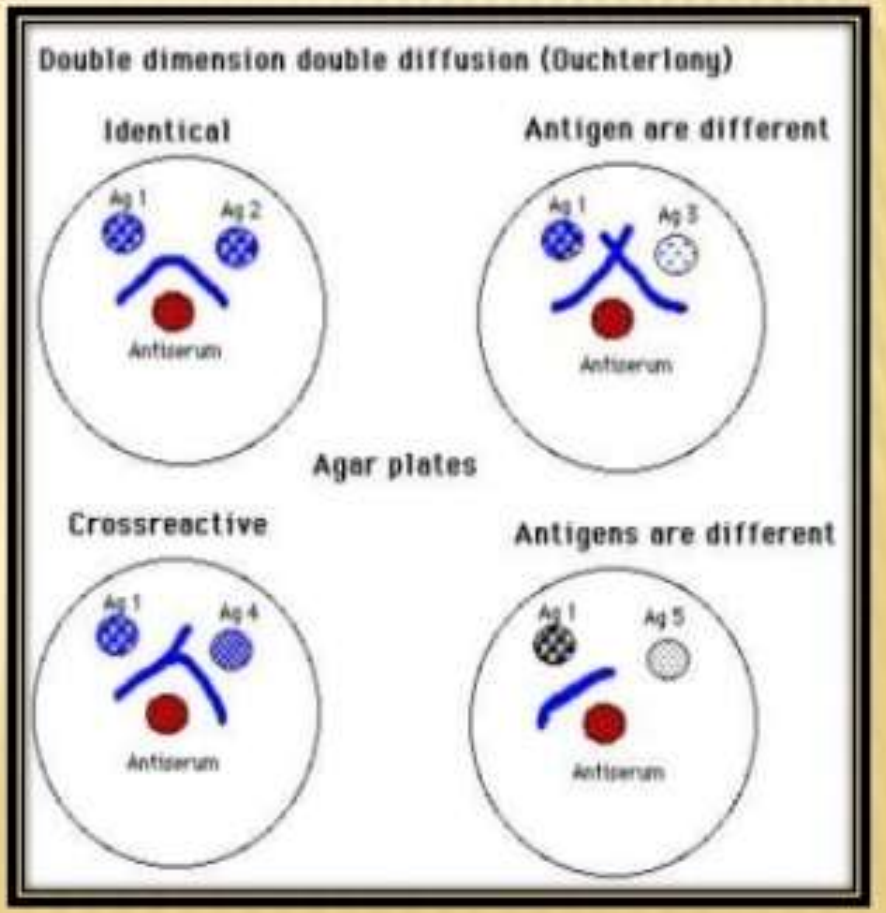
- **Crossed lines.**

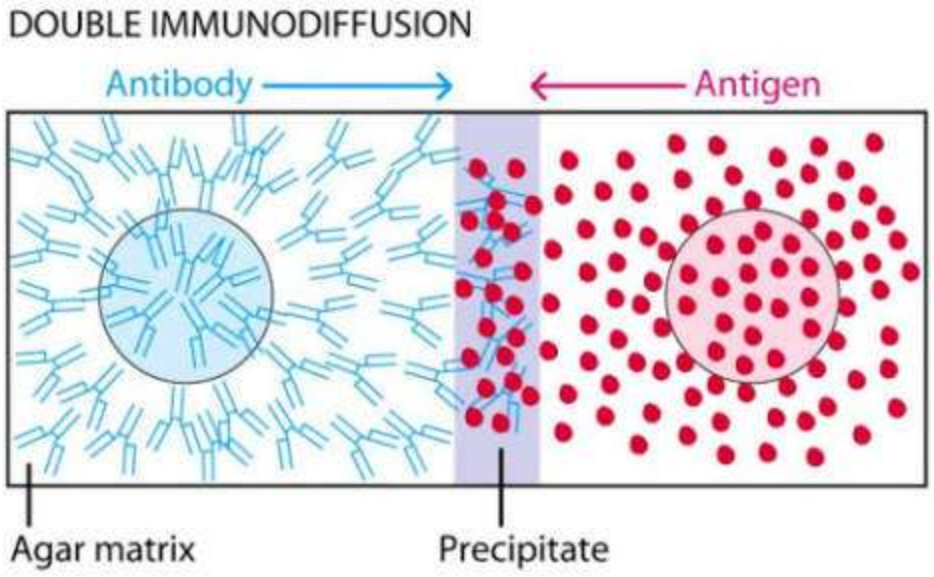
-Demonstrates 2 separate reactions.

-Compared Ag's shared no common epitopes.

- **Fusion of 2 lines with spur.**

-Indicates cross-reaction or Partial identity





Both Ag and Ab move diffuse independently in the agar gel towards each other in horizontal & vertical directions

DOUBLE DIFFUSION IN TWO DIMENSION

Uses:

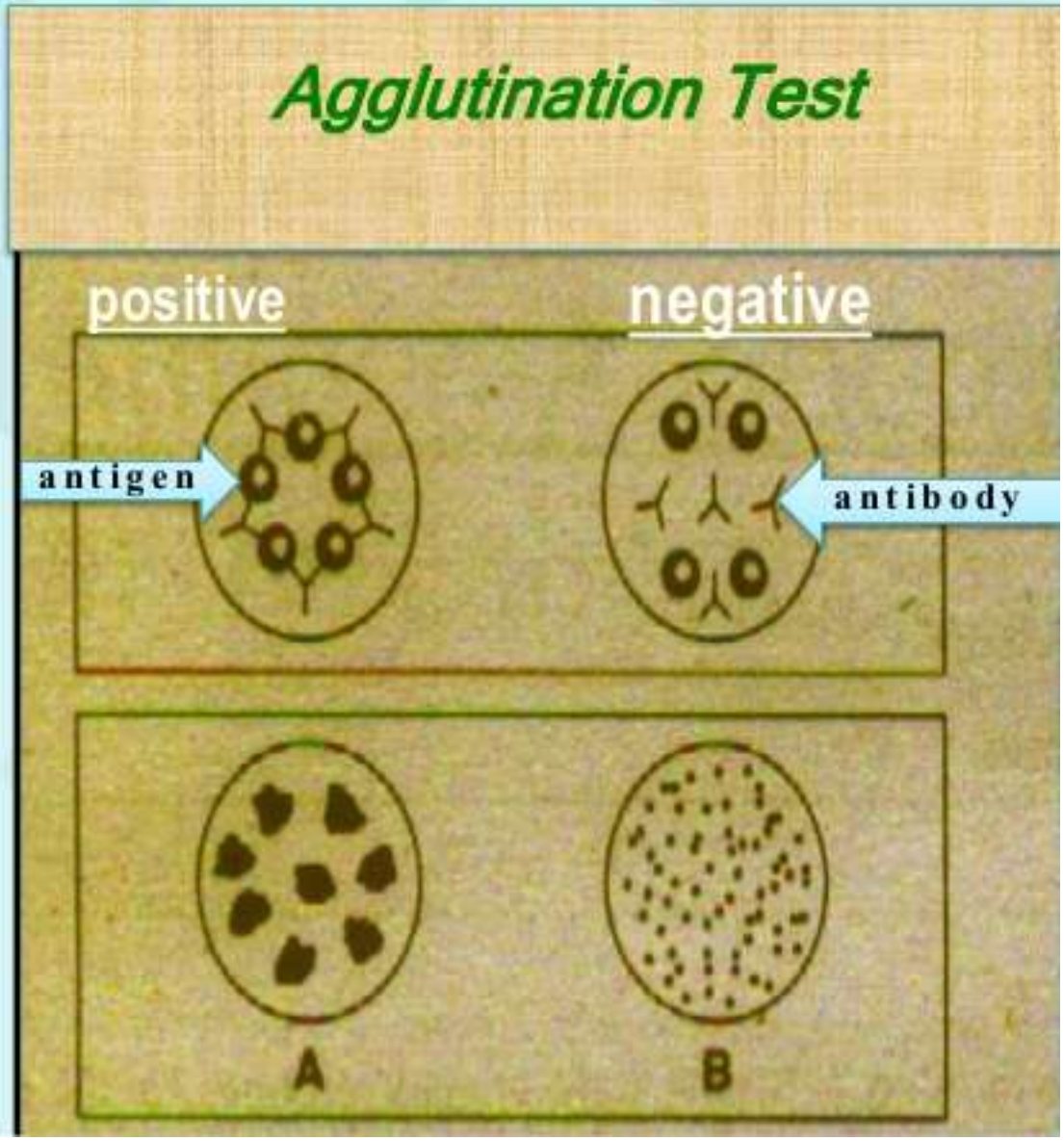
- It is an immunological technique used in the detection, identification and quantification of antibodies and antigens, such as immunoglobulins and extractable nuclear antigens.
- Demonstration of Antibodies in the serodiagnosis of small pox.
- This technique is also used for the Identification of fungal antigens.

AGGLUTINATION REACTION

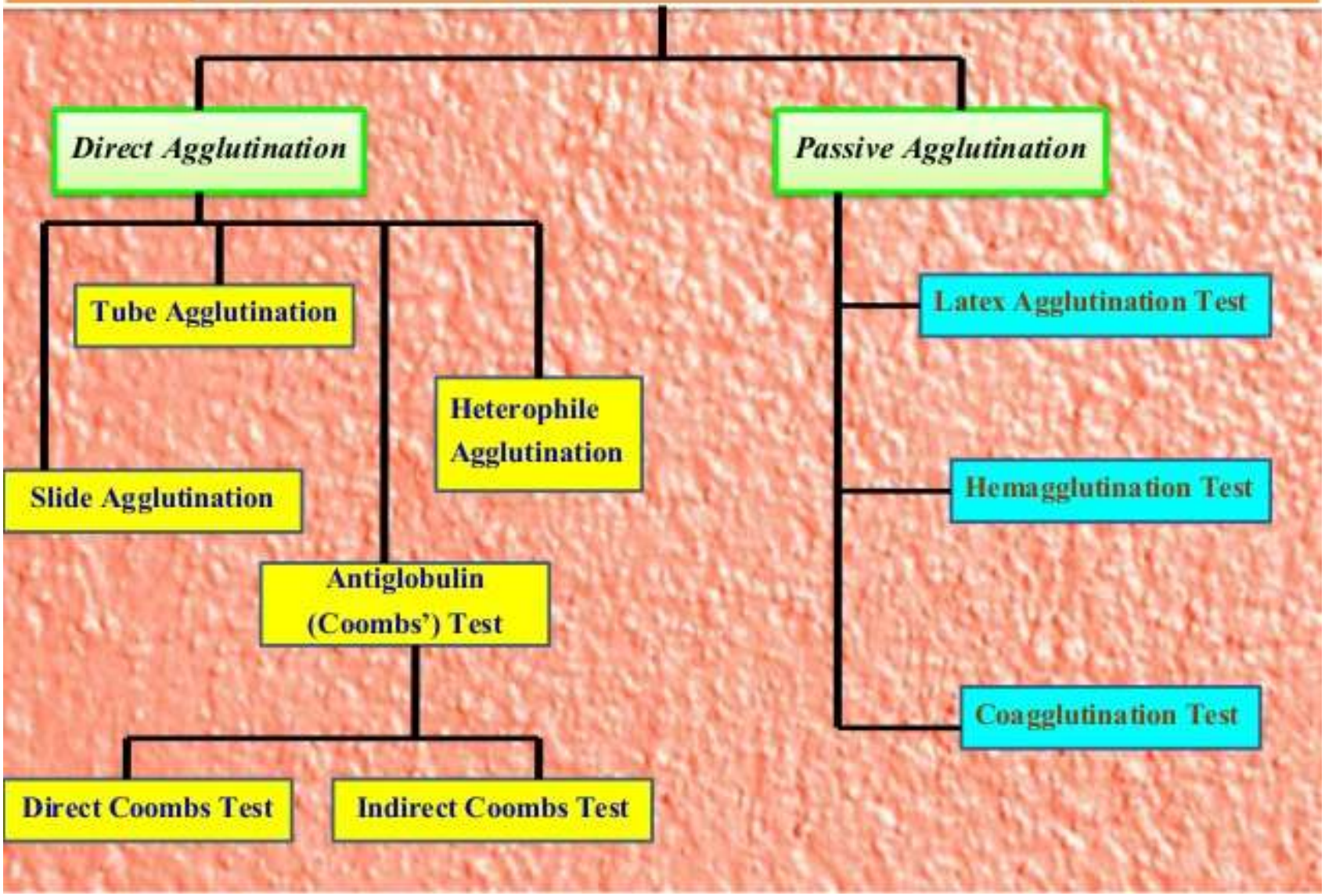
- The interaction between antibody & a particulate (Insoluble) antigen results in visible clumping called agglutination. The antibodies that cause agglutination are called Agglutinins & the particulate antigens aggregated are called Agglutinogens.

- Particulate antigen include:

a) bacteria,
b) white blood cells,
c) red blood cells,
d) latex particles .



Types of agglutination reactions



APPLICATIONS :-

- Blood typing.
- Bacterial infections.
- Viral Infections.

LIMITATIONS :-

- Time consuming (1 day)
- Cannot distinguish IgG from IgM.

Widal test

- Widal test is a serological method to diagnose enteric or typhoid fever that is cause by the infection with pathogenic microorganisms like *Salmonella typhi*, *Salmonella paratyphi* a, b and c.

- The method of diagnostic test is based upon a visible to the naked eye agglutination (clumps) reaction between antibodies of patient serum and antigens **specifically** prepared from *Salmonella sp* (KIT).

- *Salmonella* possess the following 3 antigens:
- Flagellar antigen or H antigen
- Somatic antigen or O antigen
- Surface antigen or Vi antigen

- The tests measure agglutinating antibodies directed against a *Salmonella O* somatic surface antigen and/or a *Salmonella H* flagella antigen of the suspected organism.

- The Widal test detects **antibodies** against O and H antigens.

- Type of techniques is use direct agglutination.

- Widal test, firstly descried by **Fernand Widal** in 1896.

Principle of Widal test:

- Antibody in the serum produced in the response to *Salmonella* organism, the kit contains antigen suspensions that are killed bacteria and they were stained to enhance the reading of agglutination tests.

- The **blue** stained antigens are specific to the somatic antigens (O-Ag), while the **red** stained antigens are specific to the flagella antigens (H-Ag).

Materials And Reagents Provided With The Widal Kit:

- Antigen suspensions (specifically prepared from *Salmonella* sp.) 8 Antigens: O, H, AO, AH, BO, BH, CO, CH.
- Positive control (Vial).
- Negative control (Vial).
- Instruction for use (leaflet).
- White Glass slide.
- Stirring Sticks.

Widal controls:

Widal negative control (-)

- Contains no antibodies against the specific bacteria.

Widal positive control (+)

- The widal positive control contains ready to use standardized goat antiserum with polyspecific antibodies having specific reactivity towards S. typhi O and H antigens, S. paratyphi AH and BH, S. paratyphi AO and BO, S. paratyphi CO and CH antigens and is useful in the validation of the performance of Widal reagents.

Rapid Slide (screening) Test:

- Slide Widal test is more popular as it gives rapid results.

Producers:

1- Place 1 drop (or transfer 50 µl) of the serum patient into each of circle slide.
2- Add 1 drop of well shake Ag. O, H, B (O) & B (H) respectively into each circle.
3- Spread the contents to fill the whole circle area.
4- Mix & rotate the slide for 1 minute & observe for agglutination.
5- Report the result.

Observation and Result:

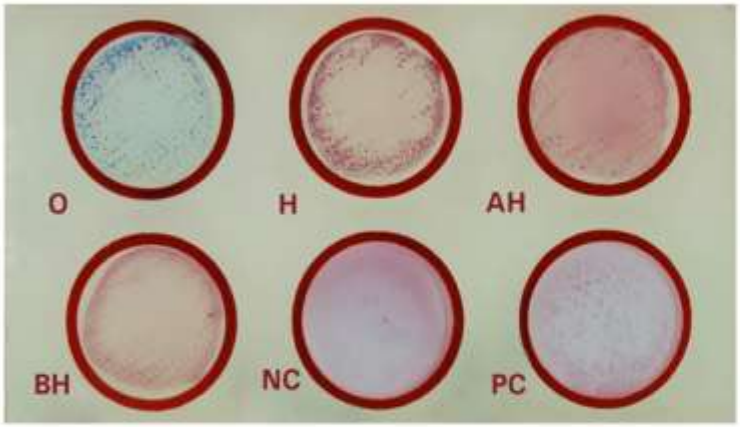
- No agglutination = Negative

- Result reported as **'titres'** : Highest dilution where agglutination is seen:

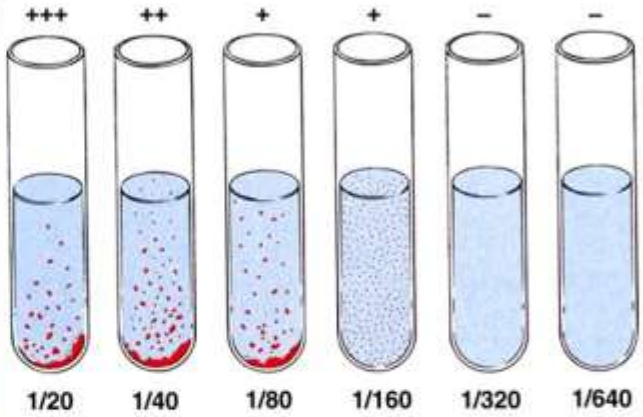
- If agglutination appear after 15 seconds = (1:640)
- If agglutination appear after 30 seconds = (1:320)
- If agglutination appear after 1 min. = (1:160)
- If agglutination appear after 1.30 min. = (1:80)

- This test is a screening test only for the detection of Widal agglutinins. If result is positive it must be confirmed by other serological tests for Widal.

Widal Test



Rapid Slide Test



Quantitative Tube Test

Interpretation

- *Salmonella typhi* O (+ve mean recent (acute) infection).
- *Salmonella typhi* H (+ve mean old (chronic) infection).
- *Salmonella paratyphi* bO,bH (+ve mean carrier can infect other).

- Test results need to be interpreted carefully in the light of past history of enteric fever, typhoid vaccination, general level of antibodies in the populations in endemic areas of the world.

Limitations of Widal test:

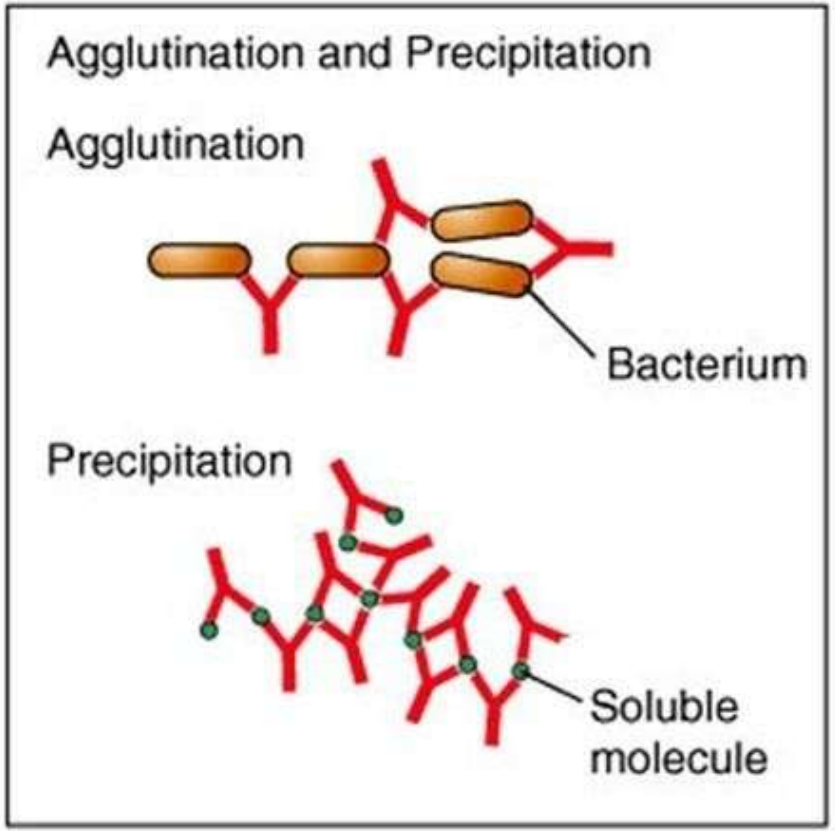
- The Widal test has a very low specificity, less sensitive, confusing and difficult to interpret for the diagnosis of typhoid f ever, because cross-agglutinating antibodies remaining from past infections with related salmonella serotypes give false-positive results.

- Furthermore, in areas where fever due to infectious causes is a common occurrence. So false positive reactions may occur as a result of non-typhoid.

- In spite of several limitation many Physicians depend on Widal Test.

Precipitation/Agglutination

- Antibody/antigen interaction
- **Similarity**: antibody crosslink antigen and form precipitate
- **Difference**: nature of antigen
  - Precipitation = soluble antigen
  - Agglutination = insoluble antigen e.g. RBC, bacteria, antigen fixed on beads (HCG, bacterial toxins, etc.)



Picture taken from: https://www.studyblue.com/notes/note/n/final-exam/deck/2914433

Complement fixation test (CFT)

- Complement fixation test is used to detect and quantify **antibody** in serum that does not form visible precipitate or agglutinate when reacted with antigen until complement is used.

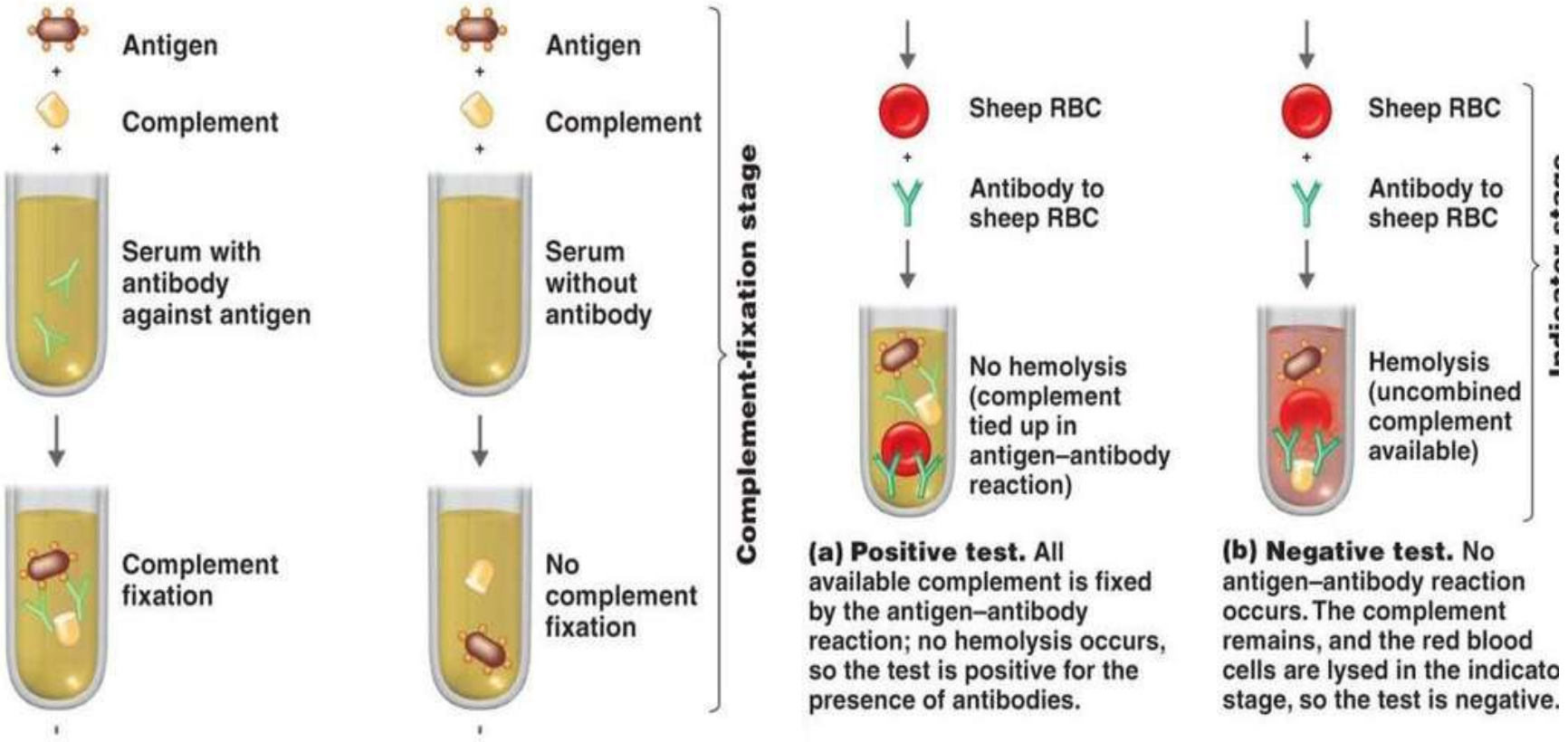
- Principle: the ability of the complement system to **fix Ag-Ab complex** forms the basis of complement fixation test.
- Sheep RBCs & its corresponding Ab act as **indicator sytem**.

- When the Ag reacts with specific antisera, **Ag-Ab complex** will be formed & the **complement** will be **unavailable** for the indicator system.

- There will be **no hemolysis**, & the test is **positive**.

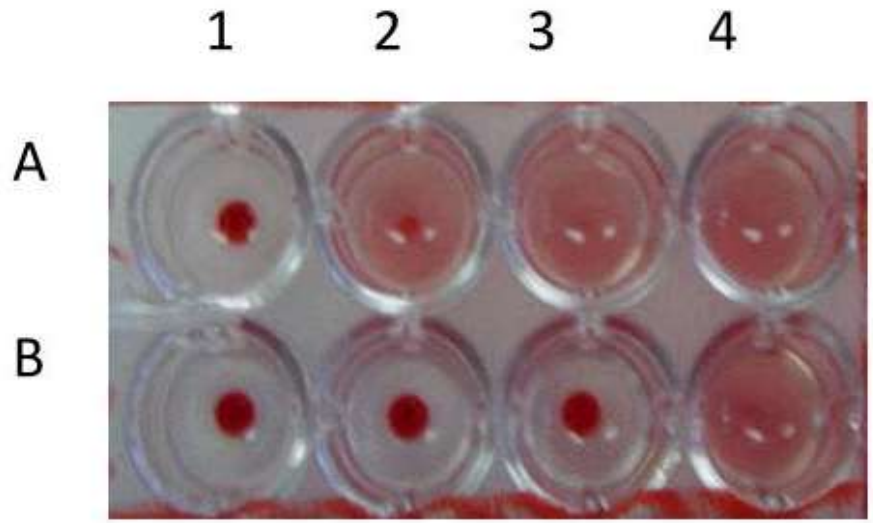
- If the antiserum is not specific, then the complement is free to fix the indicator system, resulting in hemolysis.

Complement fixation



Results and Interpretations:

- No hemolysis is considered as a **positive test**.
- Hemolysis of erythrocytes is indicative of a **negative test**.



- Microtiter plate showing
  Hemolysis (Well A2, A3 A4, B4)
  No Hemolysis (Well A1,B1,B2,B3)

Advantages

1. Well established & trusted
2. Available in most of the labs
3. Ability to identify & quantify a specific protein in a complex mixture such as a cell extract

Disadvantages

1. Lack of standardization
2. Lack of quantitation
3. Requires extensive repeat runs & controls to achieve reliable data

Applications

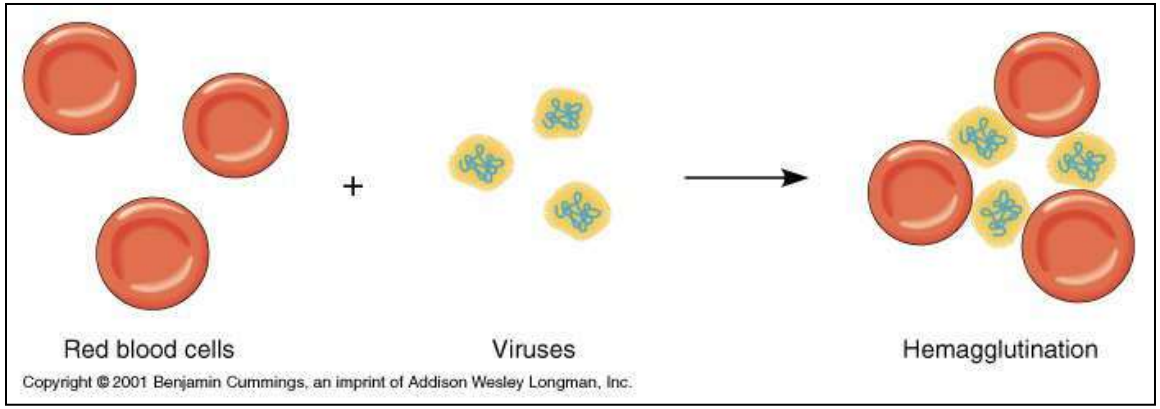
1. The confirmatory HIV test employs western blot to detect anti-HIV antibody in a human serum sample
2. Used for detection & analysis of proteins
3. Used to diagnose various diseases such as viral & autoimmune diseases (but basically used to diagnose HIV)

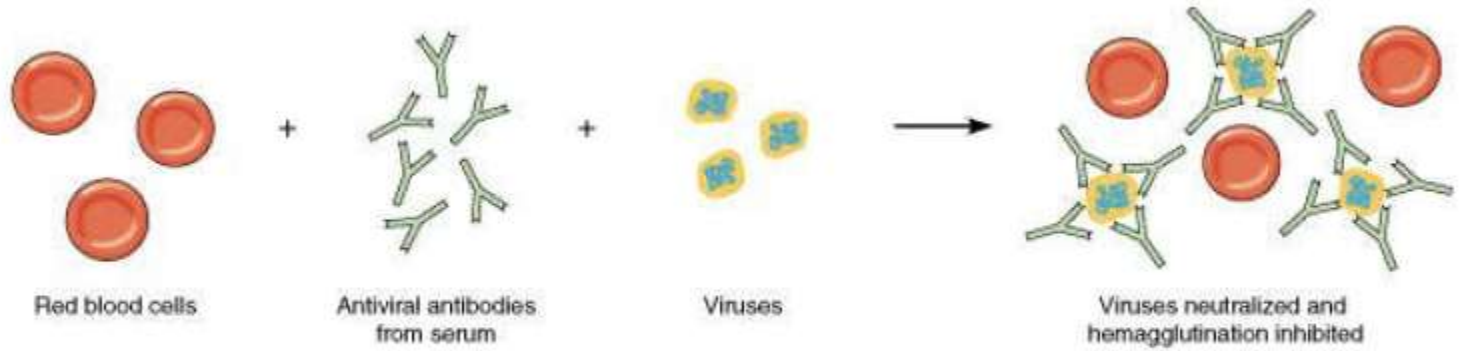
Neutralization test

- Neutralization – viral and toxins by specific Ab in the serum.
- A neutralizing antibody defends a cell from an antigen or infectious body by inhibiting or neutralizing any biological effect
- *Viral neutralization test:*
- Antibodies against the virus.
- Serum is mixed with a known viral suspension.

- If Ab to that particular virus are present in the serum, they bind to the virus, preventing its attachment to & subsequent infection of the cells.

- when virus is then added to an appropriate cell culture, it is unable to replicate & cause cell death.

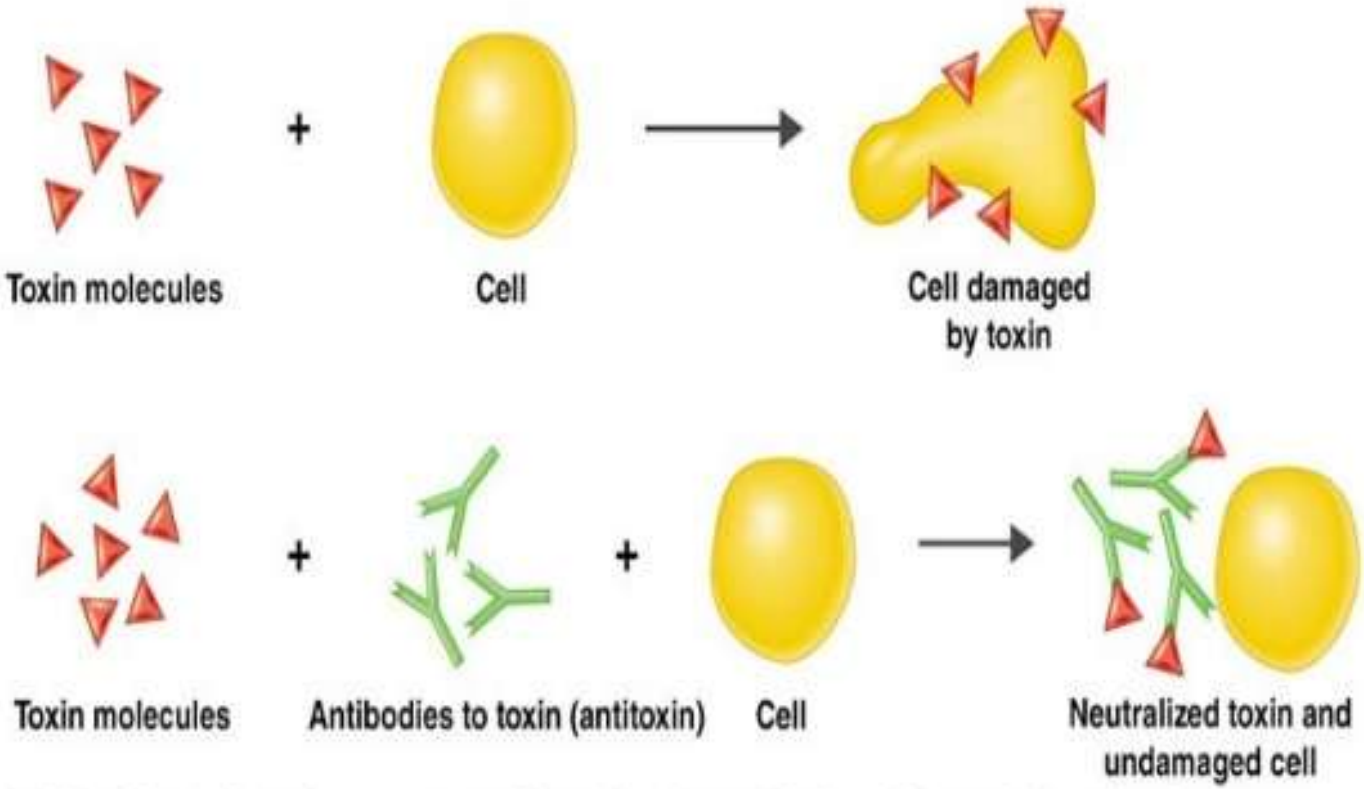




(b) Neutralization of viruses in positive hemagglutination inhibition test

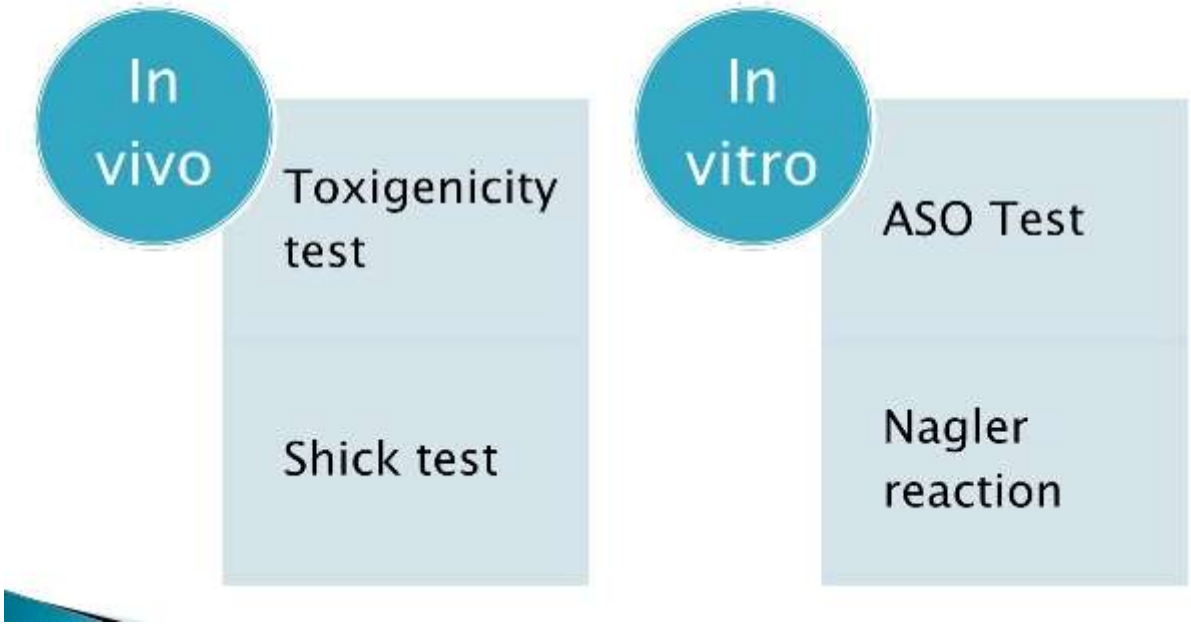
Toxin neutralization

- Bacterial exotoxins are capable of producing neutralising antibodies (antitoxins) which play a role in protection against diseases such as diphtheria and tetanus.
- The toxicity of bacterial endotoxins is not neautralised by antisera.



(a) The effects of a toxin on a susceptible cell and neutralization of the toxin by antitoxin

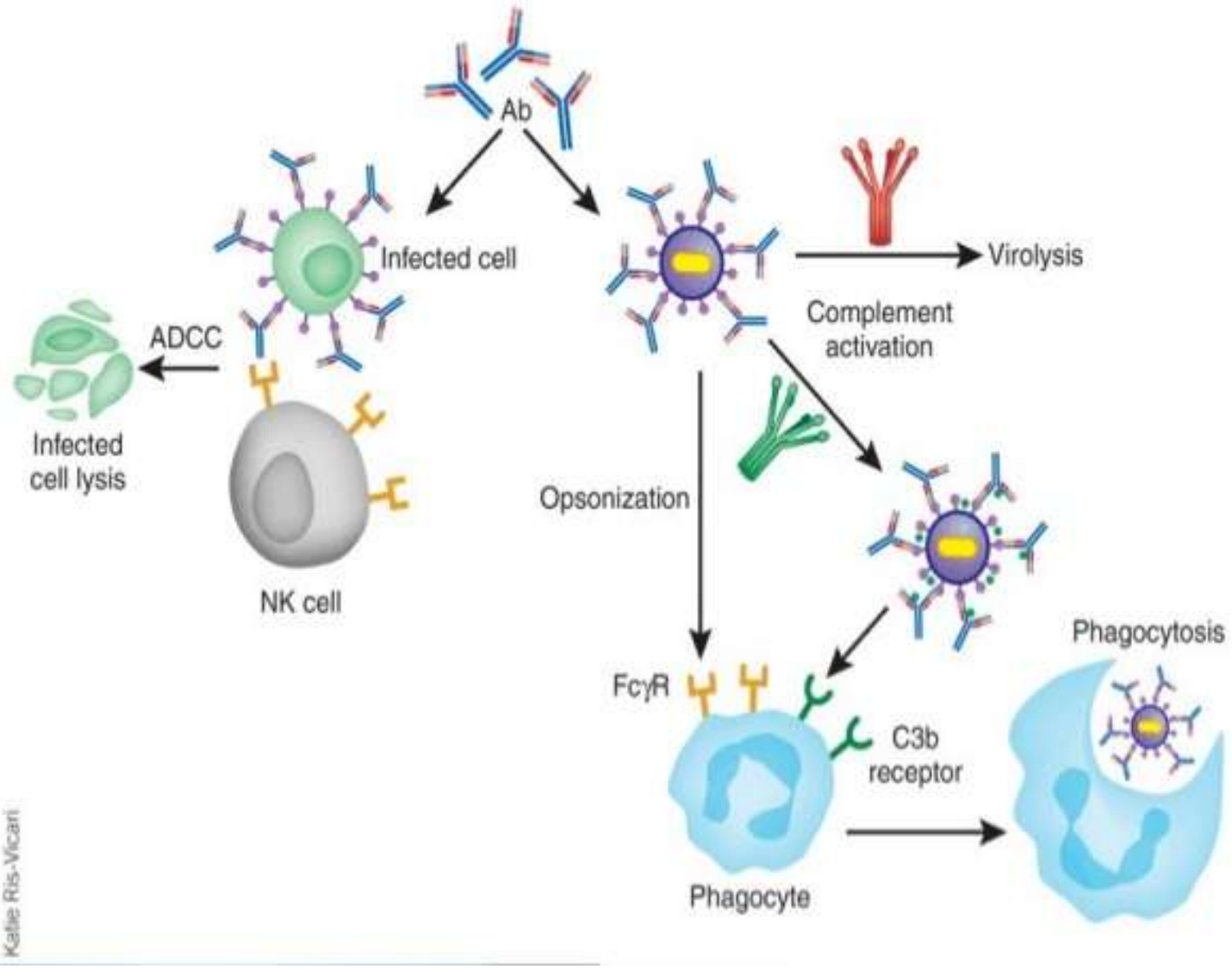
Types of neutralisation reactions



Opsonisation

- It is a process by which a particulate antigen becomes more susceptible to phagocytosis.

- Opsonic index is defined as ratio of phagocytic activity of the patient's blood for a given bacterium to that of a normal individual.
- Phagocytic index is the average number of phagocytosed bacteria per polymorphonuclear leukocytes from stained blood films.
- Phagocytic index denotes the phagocytic activity of blood and thus helps in measuring opsonic index.


Ab
Infected cell
ADCC
Infected cell lysis
NK cell
Virolysis
Complement activation
Opsonization
FcγR
C3b receptor
Phagocytosis
Phagocyte
Katie Ris-Vicari