ANTIGEN-ANTIBODY REACTIONS

The antigens and the antibodies combine specifically with each other. This interaction between them is called Antigen-Antibody reaction. It may be abbreviated as Ag-Ab reaction. These form the basis for humoral immunity or antibody mediated immunity. These reactions form the basis for detection of infectious disease causing agents and also some non-specific Ag's like enzymes. When Ag-Ab reactions occur invitro, they are known as serological reactions.

The reactions between Ag and Ab occur in three stages.

1) Primary stage- This reaction involves formation of Ag-Ab complex. The reaction is rapid and it obeys the general laws of physical chemistry and thermodynamics. The two molecules are held together by non-covalent forces, hydrogen bonding, ionic bonding and sometimes hydrophobic bonding.
2) The second stage leads to visible events like precipitation, agglutination, lysis of cells, killing of Ag, neutralization of toxins, fixation of complement and enhancement of phagocytosis.
3) The third stage includes destruction of Ag or its neutralization.

Salient features of Ag-Ab reactions:

1)**Immune complex:** Since the reaction is specific, an Ag combines only with its homologous Ab and vice versa.

2) **Specificity of Ag-Ab reaction:** An Ab will combine only with that Ag which causes its production. The specificity may be compared to a Lock and Key system.

3) **Binding sites of Ag and Ab:** The entire of the Ag participates in the reaction. But the part of the Ag that combines with the Ab is called epitope or antigenic determinant. An Ag may have 10, 50 or upto 100 antigenic determinants. The part of the Ab that combines with Ag is called paratope or antigen binding site. Most Ab's are bivalent, whereas IgM has 5 to 10 paratopes.

Neither the Ag nor the Ab denature during this reaction. But ones the reactions occur, the entire Ag molecule precipitates or agglutinates.

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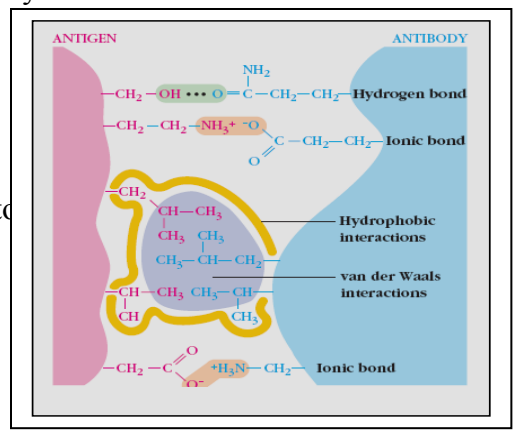
4. **Binding forces of Ag and Ab:** Three factors are responsible for Ag-Ab reactions.

a) Closeness between Ag and Ab: They must be very close to each other.

b) Intermolecular forces: These forces are of four types Electrostatic forces, Hydrogen bonding, hydrophobic bonding and Vander waal's forces.



c) Affinity of Ab: Antibodies which bind strongly to the antigenic determinant are called high affinity antibodies. Affinity refers to the intensity of attraction between an epitope and the antigen combining region of its Ab.

5) **Avidity:** It is the strength of the bond after the formation of Ag-Ab complexes. It is used to denote the overall capacity of antibodies to combine with the multivalent antigen. A multivalent Ag has many types of antigenic determinants. When injected into the blood, each antigenic determinant stimulates the production of a particular antibody. The various antibodies produced by a single Ag combine with the different antigenic determinants of the Ag. nAb+ mAg ↔ AbnAgm

Where nAb is No. of Ab's and mAg is No. of Antigenic determinants

6) **Bonus effect:** In some cases, two antigens are bridged by a single Ab. Such binding is weak and the Ag-Ab reaction may be reversible. But when two antigens are bridged by two antibodies, the binding is strong and complex will not dissociate. This phenomenon of giving extra strength to the antigen-Ab complex by the binding of two Ab's to the two Ag's is called Bonus effect.

7) **Cross reaction:** An antiserum raised against an Ag, can also react with a similar Ag of another type. This is called cross reaction and the Ag which produces the cross reaction is called **Cross reactive Ag.** But the strength of Ab raised against its own Ag is strong. The bonds involved in cross reactions are weak.

Example: The serum raised against albumin of hen's egg can cross react with albumin obtained from duck's egg.

The antiserum raised against human insulin will react with the insulin of Pig, Sheep, whale etc.

The antiserum raised against Pneumococcal polysaccharides will react with E.coli, blood group A and collagen Ag's.

Measurement of Ag-Ab reactions: Methods

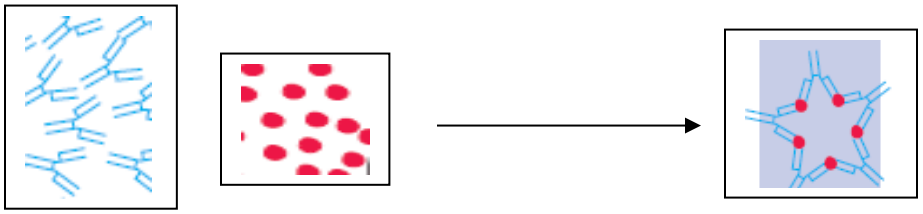
Many methods are available for the measurement of Ag's and Ab's participating in the Ag-Ab reactions. Measurement may be done interms of mass or interms of titre values. The Ab titre of the serum is the highest dilution of the serum which shows an observable reaction with the Ag. The titre value is influenced by the nature and quantity of the Ag and the type and conditions of the test. The two important parameters effecting serological tests are the specificity and the sensitivity. A sensitive test should be able to measure even very minute quantities of Ag or Ab. Specificity refers to the ability of the test to detect the reactions between homologous Ag's and Ab's and not with others. Commercial kits are available in the market to give high quality results.

The Ag-Ab reactions can be detected by the following techniques.

1) Precipitation
2) Agglutination
3) Lysis and complement fixation
4) Neutralization test
5) Flocculation
6) Opsonisation
7) Immunofluorescence
8) Enzyme Immunosorbent assay
9) Radio allegro sorbent test
10) Radio Immunosorbent test
11) Immunoblotting
12) Immunoprecipitation
13) ELISPOT

1) Precipitation reactions: When a soluble **Ag** combines with its **Ab** in the presence of an electrolyte (NaCl) at a particular temperature and pH, it forms an insoluble precipitate of **Ag-Ab** complex. The Ab causing precipitation is called **Precipitin**.

Mechanism of precipitation: Marrack (1934) proposed the lattice hypothesis to explain the mechanism of precipitation. Most of the antigens are multivalent and antibodies are bivalent. Each Ab can bridge with two multivalent antigen molecules. This bridging between Ab's and Ag's lead to formation of large **lattice**, which forms the precipitate. At optimal concentrations of Ag and Ab, maximum precipitation will occur.



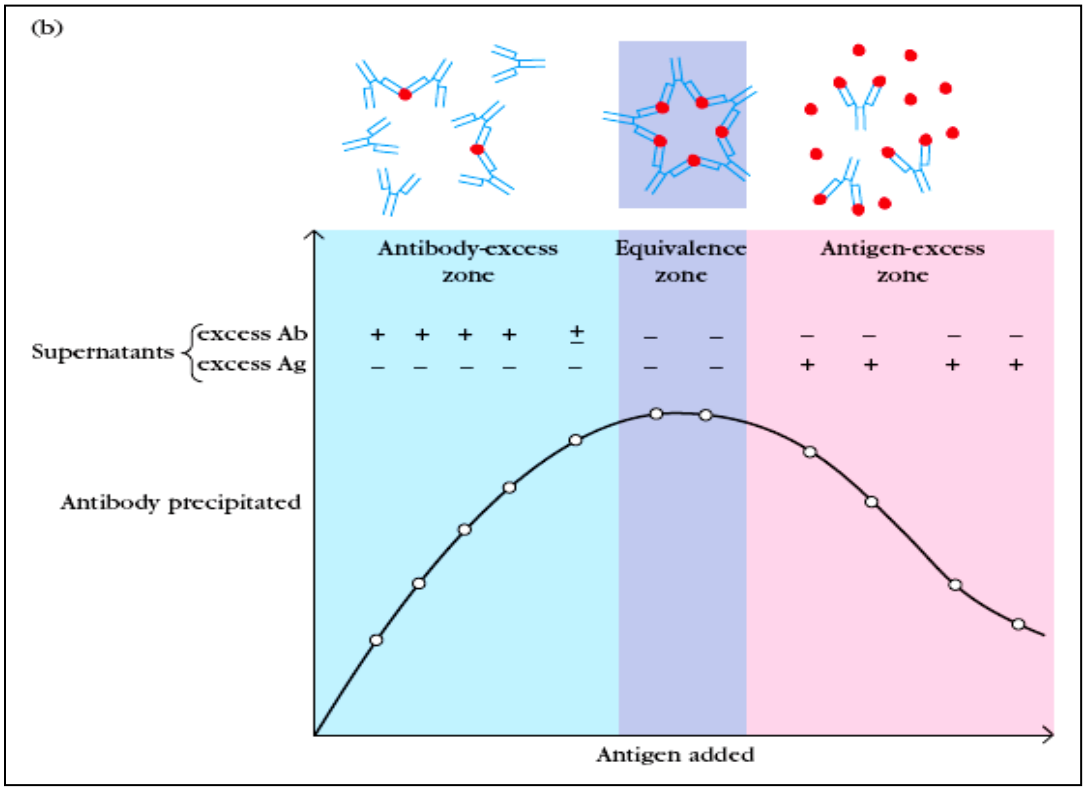
Antibodies antigens Ag-Ab complex

Precipitation Test : A quntitative precipitation reaction test can be done in a series of test tubes. Decreasing amounts of Ag are added in the test tubes to which fixed amount of antiserum is added. The Ag and Ab react resulting in a precipitate. The amount of precipitate is determined by the proportion of Ag and Ab. Maximum precipitation occurs at their optimal concentrations. When Ag or Ab are present in excess, precipitation is less.

When the amount of precipitate formed in the tubes in plotted on a graph a curve is obtained. This is called the precipitin curve. The curve shows a peak where maximum precipitation occurs and it shoes 3 zones. Namely

a) Ag excess zone also called ascending part or prozone.
b) Zone of equivalence or the peak
c) Ab excess zone or post zone or the descending zone.

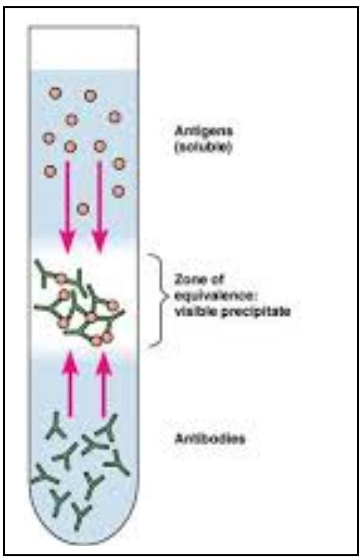
a) **Zone of excess Ag:** In the first few tubes where the Ag is in excess when compared to Ab, precipitation is less.
b) **Zone of equivalence:** Maximum precipitation occurs at optimal concentrations of Ag and Ab. All the Ag and Ab precipitate into large lattice which is insloluble.
c) **Zone of Excess Ab:** though Ag and Ab form a lattice they do not form a stable insoluble lattice and there for less of precipitate is observed.



Applications of Precipitation reactions:

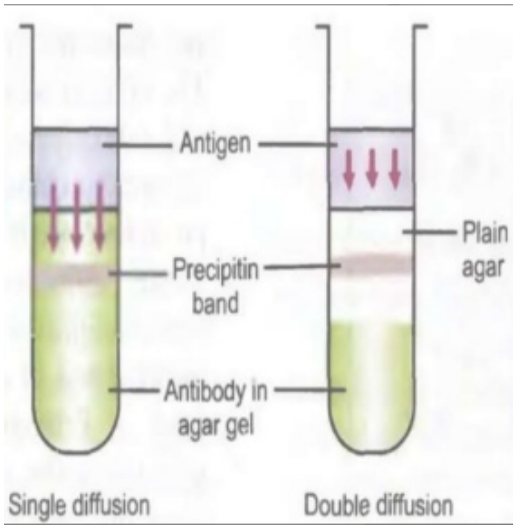
This method may be used either for quantitative or qualitative purposes. It is a very sensitive test and quantity upto 1 microgram of protein can be detected. This can be performed in a tube or can be carried out using support materials like gels.

1) Tube test(Ring test): This is a tube test and is a very rapid method for detection of Ab's or Ag. In a small test tube some solution of antiserum having Ab is taken and a layer of Ag is carefully placed above it. Within few minutes precipitation ring is observed at the interface of the two solutions.



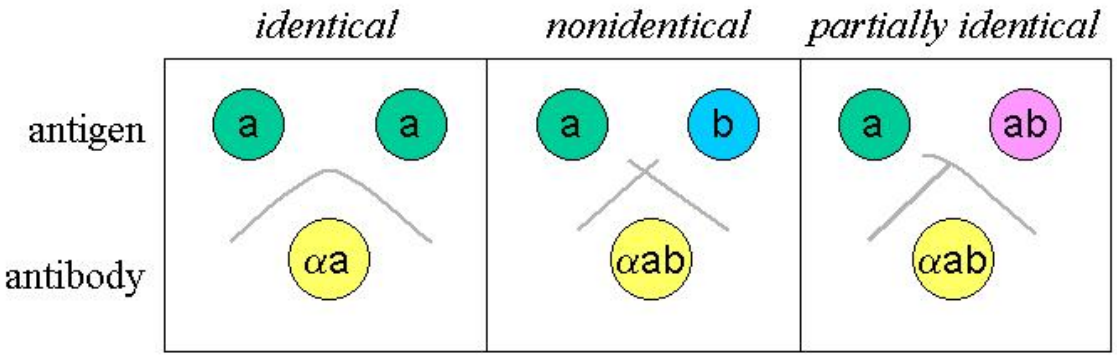
2) Gel diffusion tests: In these methods agar gel or similar gels are used on plates or petriplates. Both Ag and Ab diffuse freely in the gel system in all directions. At a certain point depending on the rate of diffusion and concentration of the reactants, a zone of equivalence will be formed, which is seen as a visible precipitation. If Ag or Ab preparpations are complex, multiple bands form. These are again of 2 types- Single diffusion methods and double diffusion methods.

a) Single diffusion in one dimension: This can be done in a tube or on a plate. In tube method Ab is incorporated in the agar gel and a solution of Ag is layered over it. Ag diffuses downwards forming a line of precipitate that moves downwards in the tube. This is because the preicipitation formed at the advancing front of the Ag get dissolved as its concentration increases by diffusion. The number of bands indicate the number of different Ag's.



b) Single diffusion in two dimensions: Also known as radial immunodiffusion: The antiserum containing Ab's is incorporated in agar gel and poured on to flat surface. The Ag is added to the wells cut on the surface of the gel. Ag now diffuses radially from the well and forms ring shaped bands of precipitation concentrically around the wells. This method is used for the estimation of Ig classes in sera and for screening sera for Ab's for influenza viruses among others.

c) Double diffusion in two dimensions (Ouchterlony procedure): This method is widely employed and helps to compare different Ag's and antisera directly. Agar gel is poured on plates and wells are cut. The antiserum is placed in the central well and different Ag's are poured in surrounding wells. If two adjacent Ag's are identical, the lines of precipitation formed by them will fuse. Cross reaction or partial identity is indicated by spur formation. If the two precipitin bands merge to form a solid chevron between the two Ag wells and Ab well then they are non identical.



d) Immunoelectrophoresis: This is one of the most sensitive methods available for the detection of a number of antigenic components in a mixture such as bacterial extract or serum. **Graber and Williams** devised this method. This involves the electrophoretic separation of a composite Ag into its constituent proteins, followed by Immunodiffusion against its antiserum, resulting in separate precipitin lines. It involves the following steps.

1. Semisolid agar is layered on the glass slide. A well for Ag and a trough for antiserum is cut out of agar.

2. Antigen well is filled with human serum.

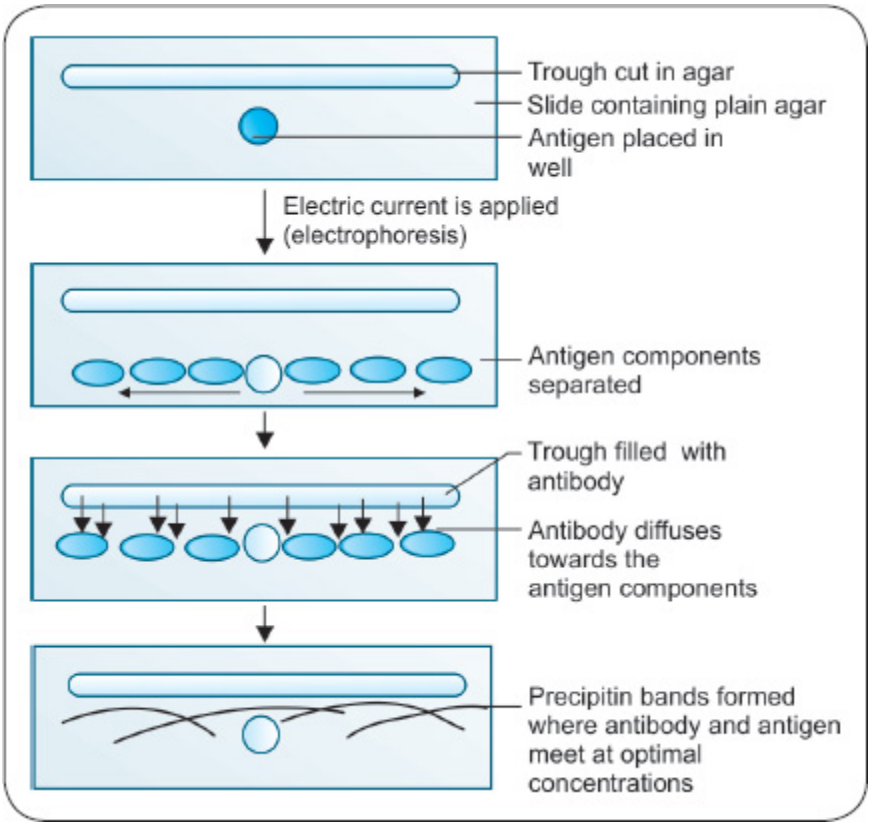
3. Serum is separated by electrophoresis.

4.Antiserum raised against human serum is filled in the trough.

5. Serum and antiserum are allowed to diffuse into agar.

6. Precipitin lines form for individual serum proteins

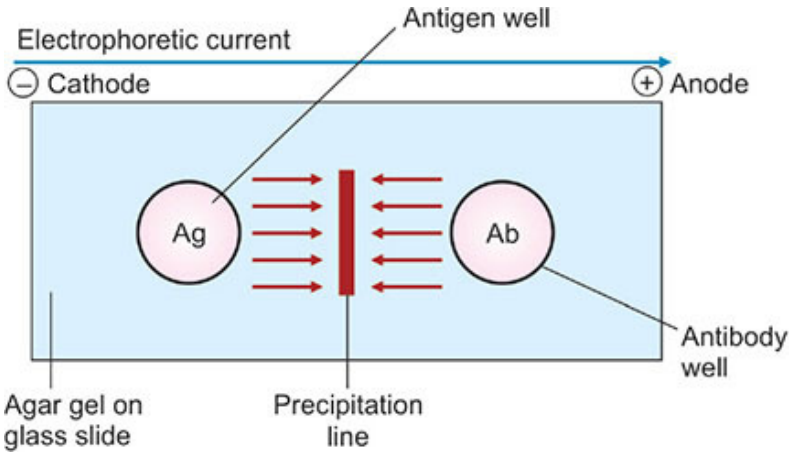
This procedure takes 18 to 24 hrs and around 30 different proteins can be identified.



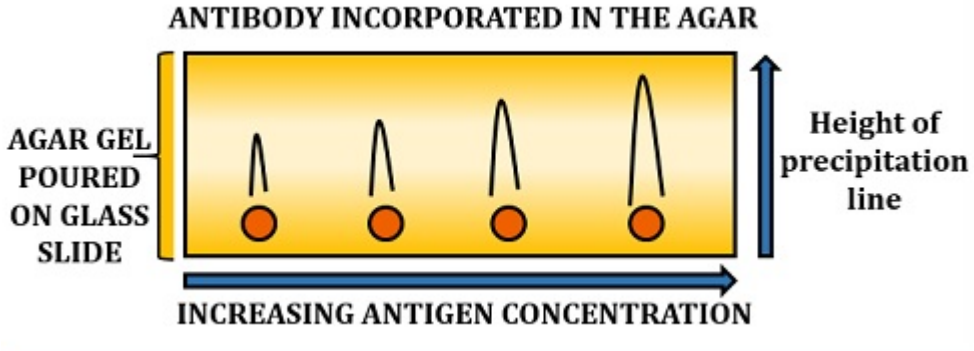
e) ElectroImmuno diffusion: The formation of the precipitin lines can be speeded up by electrically driven Ag and Ab. Two important methods may be discussed,

1) Counter immunoelectrophoresis (CIE, Counter current immunoelectrophoresis):

This involves simultaneous electrophoresis of Ag and Ab in gel in opposite directions resulting in precipitation at a point between them. This method is highly sensitive and gives results in 30 minutes. The applications of this test are, to detect Ag's or proteins present in the serum. Eg: Cryptococcus and meningocossus presence in cerebrospinal fluid is detected using this test.



2) Rocket Electrophoresis: This method is used for quantitative estimation of Antigens. The antiserum is incorporated in the gel and increased concentrations of Ag are placed in the wells of the gel. The pattern of immunoprecipitation resembles a rocket and hence the name rocket electrophoresis.



AGGLUTINATION REACTIONS:

When a particular Ag is mixed with its Ab's in the presence of electrolytes at a suitable temperature and pH, the particles are clumped or agglutinated. The Ab of the serum causes the cellular Ag's to form clumps and these are called **Agglutinins**. The particulate antigens that are aggregated are termed **Agglutinogens.** This reaction occurs optimally when Ag's and Ab's react in equivalent proportions.

Mechanism of Agglutination:

Agglutination occurs due to the linking of Ag and Ab. Most of the Antibodies having two antigen binding sites cannot form good clomps, while IgM forms very good clumps. These tests have wide applications in clinical fields. They may be classified as Macroscopic, when they are carried out in small test tubes, or they may be microscopic when the Ag and Ab are mixed on a slide and examined under a microscope.

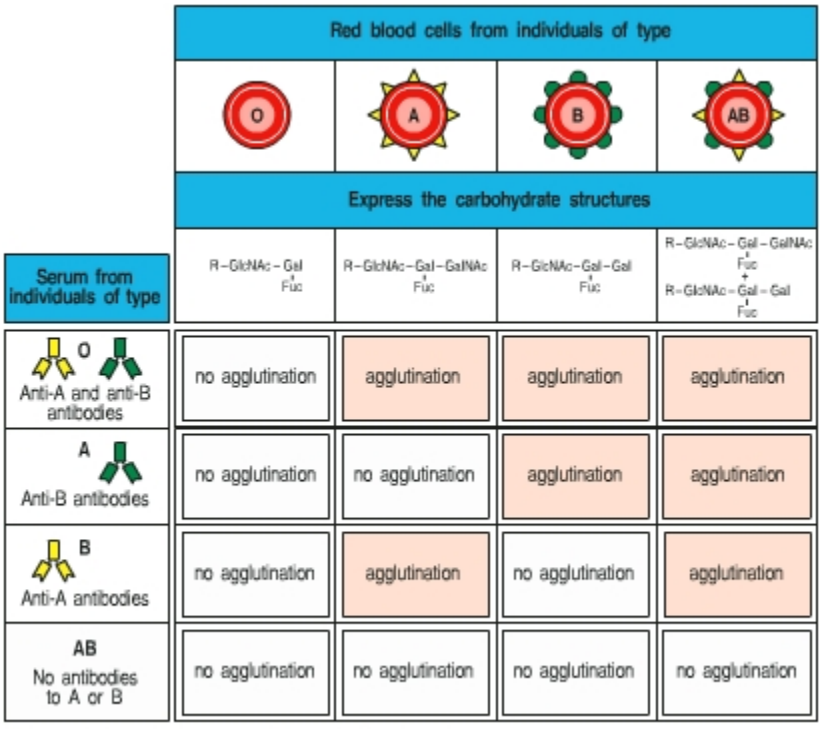
1) Slide agglutination: This is a rapid method to determine the presence of agglutinating antibodies. To a uniform suspension of particulate Ag, a drop of saline is added and then a drop of antiserum is added. The slide is gently rocked or a fine loop is used to mix the contents. If granulation occurs the test is Positive. It takes a minute for the test to complete and is visible to the naked eye. Some times confirmation may be done by observing slide under microscope.

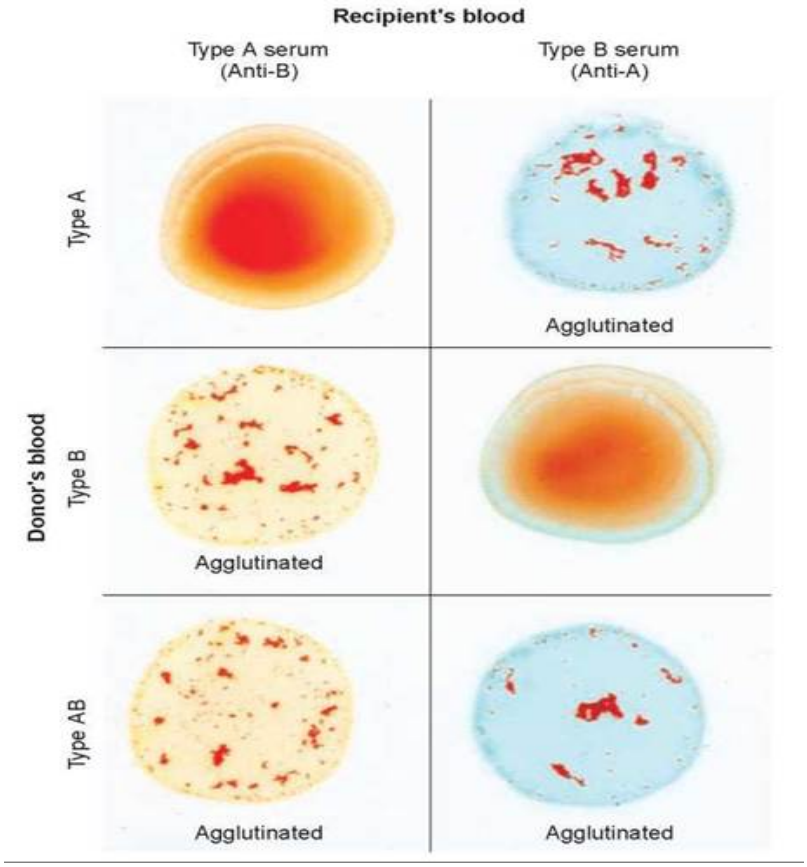
This test is used for blood grouping (Haemaglutination) and cross matching.

Haemagglutination: This is used for blood grouping. Erythrocytes when mixed with a homologous antiserum they agglutinate. This is due to the surface antigens present on the erythrocytes and the entire human population is grouped as A, B, AB or O groups.

The A group persons contain antigen A on their erythrocytes and anti-B antibodies in their serum. B blood group persons have antigen B on their RBC and anti-A antibodies in their serum. The person with blood group AB have both A and B antigens on their RBC and no antibodies in their serum, while O blood group persons do not have any antigens on their RBC and have both anti-A and anti-B antibodies in their serum.

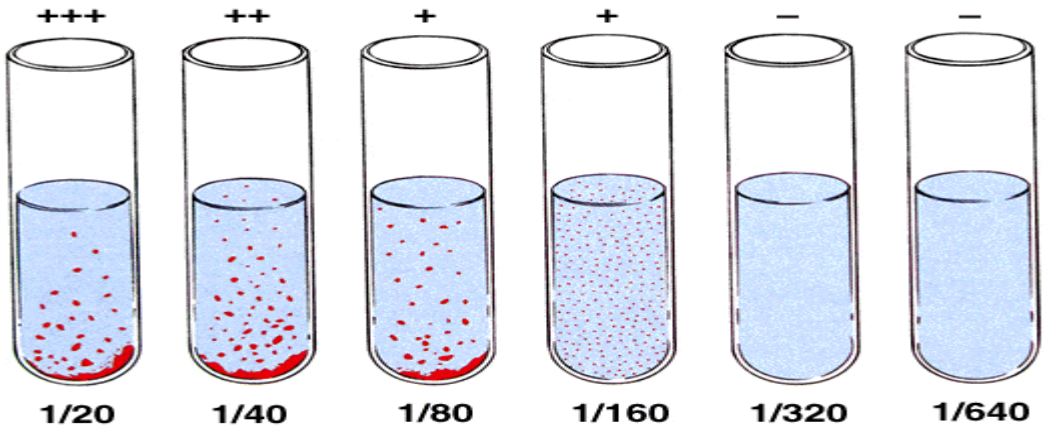
For typing blood, a drop of blood sample is mixed with a drop of antiserum A and another drop of blood sample is mixed with a drop of antiserum B on a glass slide. If the blood serum is clumped with antiserum A, the sample belongs to A blood group. If the sample clumps with antiserum B then his blood group is B. If the is clumped with both antiserum A and B, then the sample belong to AB and if no agglutination is seen then the blood group is O.



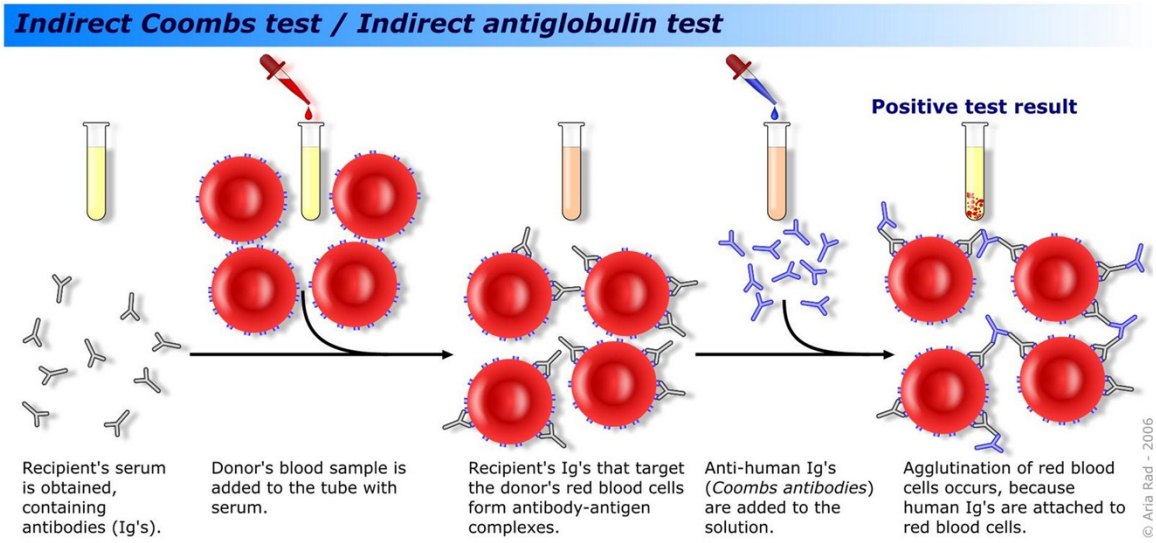


2. Tube agglutination: This is a standard method for quantitative estimation of Ab. The serum containing Ab is diluted serially with saline in several small test tubes, to which a constant volume of Ag suspension is added. A control tube is kept which has no antiserum. The tubes are incubated until visible agglutination is observed. The tube showing highest agglutination is referred to as the titre.

Tube agglutination is employed for the serological diagnosis of typhoid, brucellosis and typhus fever. **Widal test** is used for the estimation of typhoid fever. In this test Ab content of the patient's serum, is measured by adding a constant amount of antigen (Solmonella typhi) to the serially diluted serum.



3. Coombs test (anitglobulin test): This test was discovered by Coomb's, Mourant and Race in 1945. This tube test is used for the detection of anti-Rh antibodies that do not agglutinate Rh Positive erythrocytes in saline. When sera containing incomplete anti-Rh antibodies are mixed with Rh +ve red cells, the Ab globulin coats the surface of the erythrocytes and they do not agglutinate. These erythrocytes are washed free of all unattached proteins and treated with a rabbit antiserum against human Ig's. The anti-Ig Ab's reacts with the bound Ab's and bring about agglutination of the cells. This method is used to detect hemolytic disease in the new born for Rh incompatibility (Erythroblastosis foetalis).



4. Passive agglutination test: It is similar to haemagglutination test but the physical nature of the reaction is altered. The Ag is coated on the surface of a carrier particle and thereby helps to convert a precipitation reaction into an agglutination reaction making the reaction more sensitive.

The carrier particles used can be RBC, latex particles or bentonite. Some times RBC coated with polystyrene (tanned RBC) can be used. When patients serum is mixed these, it leads to agglutination. This test is used for the diagnosis of **Rheumatoid arthritis**. In this disease, patients serum has IgM antibodies raised against his own IgG. Therefore the latex particles used in this test are coated with IgG.

5. Brucella agglutination test for Brucellosis: This is a tube agglutination test for the diagnosis of brucellosis. The test is carried out in hypersaline (5% NaCl), or in saline with albumin.

6. Cold agglutinatin test for Pneumonia, Malaria and Trypnosomiasis: In case of Mycoplasmal pneumonia detection, patients serum agglutinates human O group erythrocytes at 4^0C, and is reversible at 37^oC.

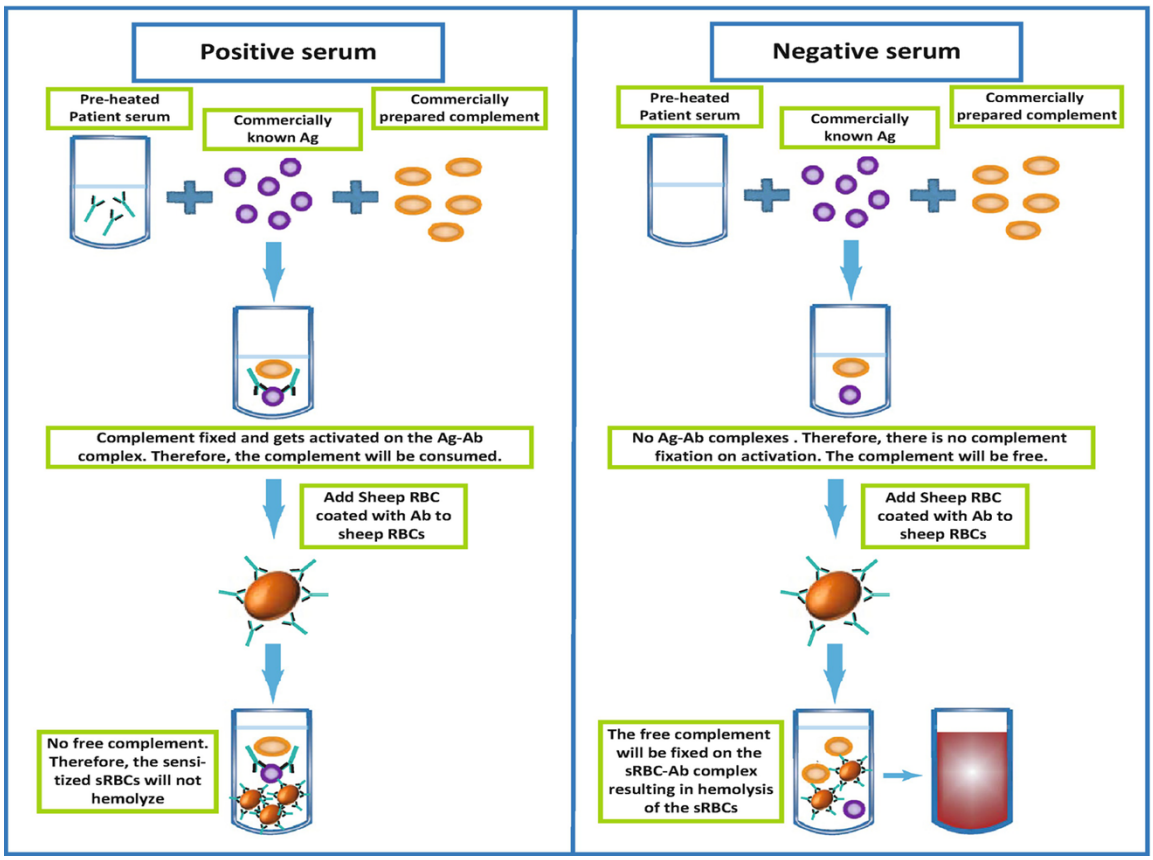
7. Weil-Felix reaction for serodiagnosis of typhus fever: This is a heterophile agglutination test and is based on sharing of a common Ag between typhus ricketesiae and some strains of Proteus bacilli.

8. Haemagglutination inhibition test: this test is used for the diagnosis of certain viruses like Influenza and Mumps causing viruses and some parasitic diseases. If a person is suspected having Influenza, his serum is mixed with a known influenza virus and then RBC cells. If Ab is present, it binds to virus and prevents agglutination of RBC. If Ab is absent, haemagglutination occurs and the person is +ve for influenza.

COMPLEMENT FIXATION TEST: Lysis of RBC or bacteria requires some non-specific unstable components of fresh serum which are called complement. This complement system comprises of 11 proteins and are present in ever individual. They bind to Fc component of Ab involved in Ag-Ab complex. This ability of the Ag-Ab complex to fix complement is used in complement Fixation tests.

In the first stage, the test Ag and the antiserum (heated to 56^{o}C to inactivate complement) are mixed in the presence of known amount of complement. This is incubated at 4^{o}C for 18h. If Ab specific for the Ag is present in the serum, Ag-Ab complex will be formed that will fix the complement.

In the second stage, indicator system is added to detect the presence of free complement. This system consists of addition of sheep red blood cells plus Ab (haemolysis) specific for sheep red cells. If all the complement has been fixed, none will be free to lyse sheep red cells (+ve complement fixation test). If no Ab is present in the patients serum, then the complement is not fixed and is free to interact with the indicator system and lyse the RBC (-ve complement fixation test).



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NEUTRALIZATION TESTS:

a) Virus neutralization test: Neutralization of viruses by their Ab's can be demonstrated in various systems. Neutralization of bacteriophages is demonstrated by **Plaque Inhibition Test.** When a bacteriophage is added to culture of susceptible bacteria on a petriplate, plaques of lysis are produced. Specific antiplaque serum inhibits plaque formation. Neutralization of animal viruses can be demonstrated in three systems- Animals, eggs and tissue culture.

b) toxin-antitoxin ractions: Microorganisms after their entry into an animal, liberate toxins into blood. These are protein Ag's and there for toxins stimulate antitoxin Ab's production which specifically combine with toxins and neutralize their effect. When carried out invitro it results in visible precipitation or flocculation.

Roman flocculation test is used to determine the concentration or strengths of either toxin or antitoxin. In a series of test tubes, varying concentrations of antisera are added to a constant concentration of toxin. The end titre point is indicated in the tube having maximum flocculation.

This test can also be done invivo. The toxin-antitoxin mixture is injected into animals and the least amount of antitoxin that prevent the animal from getting a disease is the titre value. Diphtheria toxin neutralization test is done on human skin.

FLOCCULATION: In these tests Ag-Ab reaction results in visible floccules which do not sediment but remain dispersed in the medium. Diagnosis of syphilis is based on flocculation. The spirochete of syphilis is a heterogenetic Ag and shared by beef heart. This is prepared in cardiolipin and when patients serum having Ab is added, flocculation occurs. The test may be carried out on slides.

OPSONIZATION: The name opsonin is given by Wright (1903). It refers to a heat labile substance present in fresh sera, which facilitates phagocytosis. This factor was later identified as complement. Opsonization is a process by which the particulate Ag becomes more susceptible to phagocytosis after getting coated by opsonin.

Mechanism: The Ab combines with the surface Ag of bacteria. This Ag-Ab complex activates C3 component of complement system resulting in Ag-Ab-complement complex. Phagocytic cells have receptors for C3 and also for the Fc component of the Ab. As a result the pahgocytic cells take up the Ag and cause it lysis.

IMMUNOFLUORESCENCE:

Fluorescence is the property of absorbing light rays of one particular wavelength and emitting rays with a different wave length. Fluorescent dyes show up brightly under UV light as they convert into visible light. Coons et al (1942) showed that labeled dyes can be conjugated to Ab's and these labeled antibodies can be used to detect Ag's. Dyes that are commonly used include:

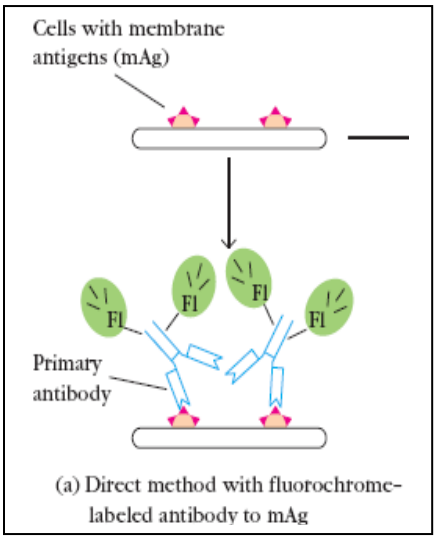
Fluorescein, an organic dye that is the most widely used label for immunofluorescence procedures, absorbs blue light (490 nm) and emits an intense yellow-green fluorescence (517 nm).

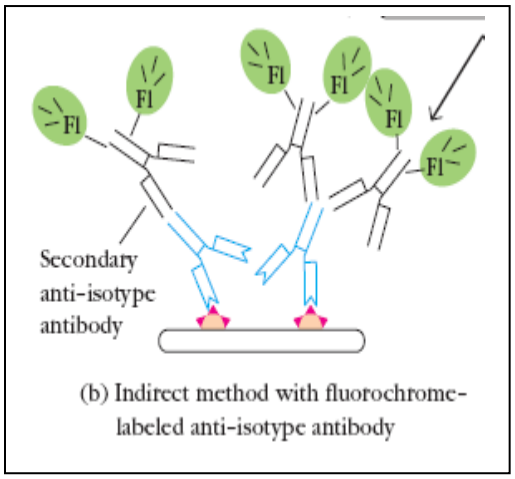
Rhodamine, another organic dye, absorbs in the yellow-green range (515 nm) and emits a deep redfluorescence (546 nm). Because it emits fluorescence at a longer wavelength than fluorescein, it can be used in two-color immunofluorescence assays. An antibody specific to one determinant is labeled with fluorescein, and an antibody recognizing a different antigen is labeled with rhodamine. The location of the

fluorescein-tagged antibody will be visible by its yellowgreencolor, easy to distinguish from the red color emitted where the rhodamine-tagged antibody has bound. By conjugating fluorescein to one antibody and rhodamine to another antibody, one can, for example, visualize simultaneously two different cell-membraneantigens on the same cell.

Phycoerythrin is an efficient absorber of light (~30-fold greater than fluorescein) and a brilliant emitter of red fluorescence, stimulating its wide use as a label for immunofluorescence.

Fluorescent-antibody staining of cell membrane molecules or tissue sections can be direct or indirect. In **direct staining,** the specific antibody (the primary antibody) is directly conjugated with fluorescein; in **indirect staining,** the primary antibody is unlabeled and is detected with an additional fluorochrome-labeled reagent. In **sandwich method** cells producing specific antibodies can be identified.

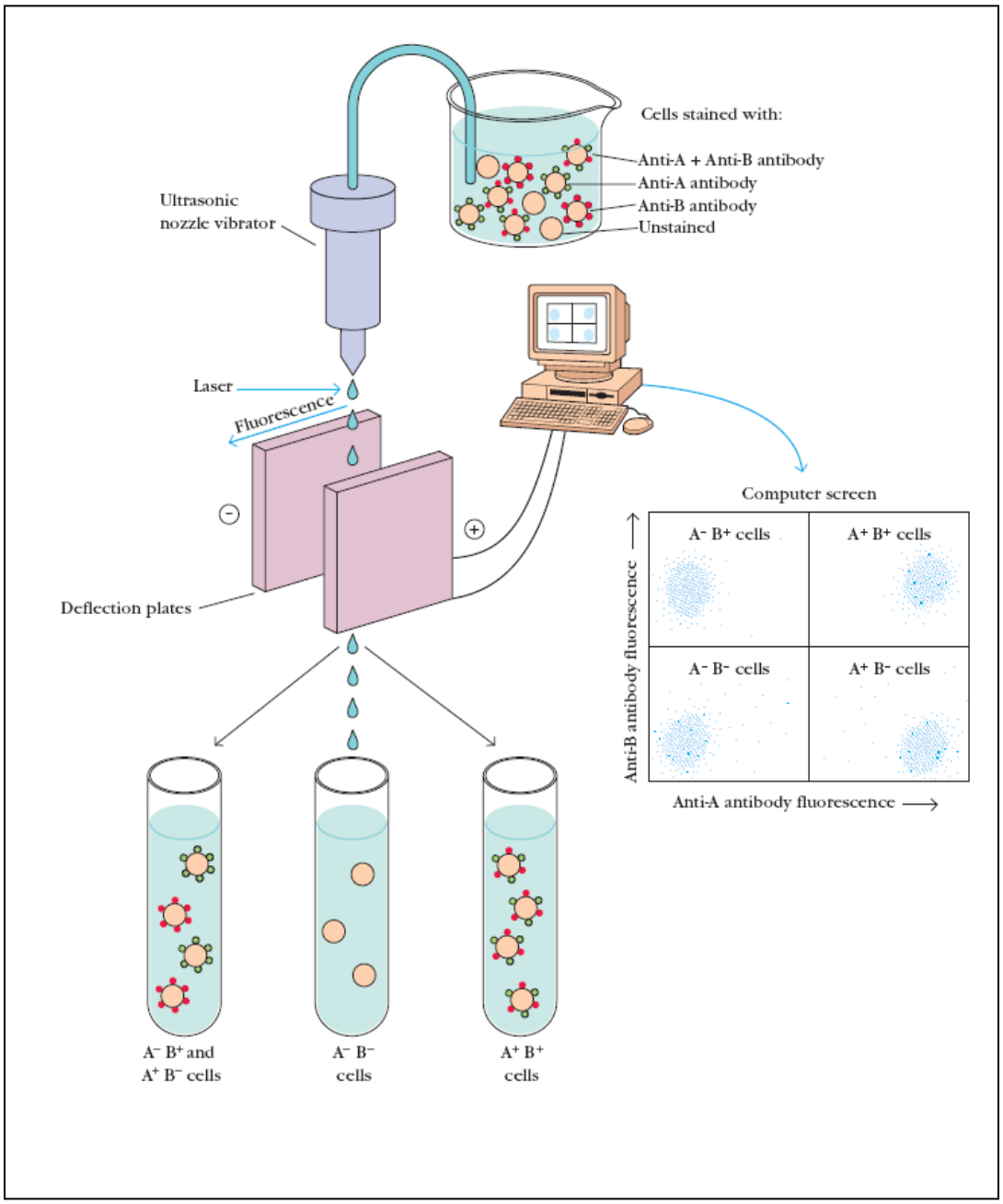




FLOW CYTOMETER: The flow cytometer was designed to automate the analysis and separation of cells stained with fluorescent antibody. It uses a laser beam and light detector to count single intact cells in suspension. Every time a cell passes the laser beam, light is deflected from the detector, and this interruption of the laser signal is recorded. Those cells having a fluorescently tagged antibody bound to their cell surface antigens are excited by the laser and emit light that is recorded by a second detector system located at a right angle to the laser beam. The simplest form of the instrument counts each cell as it passes the laser beam and records the level of fluorescence the cell emits; an attached computer generates plots of the number of cells as the ordinate and their fluorescence intensity as the abscissa. More sophisticated versions of the instrument are capable of sorting populations of cells into different containers according to their fluorescence profile. Use of the instrument to determine which and how many members of a cell population bind fluorescently labeled antibodies is called analysis; use of the instrument to place cells having different patterns of reactivity into different containers.

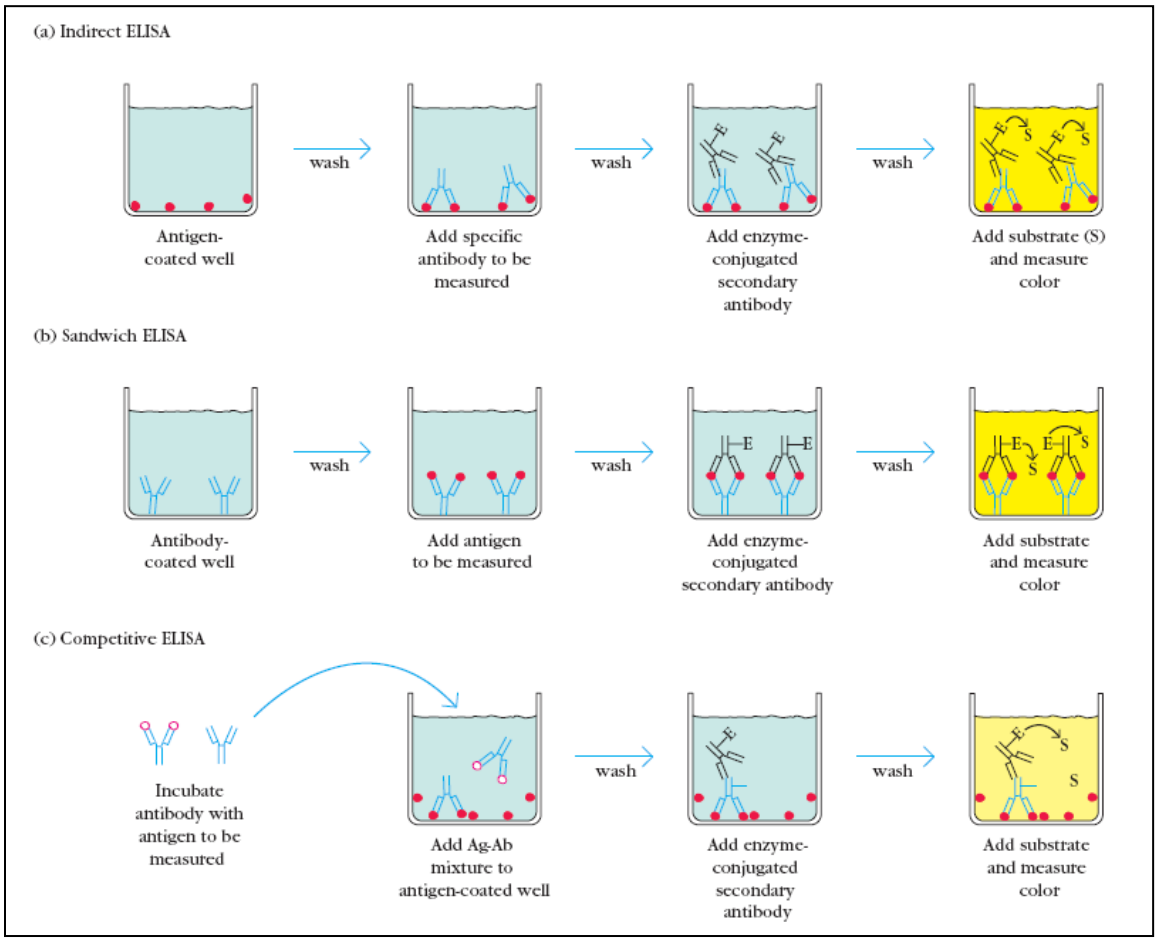
Flow cytometry also makes it possible to analyze cell populations that have been labeled with two or even three different fluorescent antibodies. For example, if a blood sample is reacted with a fluorescein-tagged antibody specific for T cells, and also with a

phycoerythrin-tagged antibody specific for B cells, the percentages of B and T cells may be determined simultaneously with a single analysis.



ELISA (Enzyme Linked ImmunoSorbent Assay): In 1971, enzyme labeled Ag's and Ab's were developed as serological reagents for the assay of Ab's and Ag's. These are very simple, sensitive, economic and less hazard when compared to RIA. The major type

of heterogenous Enzyme Linked Assays is ELISA. It is so named because this technique involves the use of an immunosorbent, an absorbing material specific for one of the components of the reaction, Ag or Ab. The material may be cellulose or agar or a solid phase such as polystyrene, polyvinyl or polycarbonate tubes or microwells or membranes or discs of polyacrylamide, paper or plastic. This technique is done in 96-welled microtitre plate suitable for automation.



The ligand used here is a molecule which can detect the Ab and is covalently coupled to an enzyme such as peroxidase, betagalactosidase, alkaline phosphatase etc. This binds the test Ab and after free ligand is washed away. The bound ligand is visualized by the addition of chromogen. A colorless substrate is acted on by the enzyme portion of the ligand to produce a colored end product. The amount of test Ab is measured by assessing the amount of colored end-product by optical density scanning of the plate. ELISA is of 3types.

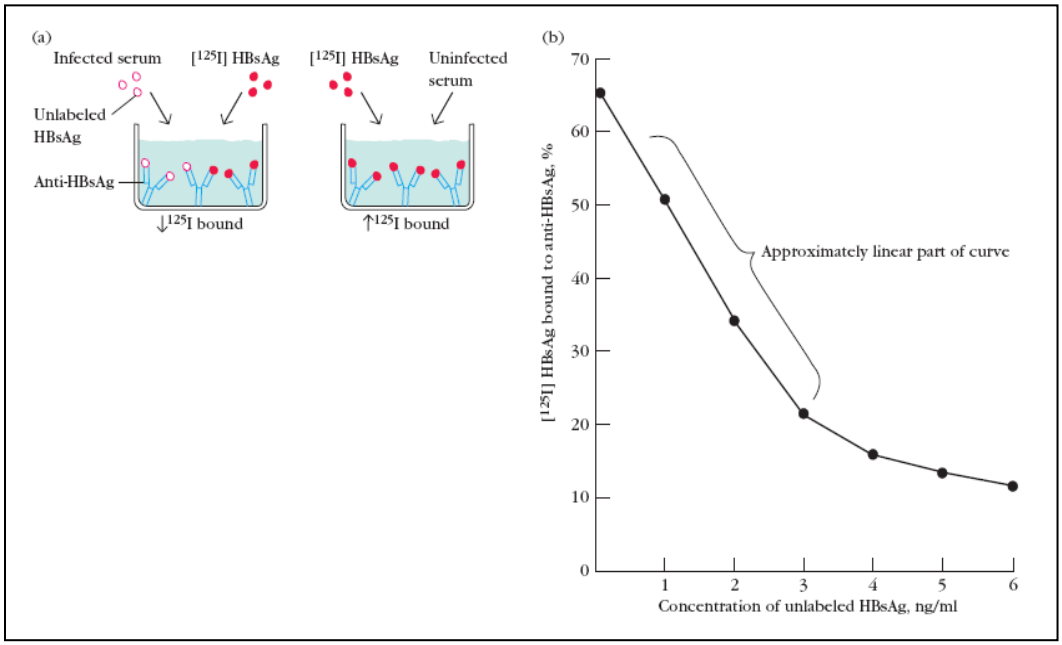
a) Indirect ELISA: This ttechnique is used for the detection of HIV. The envelop proteins are developed by recombinant technology and coated on the surface of the of microtire plates. Suspects serum is added, and unbound proteins are washed off. Then antiantibodies which are tagged with enzyme are added and then the chromogenic substrate is added. The color developed is directly proportional to the Ab present in the serum.

b) Sandwich ELISA: Used to detect the presence of Ag in a sample. The well is coated with Ab specific to the Ag and then suspect serum is added allowed to react. The wells are washed to remove unbound Ag's. Then a labeled Ab against a different epitope of the Ag is added. Unbound Ab's are removed by washing and this is followed by addition of colored substrate and development of color. The intensity of color is directly proportional to the concentration of the Ag in the serum.

c) Competitive ELISA: Another variation for measuring amounts of antigen is competitive ELISA. In this technique, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to an antigencoated microtiter well. The more antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well. Addition of an enzyme-conjugated secondary antibody (Ab2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in an indirect ELISA. The more the Ag in serum, the lesser will be the free Ab that can bind to well and therefore Secondary Ab that bind to it is less, and thereby the color intensity developed is less.

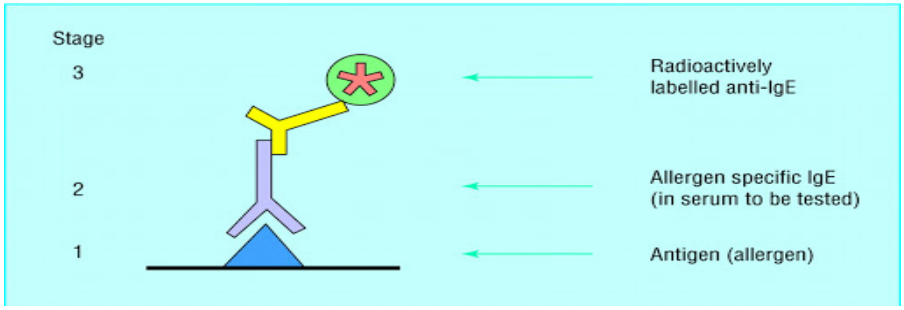
Radio Immuno Assay: The principle of RIA involves competitive binding of radiolabeled antigen and unlabeled antigen to a high-affinity antibody. The labeled antigen is mixed with antibody at a concentration that saturates the antigen-binding sites of the antibody. Then test samples of unlabeled antigen of unknown concentration are added in progressively larger amounts. The antibody does not distinguish labeled from unlabeled antigen, so the two kinds of antigen compete for available binding sites on the antibody. As the concentration of unlabeled antigen increases, more labeled antigen will be displaced from the binding sites. The decrease in the amount of radiolabeled antigen

bound to specific antibody in the presence of the test sample is measured in order to determine the amount of antigen present in the test sample. The antigen is generally labeled with a gamma-emitting isotope such as ^{125}I, but beta-emitting isotopes such as tritium (^{3}H) are also routinely used as labels. The radiolabeled antigen is part of the assay mixture; the test sample may be a complex mixture, such as serum or other body fluids, that contains the unlabeled antigen.



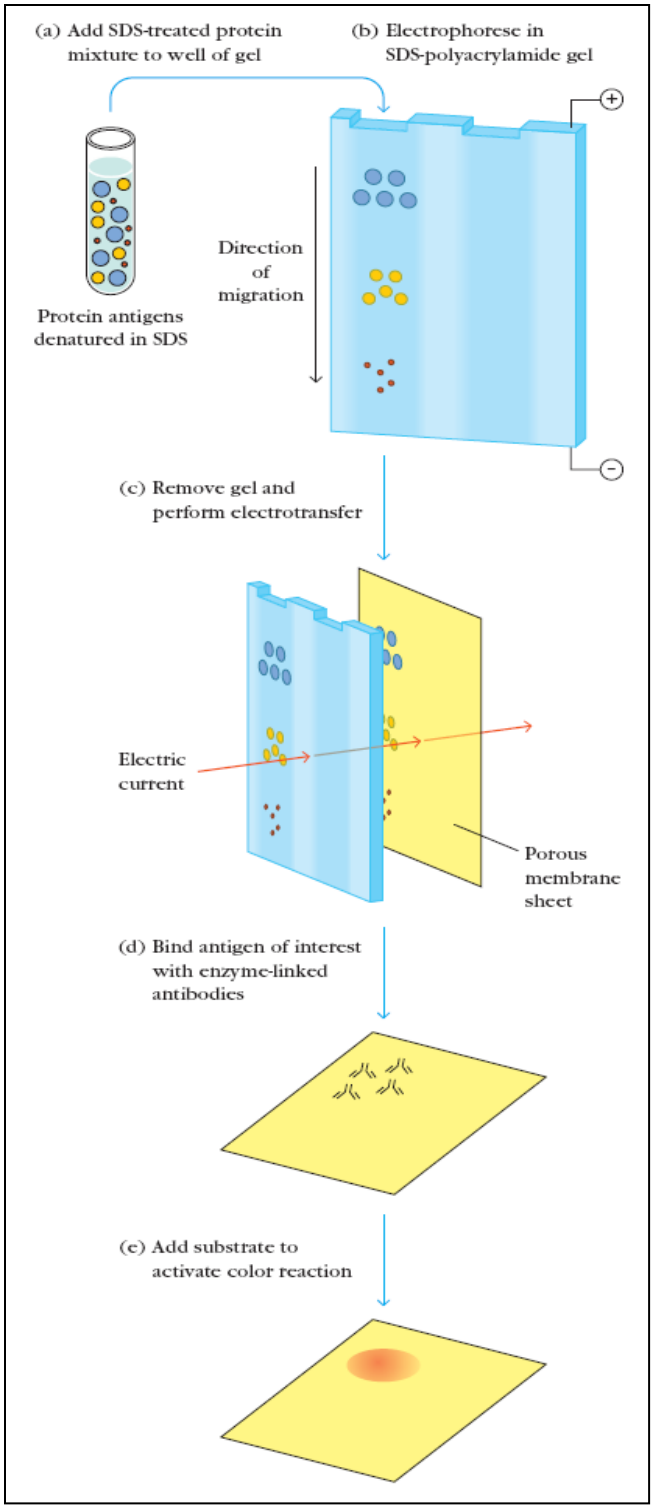
RAST (Radio allegro sorbent test):

This test measures Ag specific to IgE in a radio immunoassay. The protocol includes usage of a labeled antiIgE antibody. The allergen is first covalently bound to the surface of a cellulose disc rather than to the surface of a radioimmuno assay plate. The serum of suspect is added, and if IgE is present they bind to the Ag. Then Addition of anti-IgE Ab's is done and detected for the presence of radioactivity.



Immunoblotting or Western blotting:

The technique is used to identify and characterize an unknown Ag from a complex mixture. The protein mixture is first denatured with SDS and then the resulting fragments are separated by electrophoresis (SDS-PAGE). The proteins separate based on their molecular weight and these are now transferred onto nitrocellulose paper by electro transfer method. Now enzyme linked Ab's are added which bind to the Ag specifically. Addition of chromogenic substrate develops color at the area where the Ag is present.



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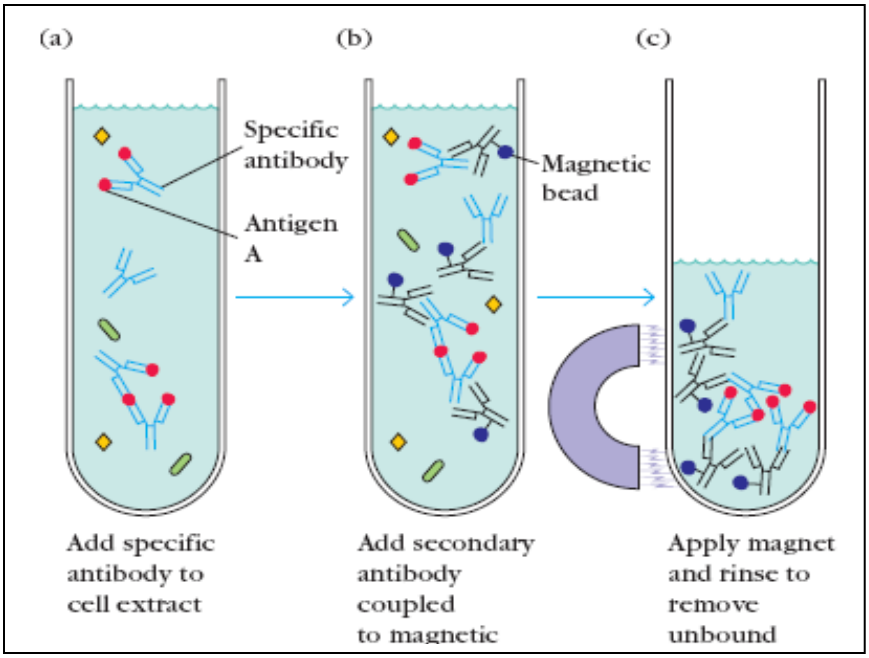
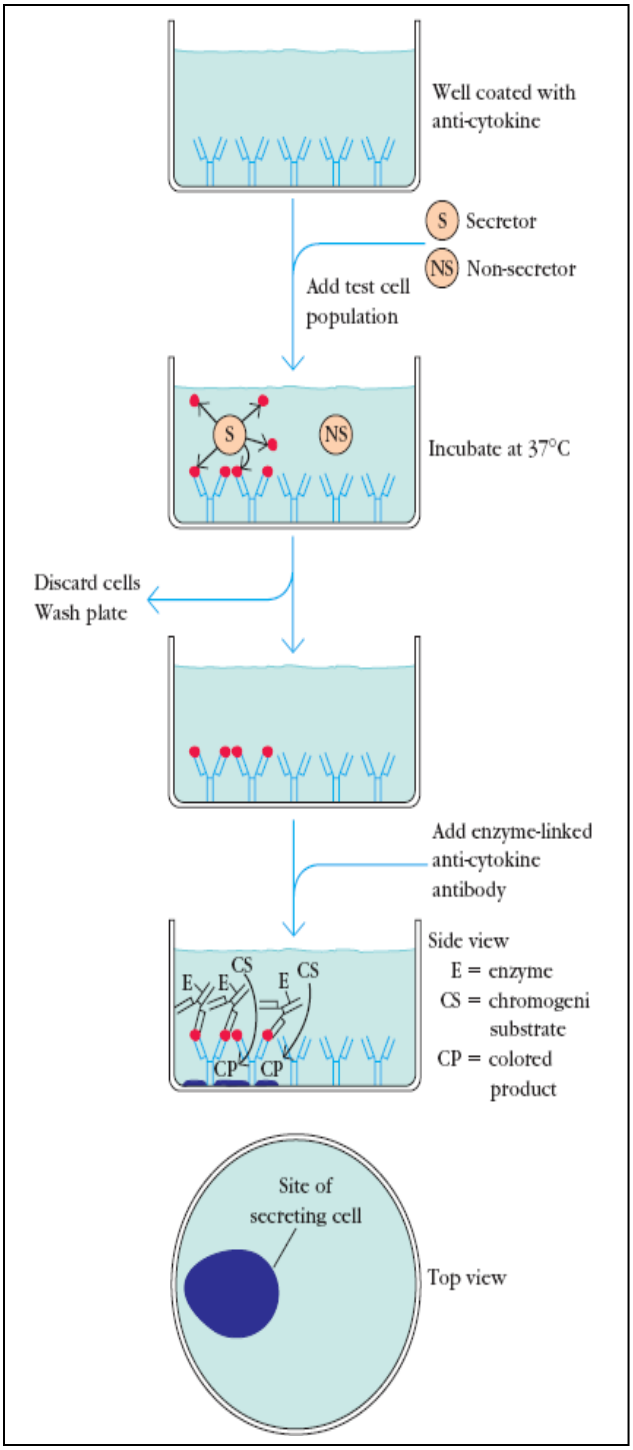
IMMUNOPRECIPITATION: the advantage with this technique it allows isolation of Ag of interest and its further use for analysis. The Ab is bound to solid beads. When a cell extract having even minute quantities of Ag is added they form Ag-Ab complexes and they precipitate. If the concentration of Ag is less then normal precipitations take several hours to days but in this technique simple centrifugation allows collection of precipitate. An alternative is to add magnetic bead coated with anti-Ab against the primary Ab. Application of magnetic beads retains the precipitate in the vial and the rest of the elements can be removed by washing. The Ag can be confirmed by subjecting it to SDS-PAGE. At times radio labeled primary Ab's may also be added, then Ag-Ab complexes are separated by SDS-PAGE and then confirmation is done by autoradiography.



ELISPOT: This allows the quantitative determination of the number of cells in a population that are producing antibodies specific for a given antigen or an antigen for which one has a specific antibody. In this approach, the plates are coated with the antigen (capture antigen) recognized by the antibody of interest or with the antibody (capture antibody) specific for the antigen whose production is being assayed. A suspension of the cell population under investigation is then added to the coated plates and incubated. The cells settle onto the surface of the

plate, and secreted molecules bind by the capture molecules in the vicinity of the secreting cells, producing a ring of antigen-antibody complexes.

The plate is then washed and an enzyme-linked antibody specific for the secreted antigen or specific for the species (e.g., goat anti-rabbit) of the secreted antibody is added and allowed to bind. Subsequent development of the assay is by addition of a suitable chromogenic antibody producing cells. Can be used to identify cytokine secreting cells and in such cases antibodies against cytokines are coated on to the well.



Important Questions:

Explain the following techniques each 5M

a) Precipitation
b) Agglutination
c) Blood grouping
d) Complement fixation
e) Fluorescent Assay
f) ELISA
g) Radio immuno assay
h) RAST
i) Western blotting or Immuno blotting
j) Radial Immuno assay
k) Immunoelectrophoresis.