Practical Microbiology

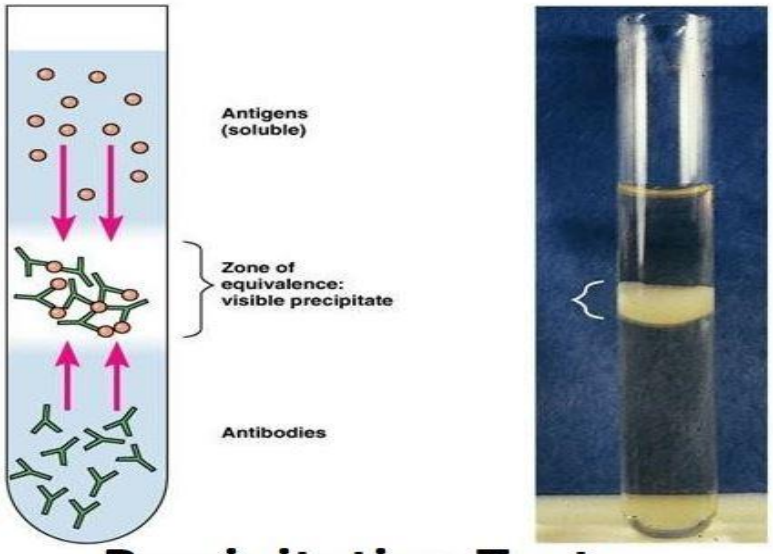
Lab (2) . Second course

Serological Tests

. Precipitation :

It is a test in which antibody interacts with the soluble antigen in the presence of electrolyte to produce a precipitate.

- **Antibody that aggregate the soluble antigens are called precipitins.**
- **So, precipitation test is a type of antigen-antibody reaction in which the antigen occurs in a solution form**



Precipitation Test

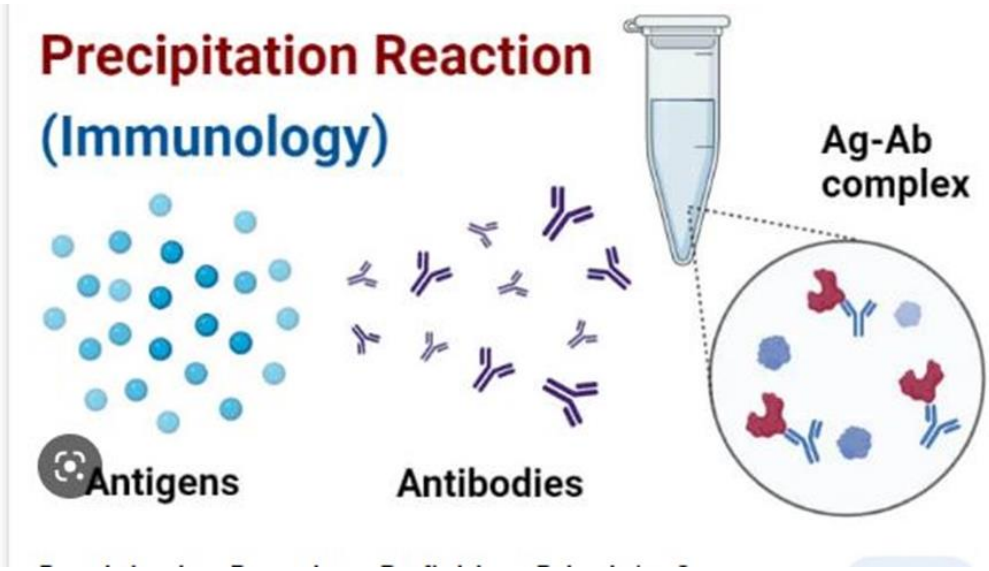


Fig.(1 A and B): Precipitation Test

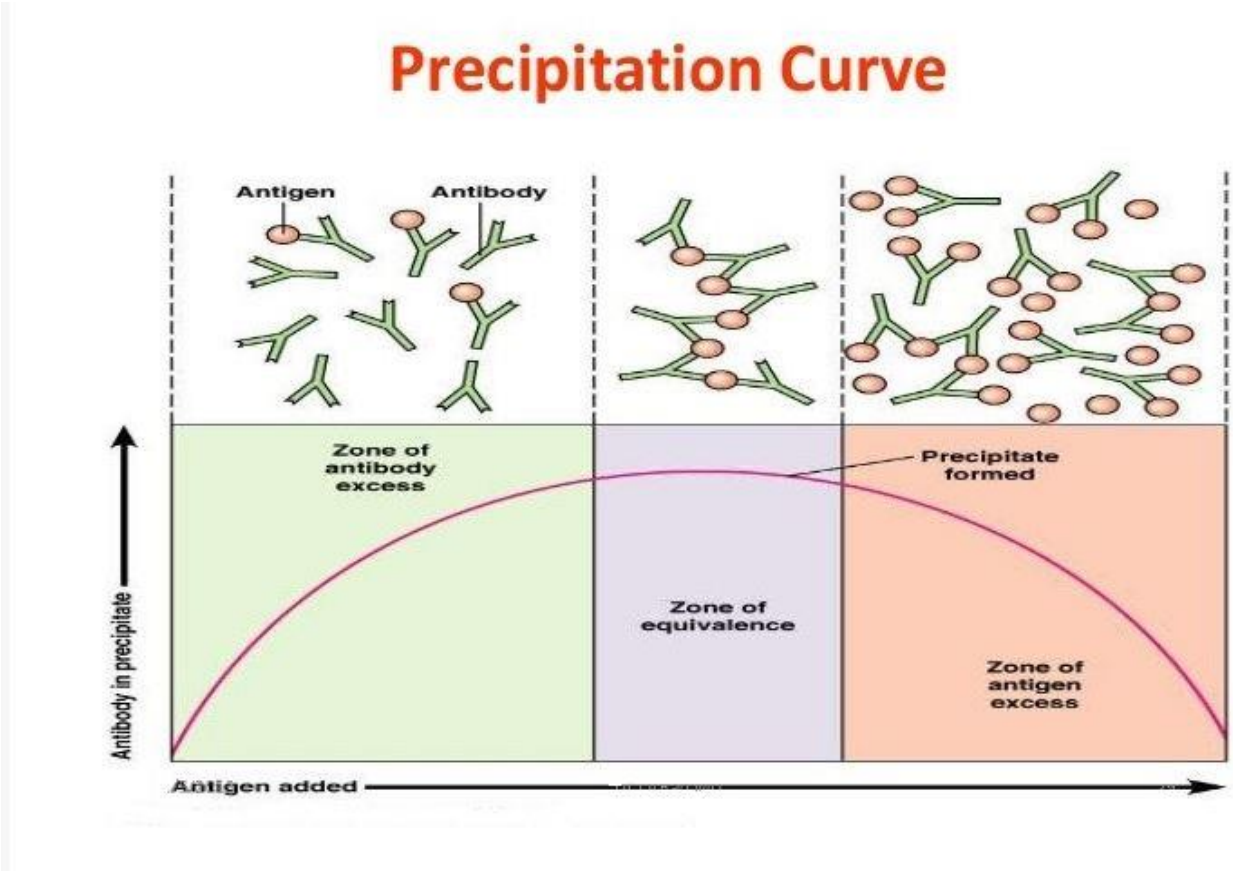


Fig.(٢): Precipitation Curve

❖ **Immunodiffusion** : precipitation in gels (semisolid medium).

Bands of precipitate form as the reactants diffuse toward each other from separate wells in the gel .

A.Double immunodiffusion method (ouchterlony technique) (fig.٣) .

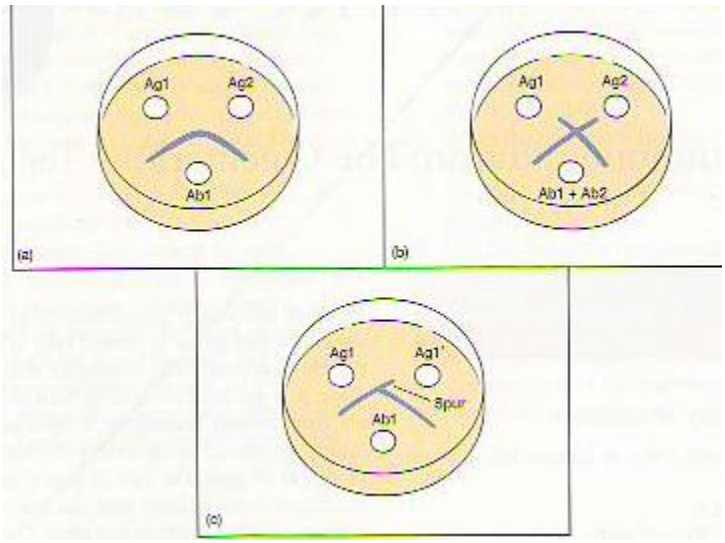
This procedure determine the antigenic relationships as following :

Identity : if the two antigens are indentical , the two precipitin bands will join to form a single band termed a line of identity .

Nonidentity : when the two antigens are completely different, the lines of precipitate cross.

Partial identity : spur formation : one of the antigens is cross- reactive with (but not identical to) the other.

The spur occurs because one of the antibody does not react with the cross-reacting antigen but migrates past that antigen until it reaches the antigen that has an epitope(antigenic determinant).



Figure(٣) : Immunodiffusion Test a-line of identity, b-lines of Nonidentity , c- lines of partial identity.

Procedure :

Prepare a 1% agar (1g agar / 99 ml 0.85 % saline), dissolve the agar by heating until boiling .
Autoclave the agar , cool at 48º to 50 ºC , add 1 ml of 1/1000 merthiolate to the agar , mix and pour the agar into plates (2mm deep agar layer) , merthiolate will prevent microbial growth.
When the agar has hardened , cut 6.0 mm diameter holes in it with a cork borer as in (fig.) .
The central hole should be filled with antiserum (or antigen) using a Pasteur pipette , the other holes with antigen (or antibody).
Seal the plate with transparent tape.
Store the plate at room temperature , right side up , and look daily for the formation of precipitin lines.

B. Single Radial Immunodiffusion :(Quantitative Method)

That can be used to quantitate antigens, such as human serum immunolobulins .
In this test , antibody to human IgG is incorporated into the agar (gel) . A serum containing a unknown concentration of IgG (Ag) is placed in wells in the agar . As the serum samples diffuse through the agar and reacts with antibody , a visible ring of precipitate appears as a halo around the well . The diameter of precipitin rings are measured . The diameter of precipitin rings produced by antigen solutions of known concentration are measured and plotted against the antigen concentrationson semilog paper to produce standard carve. In this test prepared agar for each type of IgG is used .(fig.).

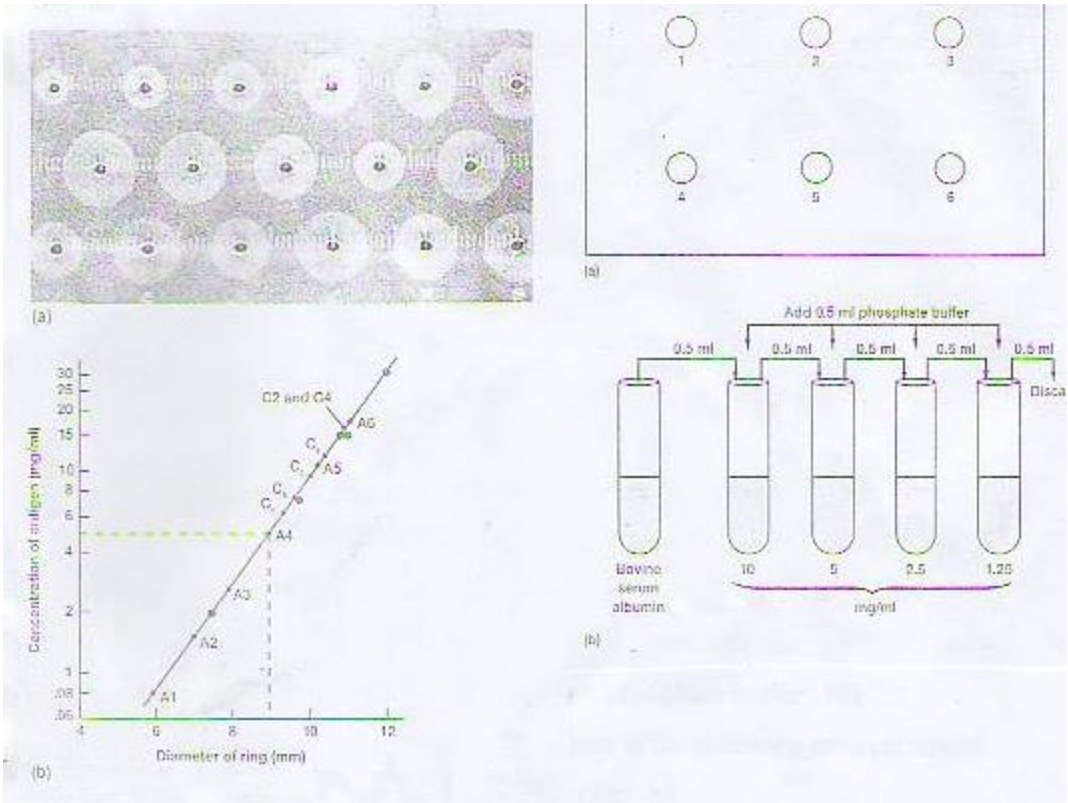


Figure- () Single Radial Immunodiffusion , a- The gel contains Antiserum specific for the antibody , b- Dilution procedure and standard curve .

Procedure :

In this test prepare a standard curve and determine the concentration of a bovine serum albumin in the unknown .

Prepare 1% agar (noble agar) with 1% sodium azide or 1/10000 merthiolate . Heat the suspension to boiling . Disperse 16 ml of melted agar into test tubes and cap the tubes .

When the agar have cooled to 48ºto 50ºC in a water bath , add 0.3 ml of antibovine whole serum to each tube (a 1/20 dilution) and mix immediately after adding the antiserum transfer the agar to a 3x2 inch glass slide.

Cover the slide with sterile cover and allow the agar to harden .Cut six , 2 to 3 mm diameter holes in the agar . This is done with either a cork borer or a sharpened piece of metal tubing. Remove the agar plug by blowing it out with the rubber tubing .
Using a 20 mg/ml bovine serum albumin stock antigen solution and 0.1 ml potassium phosphate buffer . PH 7.2 prepare a set of serial dilutions at the following concentrations : 10,5,2.5 and 1.25 mg/ml (fig. -b).
This is done by labeling four test tubes and then pipetting 0.5 ml of phosphate buffer into each tube . Remove 0.5 ml of the bovine serum albumin stock antigen solution and add it to the 10 mg/ml tube. Mix the solution and transfer 0.5 ml of the 10 mg/ml solution to the 5 mg/ml tube and mix prepare the last dilutions in the same way and discard 0.5 ml from the last tube .
Fill the first four wells on the agar slide with the four antigen standards by using capillary tube pipette.
Incubate the agar slide at room temperature for 24 to 48 hours . To prevent dryness of agar put the agar slide in moist environment.
Measure the diameter of each to the nearest 0.1 mm , and plot the standard curve to estimate the concentration of unknown antigens.

Complex Serological Procedures

Antigen-antibody reactions in which the visible manifestation requires the participation of accessory factors, indicator system and specialized equipment can be measured by several techniques .

Examples :

- **A.Hemagglutination Inhibition Test**

Hemagglutination is the agglutination of red blood cells , certain virus particles or other substances by antibodies (hemagglutinins) . Although viral hemagglutnation is a nonimmunologic phenomenon , it forms the basis for the hemagglutination inhibition test, as a viral diagnostic test. To examine the serum of a patient suspected of having influenza , the patients serum is mixed with known influenza virus and red blood cells. If antibody is present , hemagglutination normally caused by the virus will be inhibited because the antibody will bind to the virus and block its contact with the RBC.

B. Passive Agglutination Test :(Latex Agglutintion)

In this test the soluble antigen is converted to insoluble antigen.

Latex particles coated with a soluble antigen and then exposed to specific antibody become agglutinated e.g. detection of rheumatoid factor in serum.

C. Complement Fixation Test :

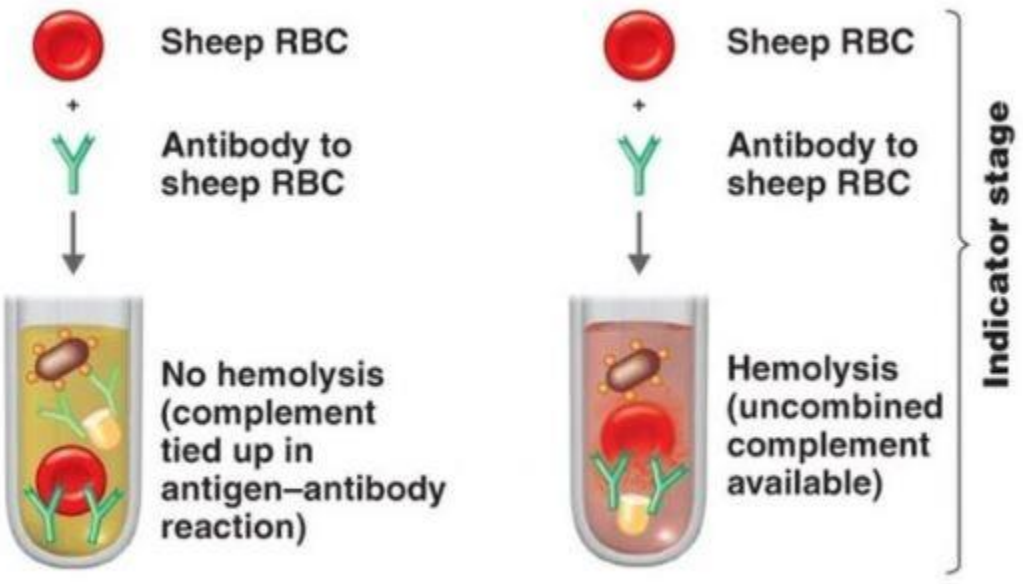
Complement is a protein constituent of normal blood serum , is consumed (fixed) during the interaction of antigens and antibodies and will not be a vailable to lyse hemolysin coated RBCs. This is the basis for the complement fixation test . It is a quantitive test (titer determination) .
The complement fixation test (CF) takes place in two phases or systems.
Test system :
Incubation of patient's serum (serum dilution) with the appropriate antigen and specified amount of complement .
Indicator system :
Red blood cells precoated with specific blood cell (sensitized cells) .
Antibody are added to the phase I and if any free complement remains it bound to the antibody-coated RBC, causing lyses of these cells .
The test includes the following controls :

Hemolysin control :

RBC + Anti-RBC + complement .
Negative and positive serum control.

Anticomplementary test :

Each serum dilution is mixed with complement and the sensitized cells (without antigen).



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D . Immunofluorescence *:*

Antibody is labeled with a fluorescent dye. If the antibody reacts with antigen (in tissues) , it is seen a green stain under ultraviolet light . This is direct immunofluorescence . In this test a fluorescent antibody directed against antibody (e.g. ,fluorescent rabbit antiserum to human gamma globulin), human gamma globulin serves as antigen . Antigen added to patient serum on slide and the slide flooded with fluorescent antibody to human gamma globulin .

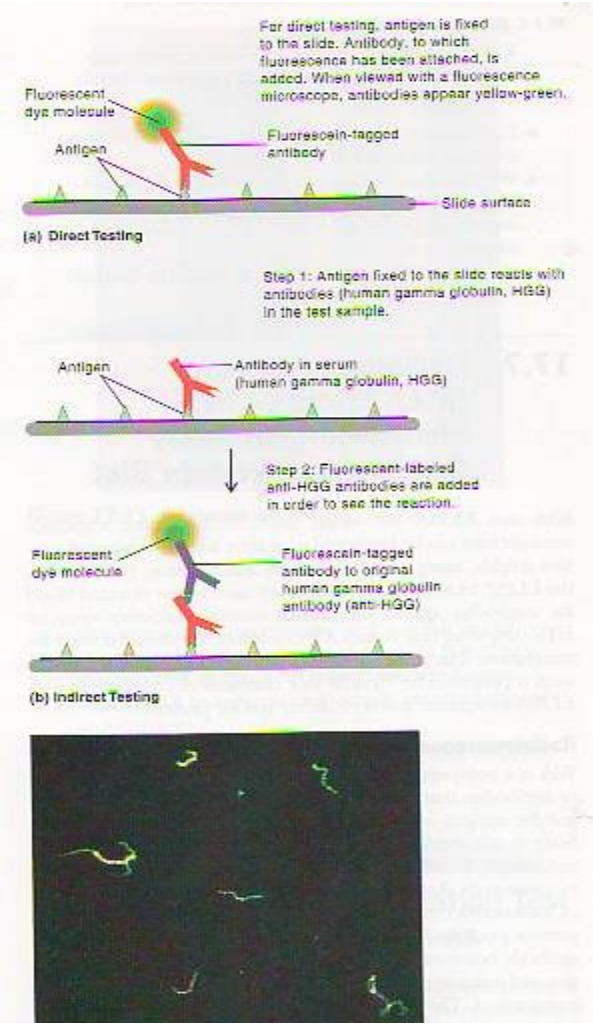

Figure () Immunoflourescence Test.

Enzyme – Linked Assay (ELIA)

Horseradis peroxidase is conjugated to the antibody this complex is allowed to react with the antigen , a substrate for the enzyme is added . Bound antibody (Ag-Ab- complex) is detected by the presence of colored precipitate .

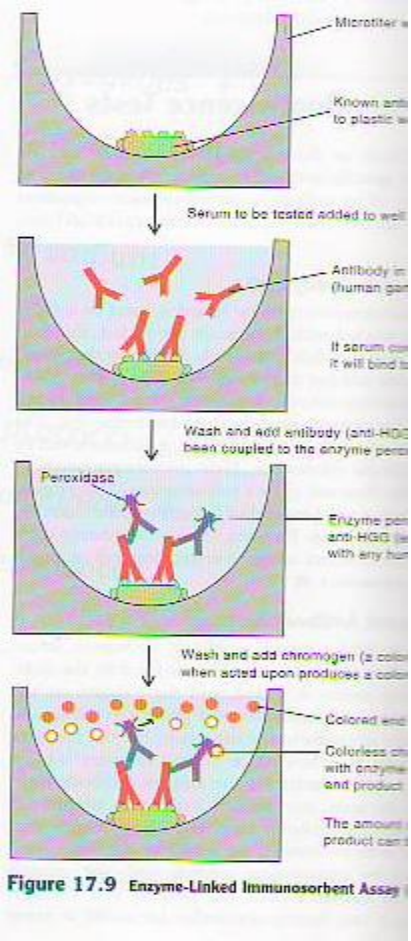

Figure 17.9 Enzyme-Linked Immunosorbent Assay

THE END …..

Figure -() ELSIA Test