Parte 2 : Immunology Techniques

Chapitre 1: Réaction de précipitation

I- Precipitation Reaction:

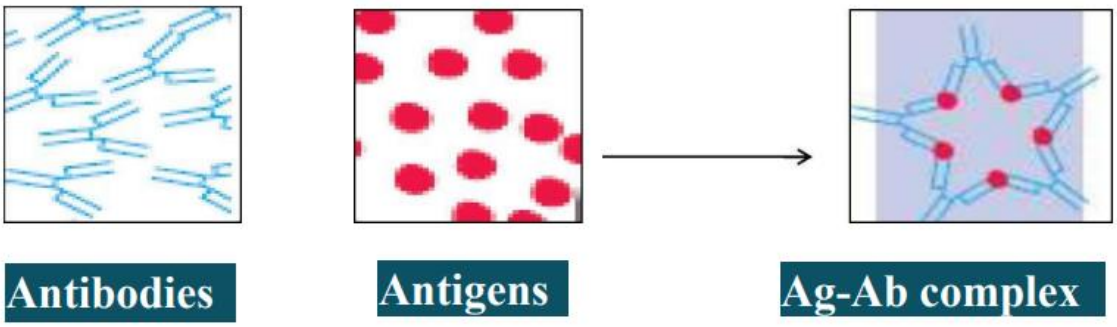
•Precipitation reactions are based on the interaction of antibodies and antigens. They are based on two soluble reactants that come together to make one insoluble product, the precipitate. These reactions depend on the formation of lattices (cross-links) when antigen and antibody exist in optimal proportions.

• There is several precipitation methods applied in clinical laboratory for the diagnosis of disease. These can be performed in semisolid media such as agar or agarose, or non-gel Excess of either component reduces lattice formation and subsequent precipitation. Precipitation reactions differ from agglutination reactions in the size and solubility of the antigen and sensitivity. Antigens are soluble molecules and larger in size in precipitation reactions. There is several precipitation methods applied in clinical laboratory for the diagnosis of disease.

• These can be performed in semisolid media such as agar or agarose, or non-gel support media such as cellulose acetate. Initially, the soluble Ag-Ab complex form within a few minutes; the formation of the visible precipitate occurs more slowly and often takes a day or two to complete.

In this reaction:

- The **antigens** are **soluble** and possess **multiple epitopes**.
- The **antibodies** are **precipitating** and **polyclonal**.



The Ag-Ab reaction is an exothermic, reversible, and specific reaction.

Exothermic: The reaction is characterized by the formation of a bond that releases energy, which consequently influences the temperature-dependent progression of the reaction.

Reversible: The bond formed between the antibody (Ab) and antigen (Ag) consists of weak interactions (electrostatic, hydrogen, hydrophobic, etc.), making it easily breakable by varying physicochemical parameters such as pH, temperature, or ionic strength.

Specific: The antibody-binding site of an immunoglobulin (paratope) can bind to only one epitope. There is a stereochemical specificity between the paratope and the epitope.

I-1- Precipitation in solution:

A- Ring Precipitate (Ring Test) (Tube Precipitation test)

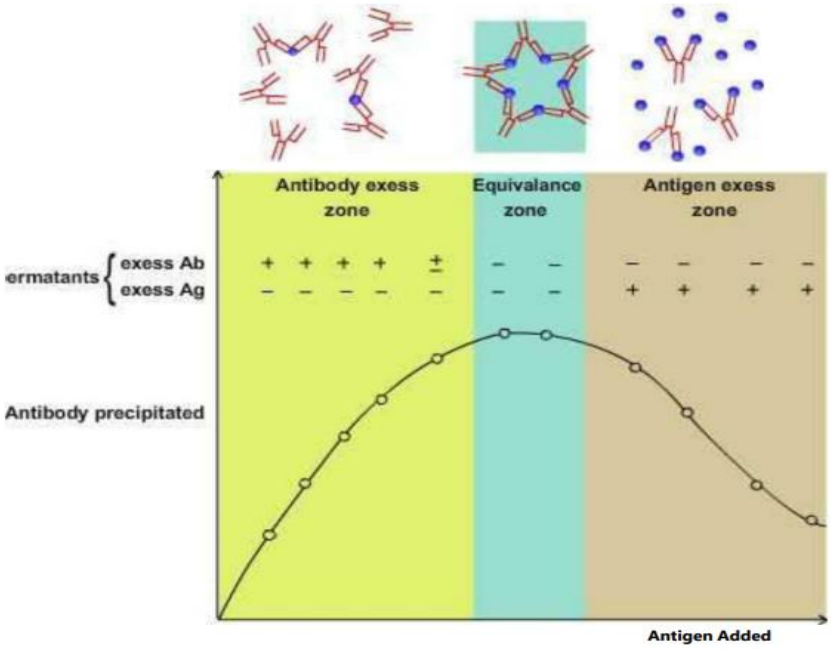
In this test, a clear solution of the test antigen is layered slowly over the clear solution of antiserum in narrow test tube. Following a period of incubation, precipitation between antigen and antibodies in the antiserum solution is marked by the appearance of a white ring at the junction of two liquid layers. C-reactive protein (CRP), Lancefield grouping of β-haemolytic streptococci, Ascoli's thermo precipitin test are the examples of the ring test.

Characteristic ring precipitate:

- Simplest test
- Qualitative

Precipitation curve shows three zones

1. Zone of Ab axis.
2. Zone of equivalence.
3. Zone of Ag axis.



B- Slide Test

This test is performed on slides such as cavity slides in the case of the VDRL test. In this test, the serum sample of the suspected patient is kept in the cavity of the slide and antigenic solution(already known) is mixed with it and shaken properly. Floccules are formed after a while in the case of the positive test.

C- Tube Test : It is a type of flocculation test in which antigens or antibodies solutions are mixed in a tube to observe the formation of floccules. It can also be used for quantitative along with the qualitative diagnosis of toxins.

I-2- Precipitation in agar

The precipitation test in agar gel is termed as an immunodiffusion test. Here, 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. An agar concentration of 0.3–1.5% allows for the diffusion of most reactants.

The reaction is visible in the form of a distinct band of precipitation.

When only antigen or antibody diffuse with a corresponding antibody or antigen being incorporated on agar gel, this is called single diffusion.

When both antigen and antibody diffuse in agar gel, it is double diffusion.

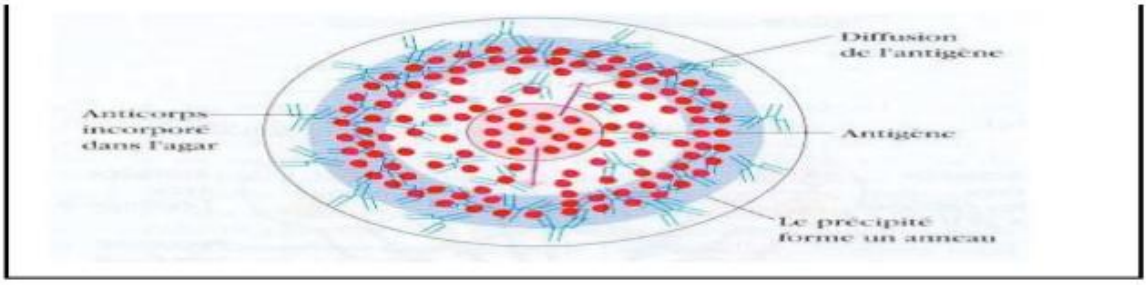
1-2-1-Type of precipitation in gel

a- Agar gel single immunodiffusion test (Mancini test)

- Radial immunodiffusion.
- Qualitative or quantitative technique.
- The gel is incorporated with the antibody (Ab).
- The antigen (Ag) is deposited in a well dug into the gel.
- After diffusion, a positive reaction is indicated by a precipitation ring.
- Can be used for the quantification of certain serum proteins.

a-1- Mancini: Qualitative aspect:

Presence or absence of the precipitation ring.



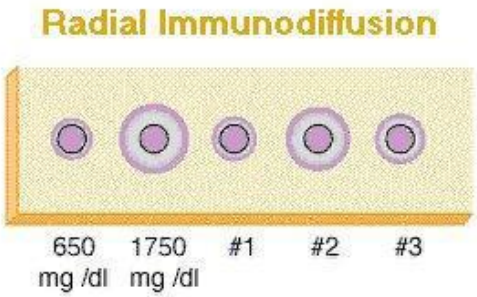
Technique de Mancini

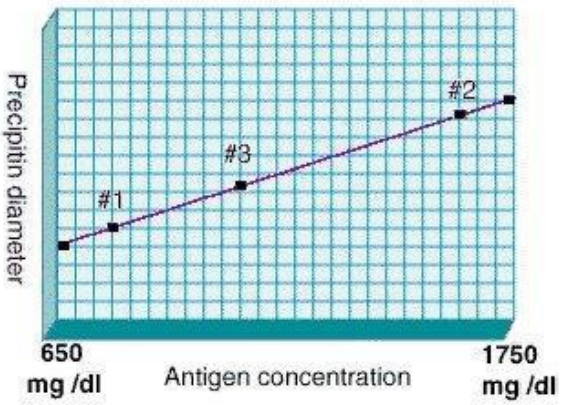
a-2- Mancini: Quantitative aspect:

• different dilutions of the Ag are placed in holes punched into the agar.

• As the Ag diffuses into the gel it reacts with the Ab and when the equivalence point is reached a ring of precipitation is formed

• The diameter of the ring is proportional to the concentration of Ag since the amount of antigen is constant.L





b- Agar Gel double immunodiffusion test (Ouchterlony test)

 It is a qualitative immunoprecipitation technique in a gel medium.

 The diffusion of the antigen (Ag) and antibody (Ac) occurs from distant wells drilled into the virgin gel.

 At the equivalence zone between the two wells, a precipitation line forms.

 There are as many precipitation lines as there are Ag-Ac systems.

 The precipitation lines of several antigenic preparations can be compared against the same antiserum, or multiple antisera can be tested against the same antigen mixture.

We observe that:

-In Agar gel double immunodiffusion test The precipitation appears as a continuous line in the form of an angle between those two wells and the C well. There are no spurs at the angle and this type of reaction is termed **a band of identity.**

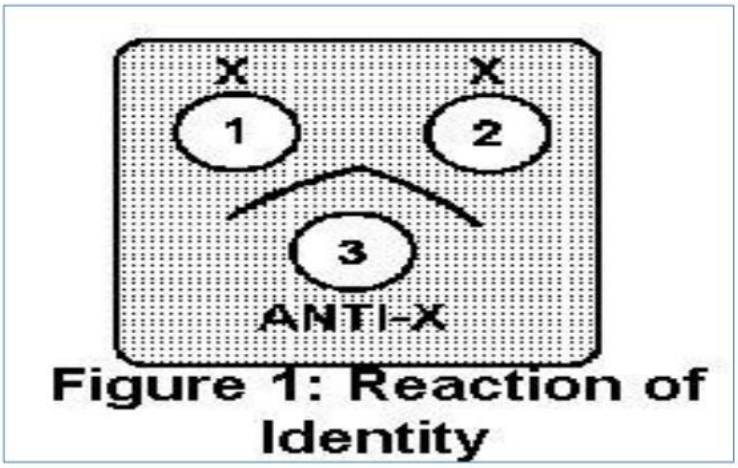


Figure 1: Reaction of Identity

- If a solution with antigens X and Y is placed in well 1, a solution with antigen X only is placed in well 2, and antiserum containing antibodies specific for both X and Y is placed in well 3, a reaction similar to that appearing in Fig. 2 will occur. Notice that there is a spur reaction towards the XY well. This indicates that the two antigenic materials in wells 1 and 2 are related, but that the material in well 1 possesses an antigenic specificity not possessed by

the material in well 2. Such a reaction with spur formation **indicates partial identity**

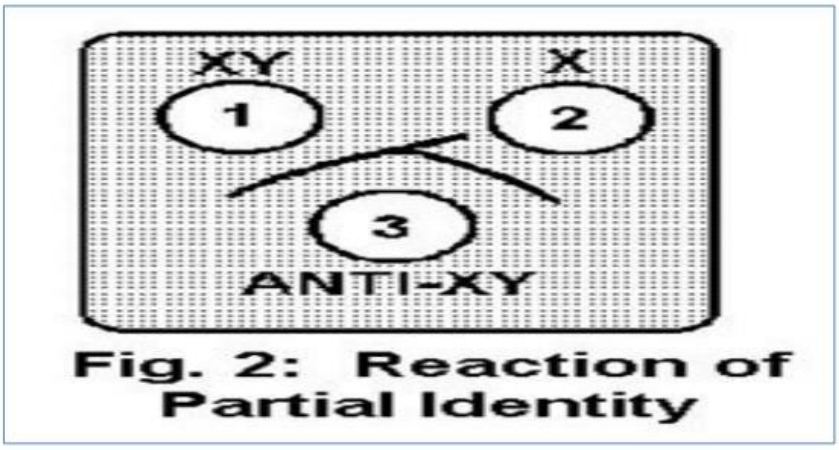


Fig. 2: Reaction of Partial Identity

If the material in wells 1 and 2 do not possess common antigens and the antiserum in well 3 possesses specificities for both materials, the reaction will appear as two crossed lines as in Fig. 3

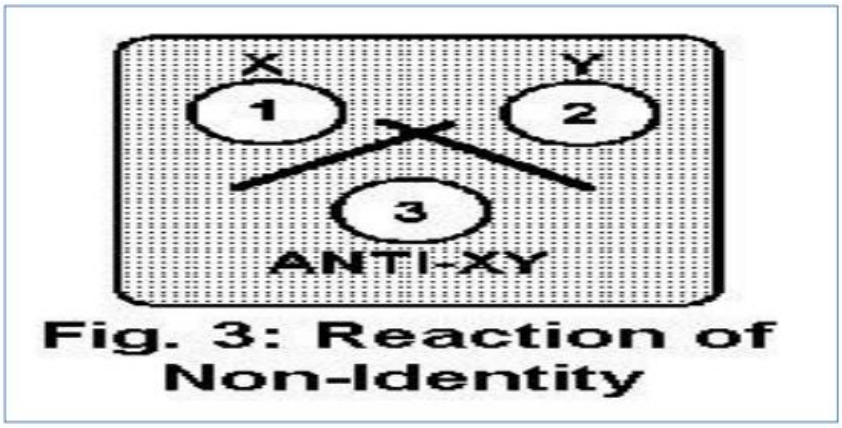
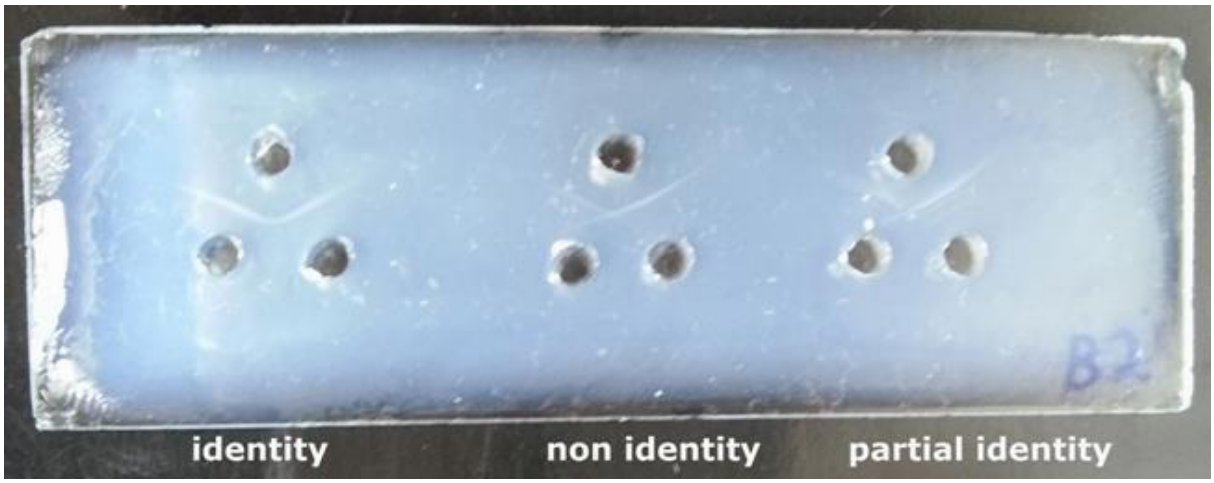


Fig. 3: Reaction of Non-Identity



It is therefore a qualitative and comparative technique with diffusion over several days that allows:

- Verifying the purity of a solution.
- Detecting a protein in a mixture.
- Checking for the possible presence of common epitopes between two proteins.

c- Immunoelectrophoresis

- Immunoelectrophoresis (IEP) allows the identification of antigens in a mixture based on their electrophoretic mobility. It is a qualitative technique.

- Immunoelectrophoresis is a two-step technique:

• First step: Protein electrophoresis

• Second step: Immunoprecipitation

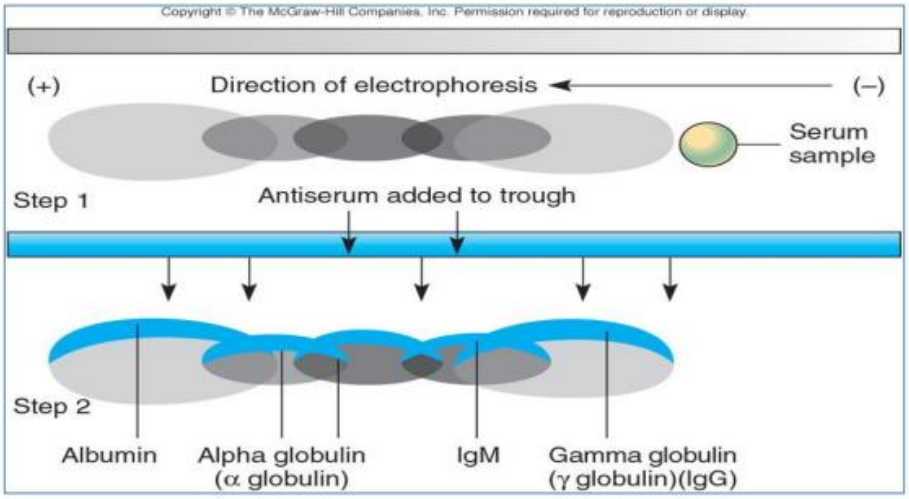
Each protein in the mixture contains a specific net electrical charge. Depending on its molecular size and solubility, each protein migrates differently in an electrical field .

 Animal serum is separable into albumin, alpha globulins, beta globulins, and gamma globulins .

 Each of these fractions, except albumin, contains many different proteins .

 Multiple precipitin arcs are discernible in each of the globulin fractions

 multiple bands are visibles



D- Electrosyneresis or Counter-Immunoelectrophoresis:

This is an immunoprecipitation technique in a gel medium.

It follows the same principle as the Ouchterlony technique, but the reaction is accelerated by an electric field.

It is a qualitative technique used, for example, to detect autoantibodies.

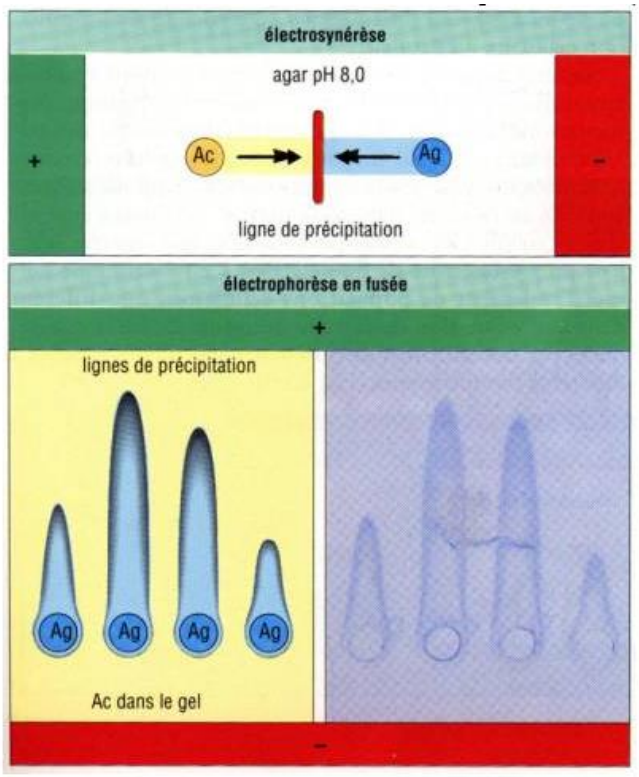
Antigens (Ag) and antibodies (Ac) are placed in separate wells in the gel, where they migrate toward each other under the influence of an electric field.

This forced migration speeds up and enhances the formation of precipitation lines.

It is more sensitive than the Ouchterlony technique.

E- Rocket Immunoelectrophoresis (Laurell Technique)

In this technique, a specific antibody for the antigen to be measured is incorporated into a gel plate. Different dilutions of the antigen preparation to be quantified are placed in aligned wells. An electric current is then applied perpendicularly to the line of wells. A rocket-shaped halo forms and progresses as long as the antigen is in excess. The existence of a linear relationship between the antigen concentration in the wells and the height of the precipitate allows this technique to be used for highly precise and sensitive antigen quantification.



Summary of Differences:

1️⃣ **Laurell Technique (Rocket Immunoelectrophoresis) → A quantitative and precise technique** for measuring antigen concentrations. Uses an **electric field** to push antigens through a gel containing antibodies, forming a **rocket-shaped precipitate** proportional to antigen levels.

2️⃣ **Ouchterlony Technique (Double Immunodiffusion) → A qualitative technique** that relies on passive diffusion of antigens and antibodies through a gel. It forms **precipitation lines** at the equivalence point, but it is **slow and less sensitive** than other methods.

3️⃣ **Electrosyneresis (Counter-Immunoelectrophoresis) → A rapid qualitative technique** that uses an **electric field** to force antigen and antibody migration, speeding up **precipitation line formation** compared to Ouchterlony.