Immunodiffusion Technique

Immunodiffusion is a technique for the detection or measurement of antibodies and antigens by their precipitation which involves diffusion through a substance such as agar or gel agarose.

It refers to any of the several techniques for obtaining a precipitate between an antibody and its specific antigen.
This can be achieved by:
a) Suspending antigen/antibody in a gel and letting the other migrate through it from a well or,
b) Letting both antibody and antigen migrate through the gel from separate wells such that they form an area of precipitation.

Based on the method employed, immuno-diffusion may be following types:

Radial Immunodiffusion (Mancini Method)
Double Immunodiffusion (Ouchterlony method)

Radial immunodiffusion (RID) or **Mancini method** is also known as **Mancini immunodiffusion** or **single radial immunodiffusion assay**. It is a single diffusion technique whereby a solution containing the antigen is placed into wells in a gel or agar surface evenly impregnated with antibody.

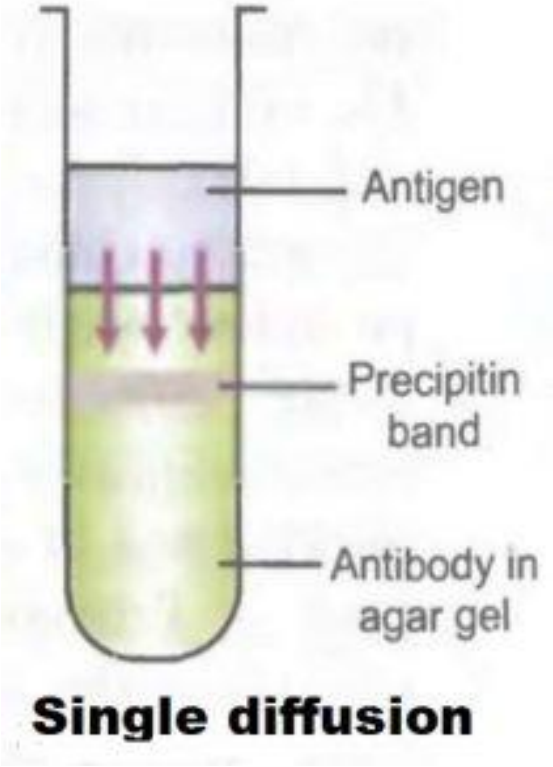
The diameter of the ring that precipitates around the well as a result of antigen antibody reaction corresponds to the amount of antigen in the solution.

Single Diffusion in One Dimension (Oudin Procedure)

The antibody is mixed with agar in a test tube and the antigen solution is added over it.

As a result, antigen diffuses downward through agar gel and a line of precipitation is formed.

The number of different precipitate bands will indicate different types of antigens.



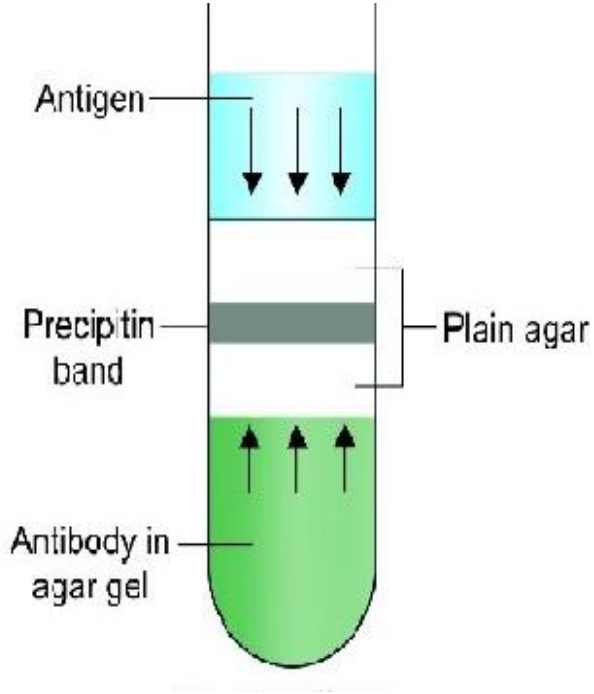
Double Diffusion in One Dimension (Oakley- Fulthrope Procedure)

The antibody is mixed with agar in a test tube.

A column of plain agar is added on top of the antibody solution.

The antigen is poured on the plain agar column.

The antigen and antibody move toward each other through the intervening column of plain agar and a precipitate band will form when at the optimum concentration of the antigen and antibody.



Single diffusion in two dimensions (Radial immunodiffusion)

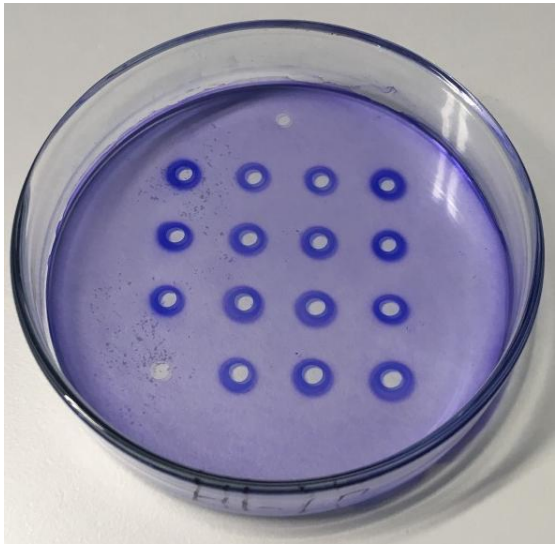
The antibody is mixed with agar gel and a layer of this mixture is formed on a glass slide.

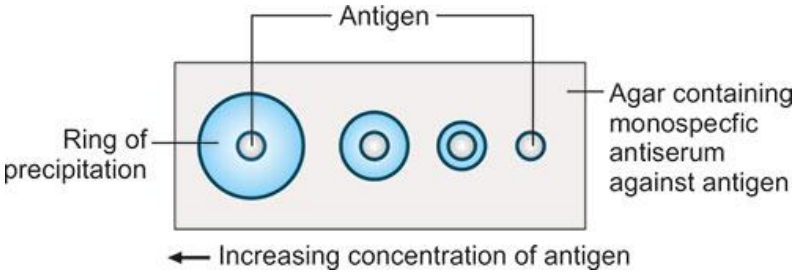
Wells are cut on the surface of the gel.

The antigen solution will be added to the wells. As a result, it diffuses and a ring-shaped precipitation band is formed.

The diameter of the band is proportional to the concentration of the antigen.

This technique was used for the estimation of IgG, IgM, IgA in sera and for the screening of antibodies of influenza virus.





Double diffusion in Two Dimension (Ouchterlony Procedure)

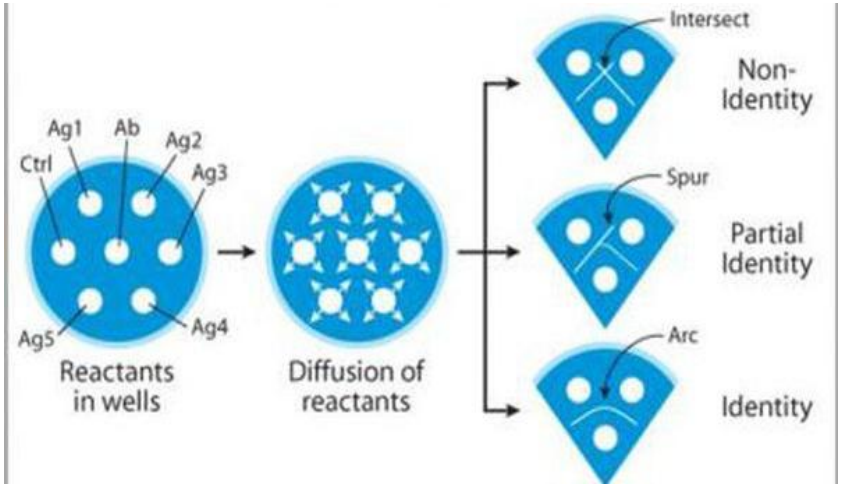
A layer of agar gel is formed on a Petri plate. Then wells are formed by using the template.

Antibody solution is added on the central well and different antigens are added in the surround wells.

If two adjacent antigens are identical, the precipitation lines will fuse.

If two adjacent antigens are unrelated, the precipitation lines will cross.

In case of partially related antigens, spur formation will be observed.
This technique is used for the toxicity test of C. diphtheria (Elck's Test).



Principle of Radial Immunodiffusion

Radial immuno-diffusion is a type of precipitation reaction. It is thus based on the principles of the precipitin curve which states that antigen-antibody interact forming visible cross-linked precipitate when the proper ratio of antigen to antibody is present.

In the test, antibody is incorporated into agar and poured into a glass plate to form a uniform layer.

Circular wells are cut into the agar and antigen is introduced into the wells. Specific antigens to the impregnated antibodies diffuse through the agar in all directions from the well and react with the antibody present forming visible precipitate or a precipitin ring.

Ring shaped bands of precipitates from concentrically around the well indicating reaction. The diameter of the precipitate ring formed, corresponds to the amount of antigen in the solution.

Applications of Radial Immunodiffusion

Immuno-diffusion techniques are mostly used in immunology to determine the quantity or concentration of an antigen in a sample.
Estimation of the immunoglobulin classes in sera.
Estimation of IgG, IgM antibodies in sera to influenza viruses.

Other applications include:
To determine relative concentrations of antibodies in serum.
Estimate serum **transferrin and alpha-fetoprotein**.
To compare properties of two different antigens.
To determine the relative purity of an antigen preparation
For disease diagnosis
Serological surveys

Principle of Ouchterlony Procedure

In the test, an antigen solution or a sample extract of interest is placed in wells bore on gel plates while sera or purified antibodies are placed in other remaining wells (Mostly, an antibody well is placed centrally).

On incubation, both the antigens in the solution and the antibodies each diffuse out of their respective wells.

In case of the antibodies recognizing the antigens, they interact together to form visible immune complexes which precipitate in the gel to give a thin white line (precipitin line) indicating a reaction.

In case multiple wells are filled with different antigen mixtures and antibodies, the precipitate developed between two specific wells indicate the corresponding pair of antigen-antibodies

A full identity (i.e. a continuous line): Line of precipitation at their junction forming an arc represents serologic identity or the presence of a common epitope in antigens.

Non-identity (i.e. the two lines cross completely): A pattern of crossed lines demonstrates two separate reactions and indicates that the compared antigens are unrelated and share no common epitopes.

Partial identity (i.e. a continuous line with a spur at one end): The two antigens share a common epitope, but some antibody molecules are not captured by the antigen and traverse through the initial precipitin line to combine with additional epitopes found in the more complex antigen.