

## Basic antigen-antibody reactions

### Precipitation Reactions

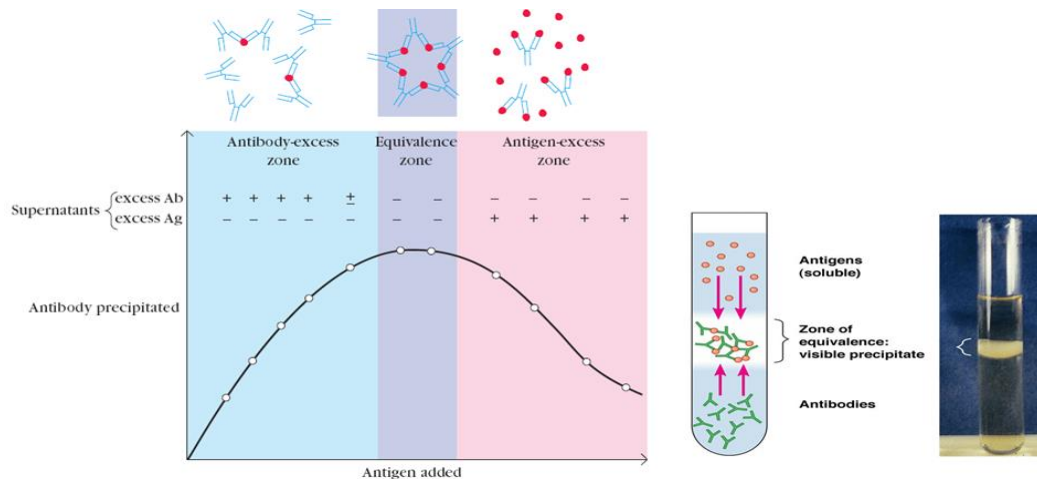
Antibody and soluble antigen interacting in aqueous solution form a lattice that eventually develops into a visible precipitate. Antibodies that aggregate soluble antigens are called **precipitins**. Formation of the visible precipitate occurs more slowly and often takes a day or two to reach completion. 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.

## Type of Precipitation Reactions

### 1- Precipitation Reactions in Fluids Yield a Precipitin Curve

A quantitative precipitation reaction can be performed by placing a constant amount of antibody in a series of tubes and adding increasing amounts of antigen to the tubes. After the precipitate forms, each tube is centrifuged to pellet the precipitate, the supernatant is poured off, and the amount of precipitate is measured. Plotting the amount of precipitate against increasing antigen concentrations yields a precipitin curve.



A precipitation curve for a system of one antigen and its antibodies. This plot of the amount of antibody precipitated versus increasing antigen concentrations (at constant total antibody) reveals three zones: a zone of antibody excess, in which precipitation is inhibited and antibody not bound to antigen can be detected in the supernatant; an equivalence zone of maximal precipitation in which antibody and antigen form large insoluble complexes and neither antibody nor antigen can be detected in the supernatant; and a zone of antigen excess in which precipitation is inhibited and antigen not bound to antibody can be detected in the supernatant.

## 2- Precipitation Reactions in Gels Yield Visible Precipitin Lines

Immune precipitates can form not only in solution but also in an agar matrix. When antigen and antibody diffuse toward each other in agar, or when antibody is incorporated into the agar and antigen diffuses into the antibody-containing matrix, a visible line of precipitation will form. As in a precipitation reaction in fluid, visible precipitation occurs in the region of equivalence, whereas no visible precipitate forms in regions of antibody or antigen excess.

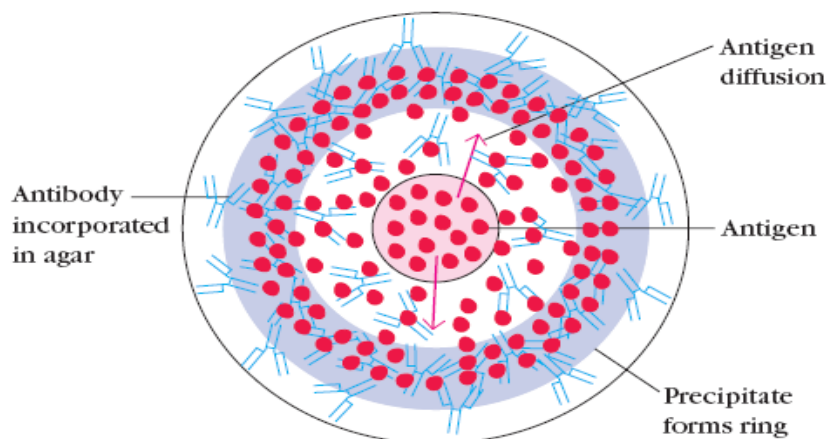
Two types of *immunodiffusion reactions* can be used to determine relative concentrations of antibodies or antigens, to compare antigens, or to determine the relative purity of an antigen preparation. They are

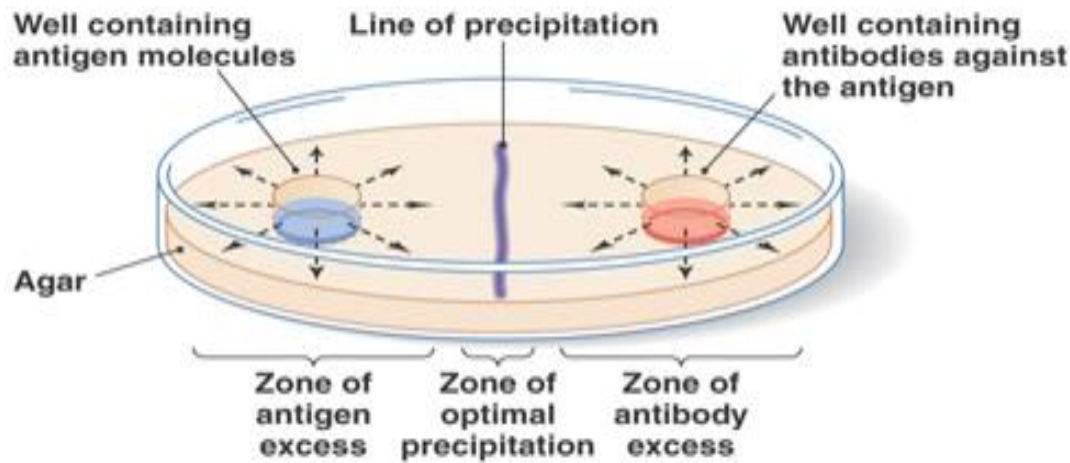
a- **Radial immunodiffusion**

b- **Double immunodiffusion**

**both are carried out in a semisolid medium such as agar.**

### RADIAL IMMUNODIFFUSION





### Ouchterlony method

#### Ouchterlony method

Both antigen and antibody diffuse radially from wells toward each other, thereby establishing a concentration gradient. As equivalence is reached, a visible line of precipitation, a precipitin line, forms. The concentration of the antigen and antibody in the agar. The local concentration of each reactant depends on: (a) absolute concentration in the well; (b) its molecular size; and (c) the rate at which it is able to diffuse through the gel. Multiple lines of precipitation will be present if the antigen and antibody contain several molecular species.

#### Advantages:

1. The method is sensitive .
2. It can detect more than one antigen-antibody system in mixture.
3. It detect similarities of components.
4. It is used for detecting about IgA, IgG, IgM, C3, C4 in patients with autoimmune disease .

**Identity**

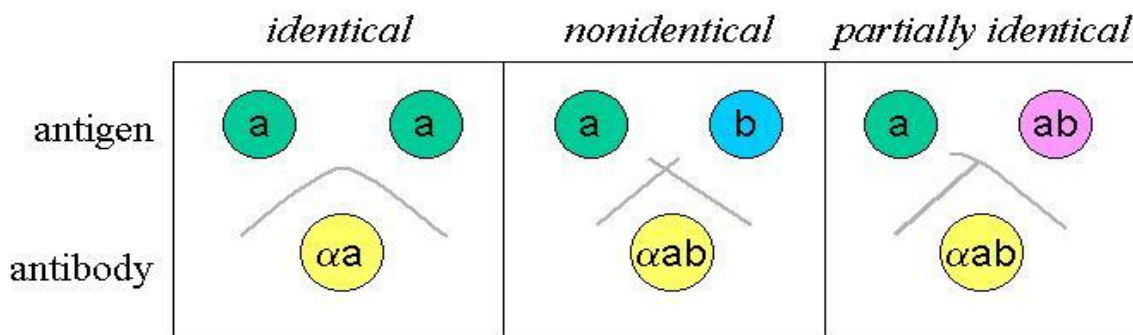
An identity reaction is indicated when the precipitin band forms a single smooth area. This precipitin is formed between the antibody and the two test antigens fuses, indicating that the antibody is precipitating identical antigen specificities in each preparation.

**Nonidentity**

A non-identity pattern is expressed when the precipitation line cross each other. They intersect or cross because the sample contain no antigenic determinants in common.

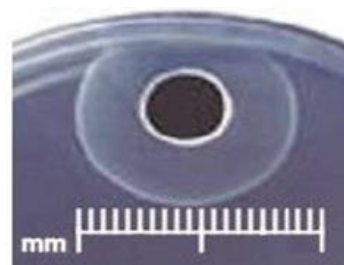
**Partial Identity**

In a partial identity pattern, the precipitation lines merge with spur formation. This merger indicated that the antigen are non identical but possess common determinants. insofar as the antibody can distinguish the difference.



**Procedure (1)**

1. The technique based on the reaction between an Ag, and a specific Ab.
2. Dissolve agarose gel powder (20 g/l) in boiling phosphate buffered saline PBS, pH 7.4, after cool ( $50^{\circ}\text{C}$ ) then pour into special plates, with a specific antigen, thickness of less than 1mm.
3. After the gel has set, cut out wells about 2mm in diameter.
4. Extract the core by pipette tip with a negative pressure pump.
5. Cover plates with fitted lids, and store in sealed packets at refrigerator ( $4^{\circ}\text{C}$ ) until used.
6. Application of serum and commercial prepared bovine albumin antibodies using micropipette 10  $\mu\text{per}$  well for both.
7. Incubation for 24-48 hr. at  $37^{\circ}\text{C}$ . then read results.
8. A ring of precipitation around the Ab well will be formed after the Ag-Ab reaction. This ring represents the immunocomplex that formed at equivalence zone.
9. After incubation time, calculating rings diameters, then a standard curve is plotted to determine the concentration of unknown sample Ab.



**Procedure (2)**

1. Holes are cut in the agar, one central hole surrounded by other wells.
2. Antibody is added to the central well, antigens are added to the outer wells, the position of the bands formed between the antigens allows for comparison of the antigens to each other.
3. Suggested antibody and antigen patterns: A straightforward demonstration of identity and non-identity can be shown using the antigen mixtures.

