

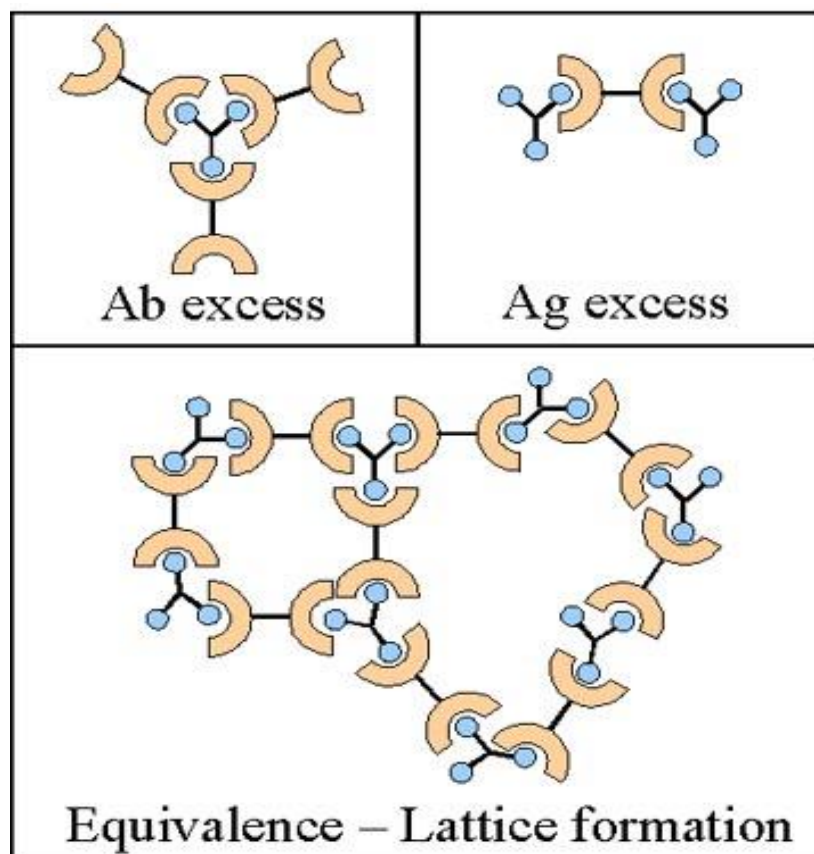


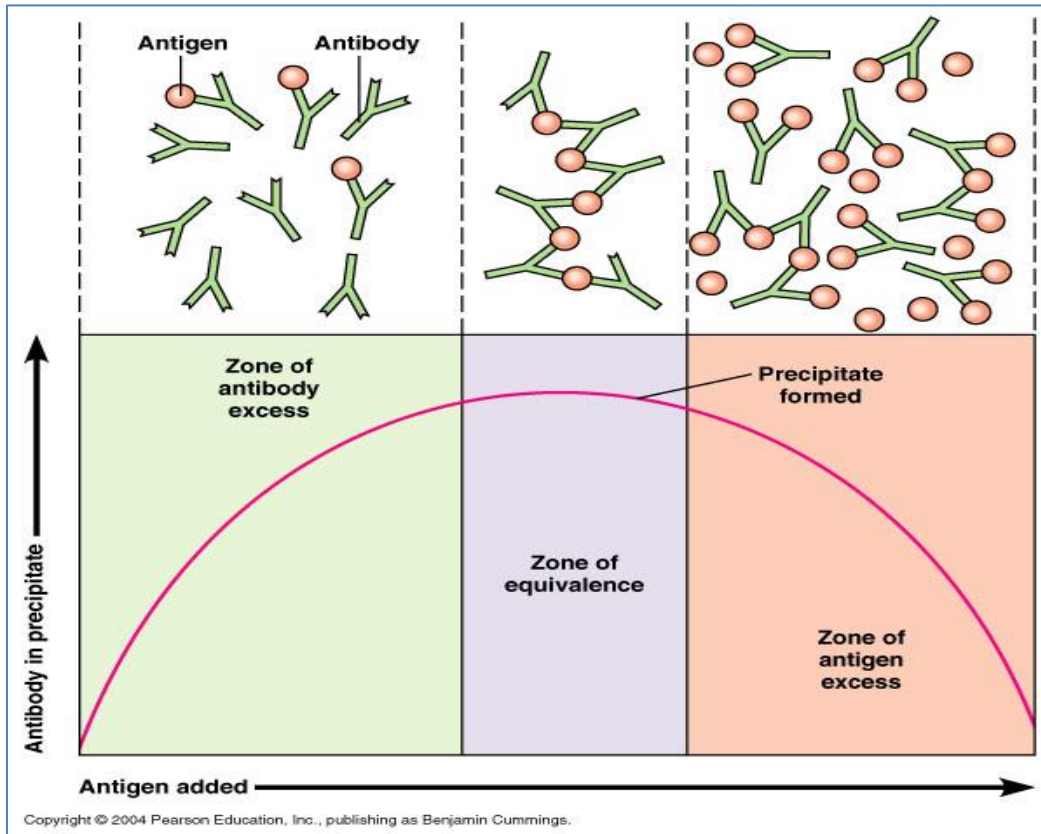
Precipitation test

The precipitation reaction occurs when certain soluble antigens are in contact with the homologous antibodies . In titration of precipitating antigen, three general zones of reaction may be observed, three are

- 1- the zone of antibody excess
- 2- the zone of equivalence
- 3- the zone of antigen excess

in the equivalence zone the proportion of antigen to antibody is optimal for maximal precipitation, in the zone of antibody excess or antigen excess the proportions of the reactants do not lead to efficient cross-linking and formation of precipitate





Precipitation curve

The suitable requirements for the test

- Temperature 22-23°C (room temperature)
- pH: 7.2-8.7.
- Buffer (normal saline, PBS)
- Humidity.
- Zone of equivalence (equal amount of antigen and antibody)

Types of precipitation test

Reaction in Gels and this classified into:

A) Gel diffusion.

i) Agar gel single immunodiffusion test (Mancini test)

ii) Agar Gel double immunodiffusion test (Ouchterlony test)

B) Immunoelectrophoresis



Agar gel Double immunodiffusion test (Ouchterlony test): This method is used to determine whether antigens are related, identical or non-identical or partial identical

Materials and Methods

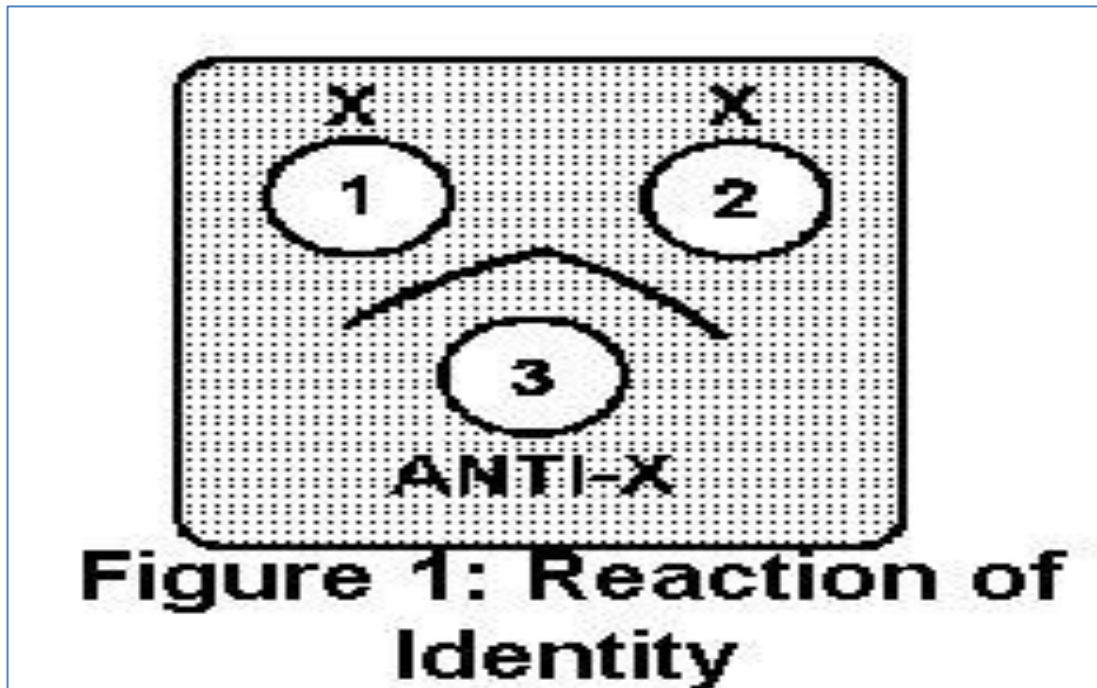
- 2% agar
- Antiserum
- Standard antigen
- PBS
- Flat level surface (Petri dish)
- Gel punch (Pasture pipette)
- Humid chamber (plastic box with wet filter paper)

Procedure:

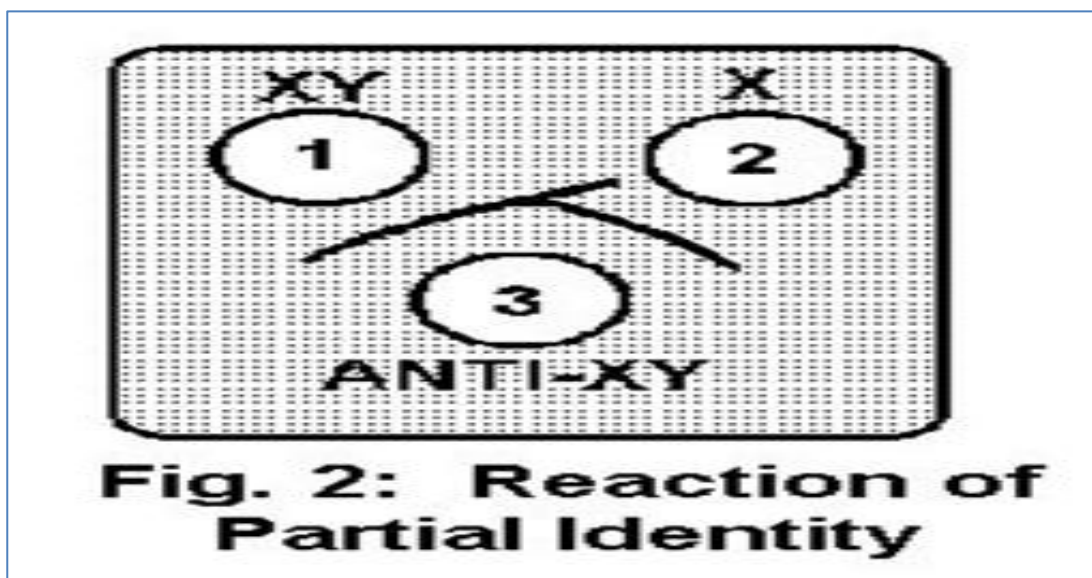
- Melting 2 g of agarose in 100 ml PBS using heater with magnetic stirrer.
- Sterilizing the agar by autoclave (121°C under 1.5 P in 30 minutes).
- Using water bath for cooling the agar until 65°C.
- Pour 3-5 ml of liquid agar in petri dish, let it until rigidity.
- Wells made in the agar by using pasture pipette.
- Put the standard antigen in the central well and the antiserum in the surrounded wells by using micropipette.
- Incubate the dish in incubator in 37°C with humidity for 24-48 hrs



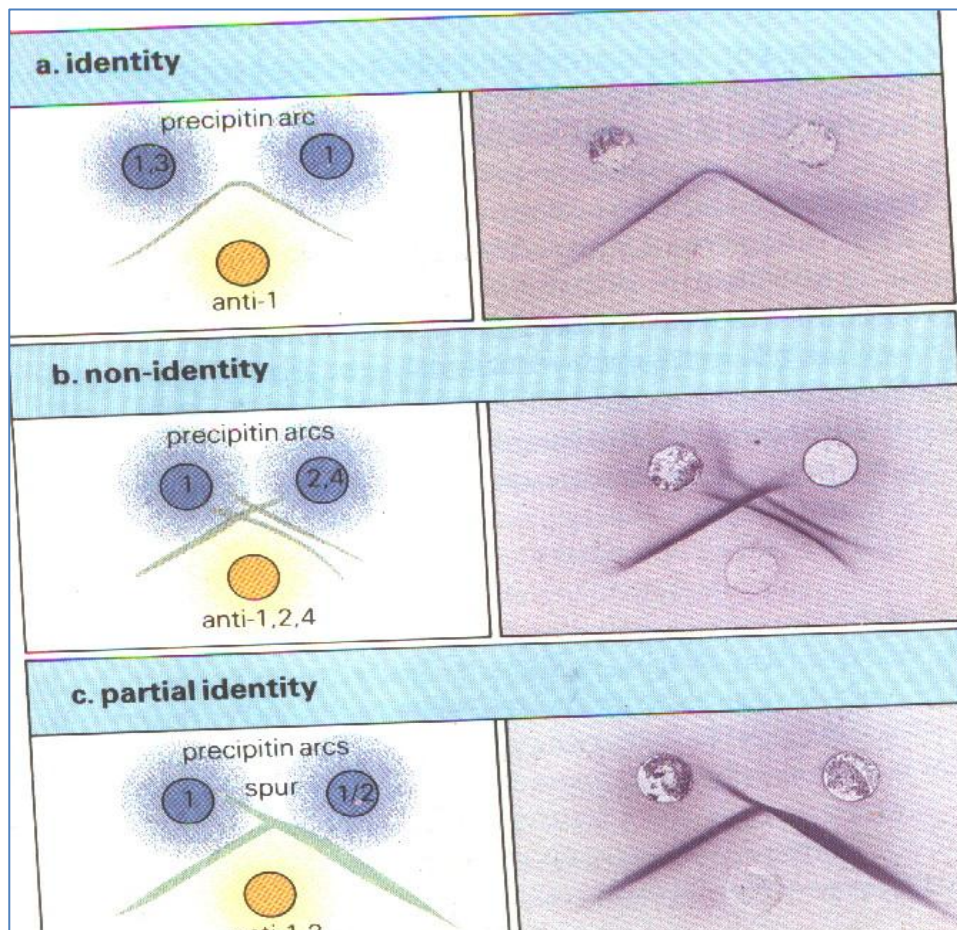
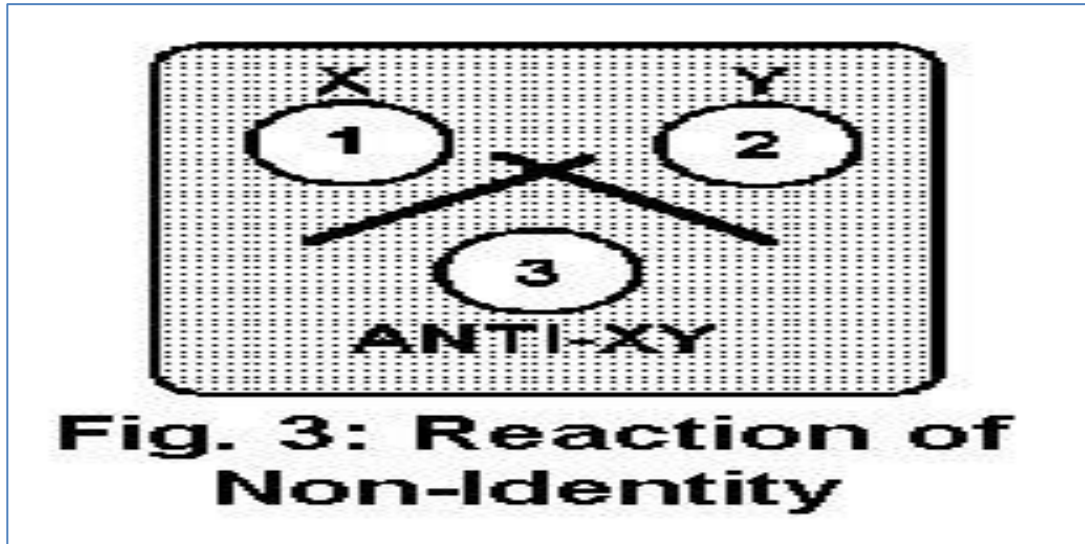
In Agar gel double immunodiffusion test The precipitation appears as a continuous line in the form of an angle between those two wells and the C well. There are no spurs at the angle and this type of reaction is termed a band of identity.



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

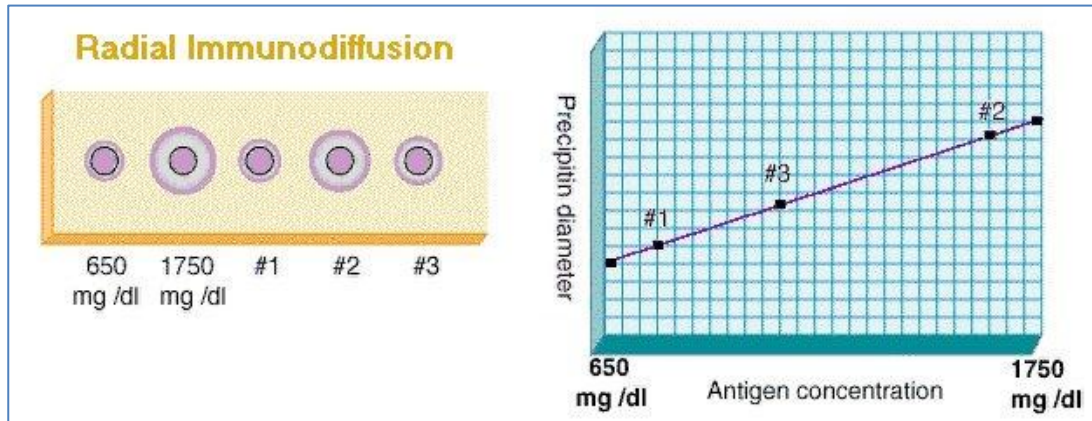


If the material in wells 1 and 2 do not possess common antigens and the antiserum in well 3 possesses specificities for both materials, the reaction will appear as two crossed lines as in Fig. 3



Agar gel single immunodiffusion test (Mancini)

- In radial immunodiffusion Antigen is incorporated into the agar gel
- different dilutions of the antibody are placed in holes punched into the agar.
- As the antibody diffuses into the gel it reacts with the antigen and when the equivalence point is reached a ring of precipitation is formed
- The diameter of the ring is proportional to the concentration of antibody since the amount of antigen is constant.



Immuno-electrophoresis

- 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 visible

