Immunodiffusion Techniques

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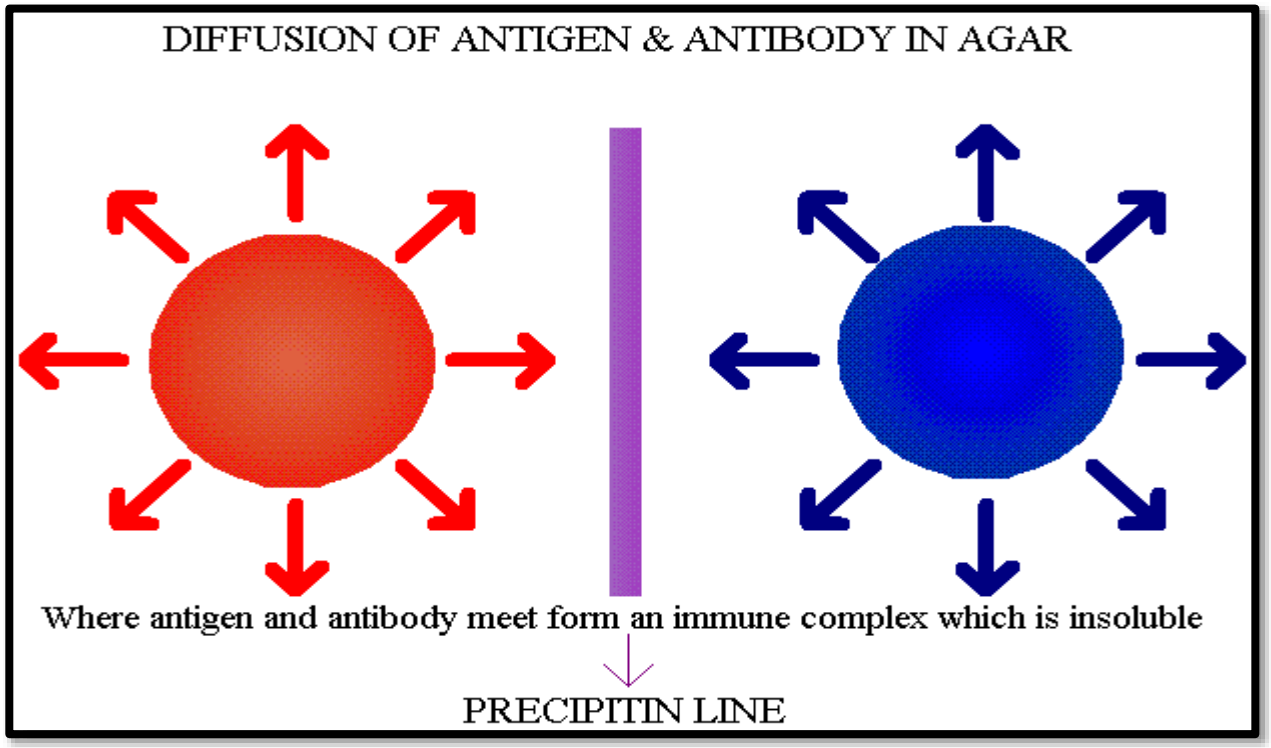
IMMUNODIFFUSION

A technique for studying reactions between antigens & antibodies by observing precipitates formed by the combination of specific antigens & antibodies diffused in gel in which they have been separately placed.



ADVANTAGES:

a)Precipitin band is visible which can be stained for preservation.

b)It can be used to detect identity, cross-reaction & non-identity b/w antigens in a mixture.

TYPES OF IMMUNODIFFUSION

They are classified on the basis of:

a) Number of reactants diffusing
b) Direction of diffusion

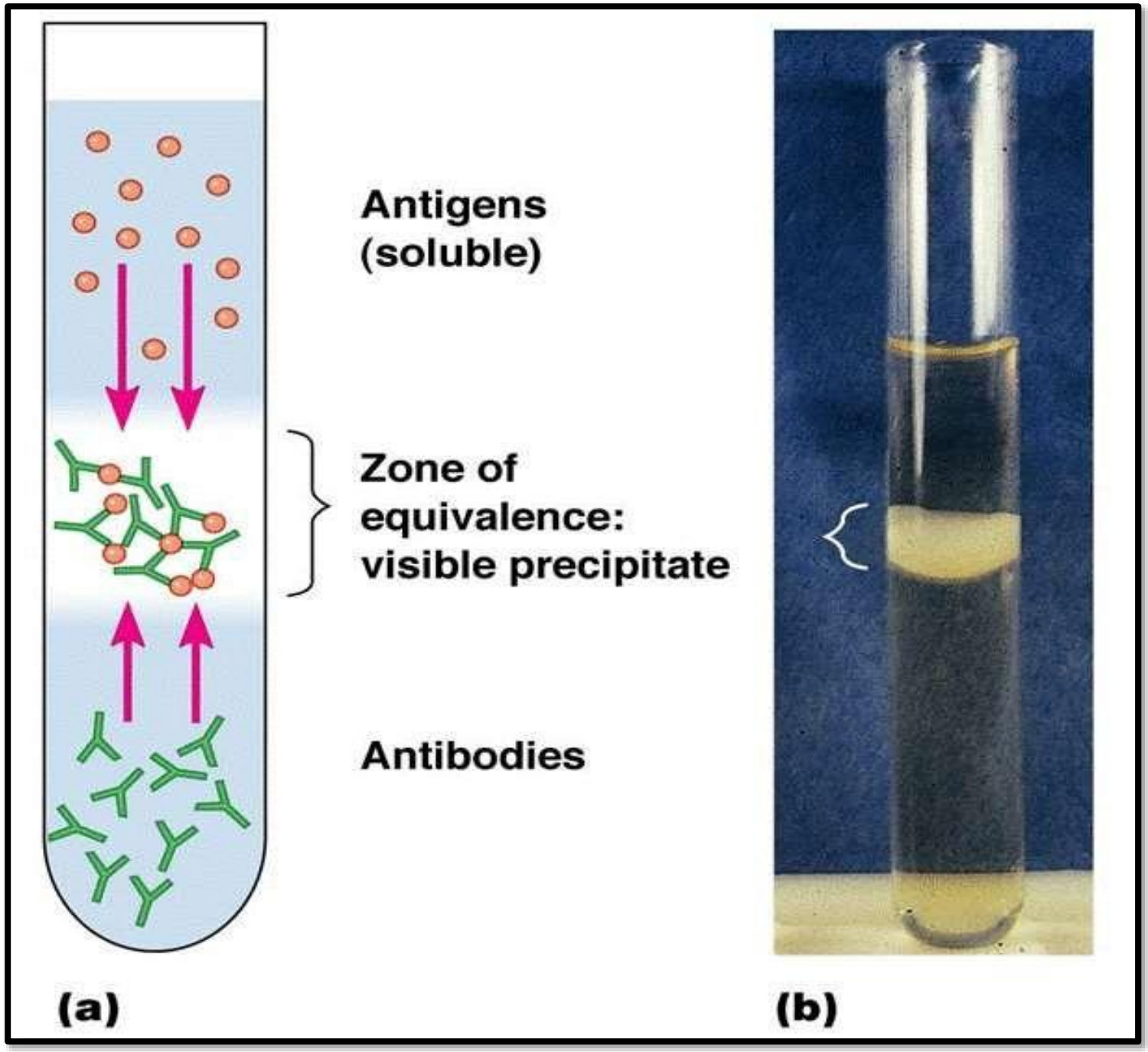
1. SINGLE DIFFUSION IN ONE DIRECTION

As the name suggests it is the single diffusion of antigen in agar in one direction.

This technique was pioneered by 'Oudin' who first time used gels for precipitation.

Procedure:

1. Ab is added in agar gel in test tube
2. Ag solution is poured over it
3. Ag diffuses downward towardsAb
4. Line of precipitation is formed
5. The number of bands shows number of Ag present in solution

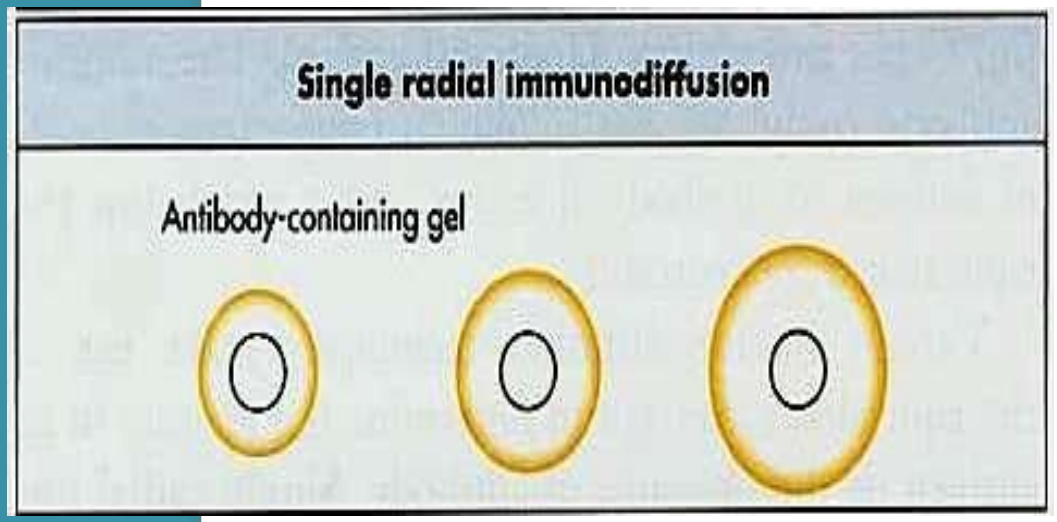

Antigens
(soluble)
Zone of
equivalence:
visible precipitate
Antibodies
(a)
(b)

2. SINGLE DIFFUSION IN TWO DIMENSIONS

Radial Immunodiffusion

Single diffusion in 2 dimensions is also called 'radial immunodiffusion'.

It is used in immunology to detect quantity of Ag by measuring the radius surrounding samples of the Ag, marking the boundary b/w it & A



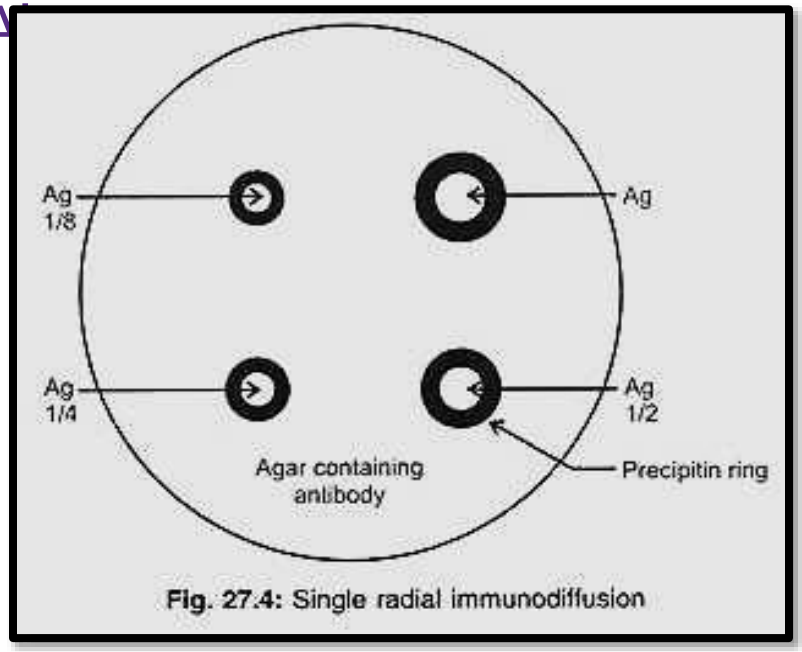
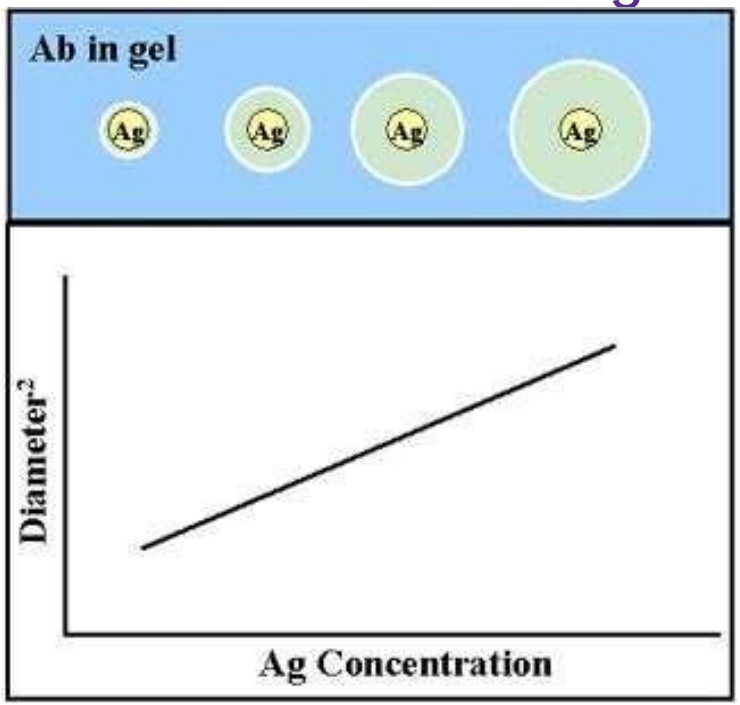
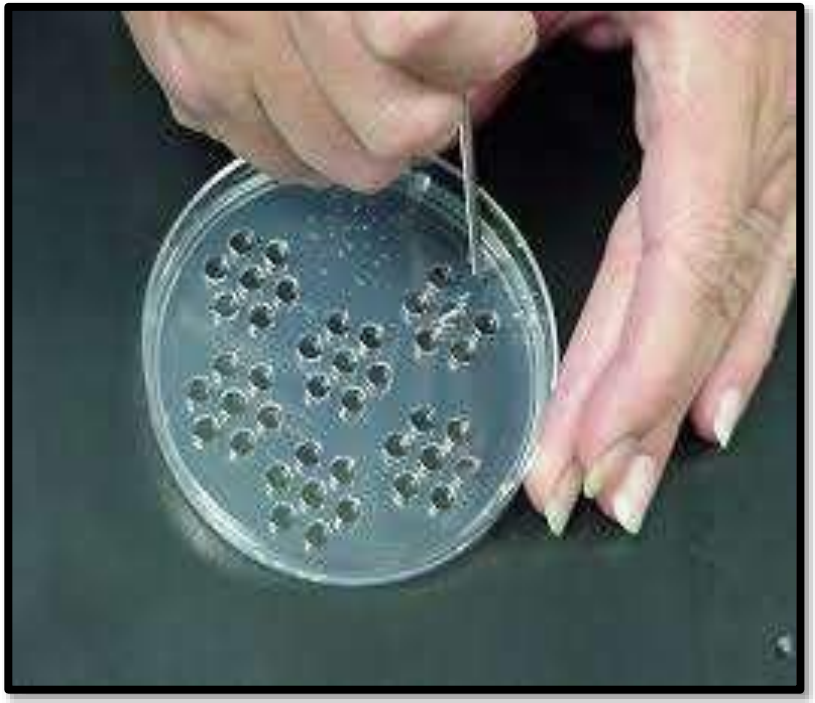


Fig. 27.4: Single radial immunodiffusion

Procedure:

1. Anti-sera sol containing Ab in agar sol is placed on a slide/petri dish.
2. Ag is added to the wells cut on the surface of the gel
3. Ab present in the gel reacts with Ag which diffuses radially from well & forms a ring shaped band of precipitation
4. Diameter of the ring is directly proportional to the concentration of the Ag



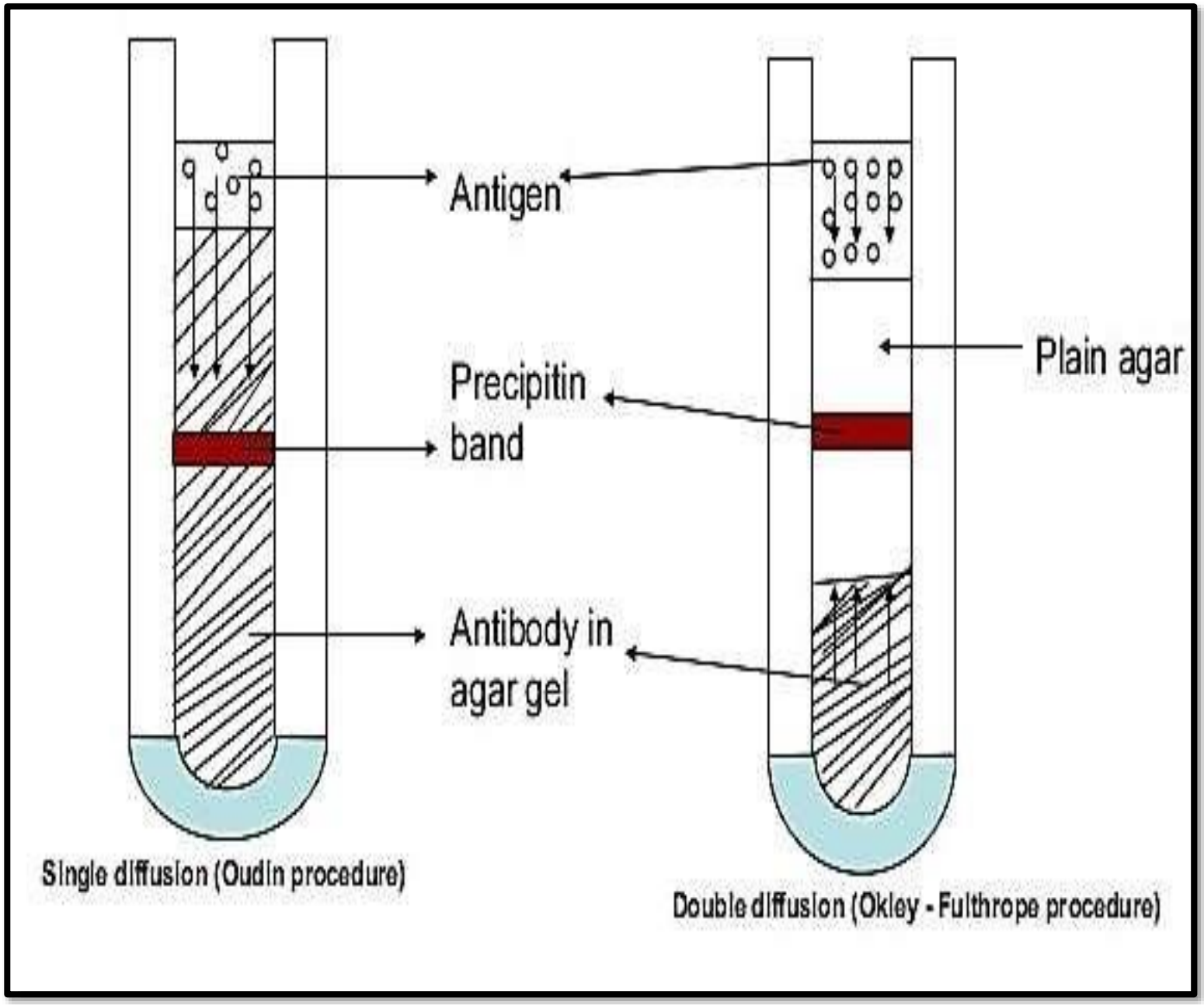


3. DOUBLE DIFFUSION IN ONE DIMENSION

This method is also called 'Oakley-Fulthrope' procedure & is performed in a test tube.

Procedure:

1. Ab's are incorporated in gel in test tube
2. Above which a layer of plain agar is placed.
3. Ag layer is poured on top of this plain
4. Ag's & Ab's moves towards each other through intervening layer of plain agar.
5. Ag & Ab reacts to form precipitin ring at optimum concentration.


Antigen
Precipitin band
Antibody in agar gel
Plain agar
Single diffusion (Oudin procedure)
Double diffusion (Okley - Fulthrope procedure)

4. DOUBLE DIFFUSION IN TWO DIMENSIONS

This method is also called 'Ouchterlony's procedure'. In this both Ag & Ab diffuse independently through agar gel in 2 dimensions i.e. horizontally & vertically (radially)

Procedure:

1. Wells are cut in agar gel poured in glass slide or petri dish
2. Anti-serum consisting of Ab's are poured in the central well
3. Different Ag's are added to it surrounding the central well
4. After incubation the lines of precipitin are formed at the sites of combination of Ag & Ab.

3 types of lines can be formed:

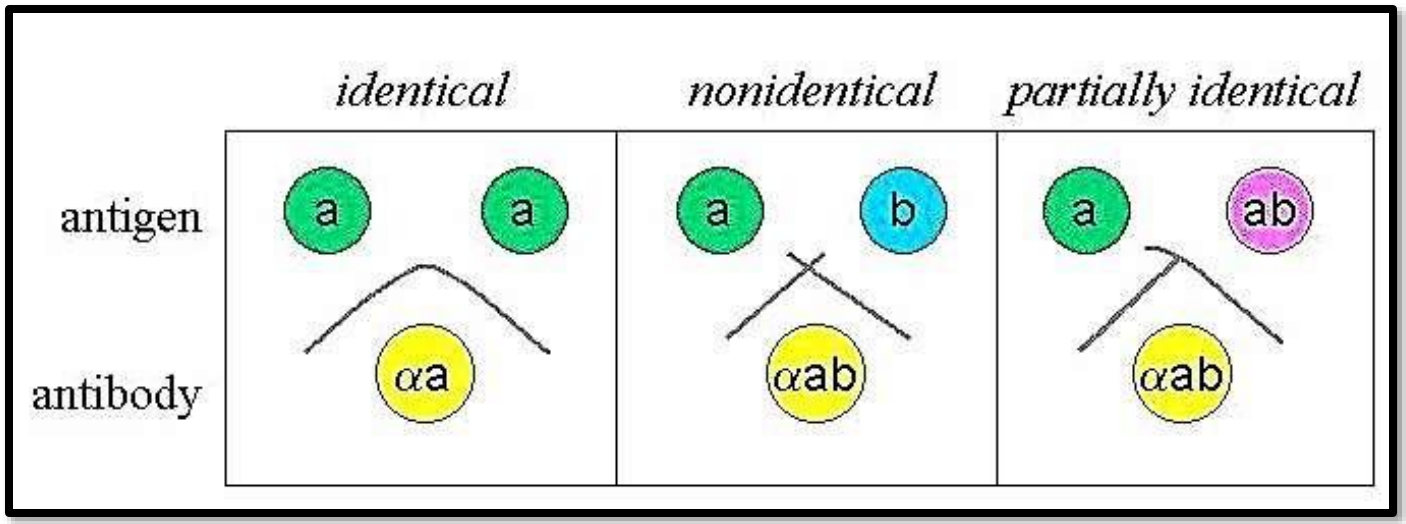
a) At junction forming an arc => presence of common epitope in Ag.

b) Crossed lines => no common epitope b/w compared Ag's

c) Fusion of lines with a spur => cross-reaction or partial identity

USES:

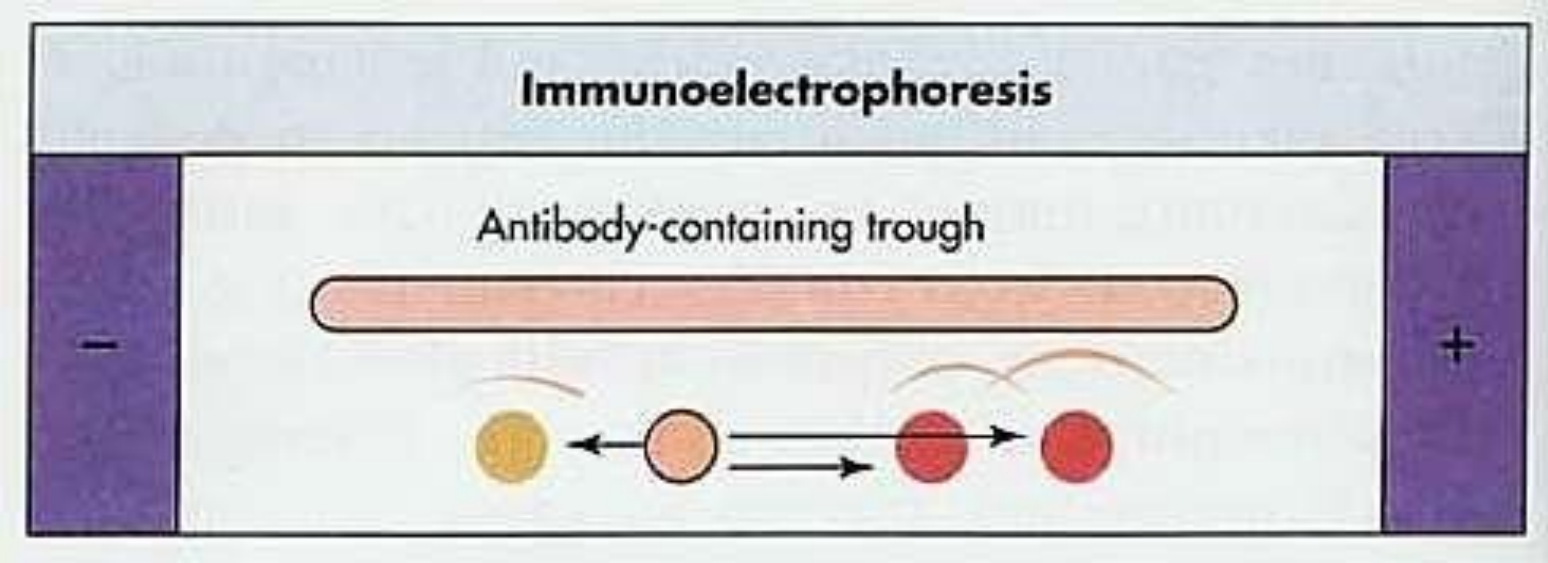
a) Demonstration of Ab's in diagnosis of small pox
b) Identification of fungal Ag's
c) Detection of Ab's to extract nuclear antigens.



IMMUNO-ELECTROPHORESIS

It is a method in which different antigens are separated according to their charge by the presence of electrical field.

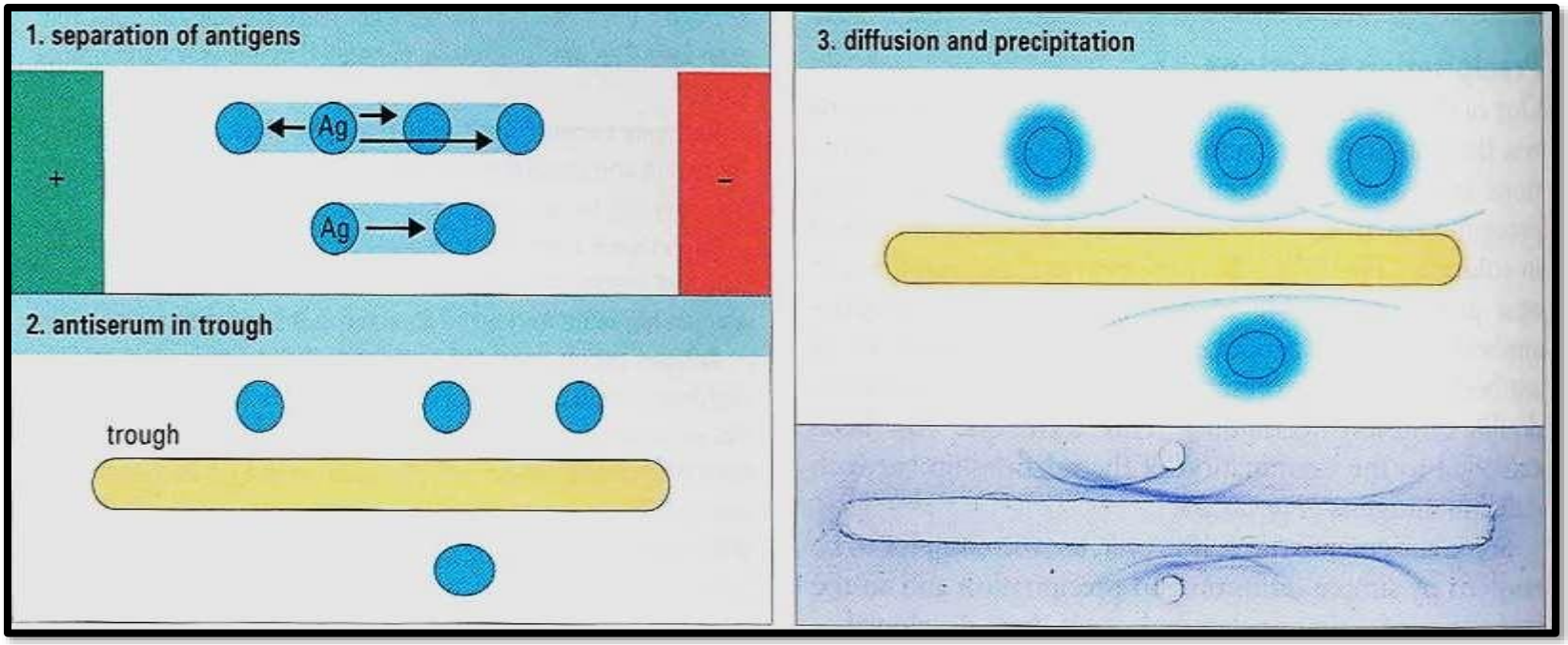
It is a process of combination of immunodiffusion & electrophoresis.



Procedure:

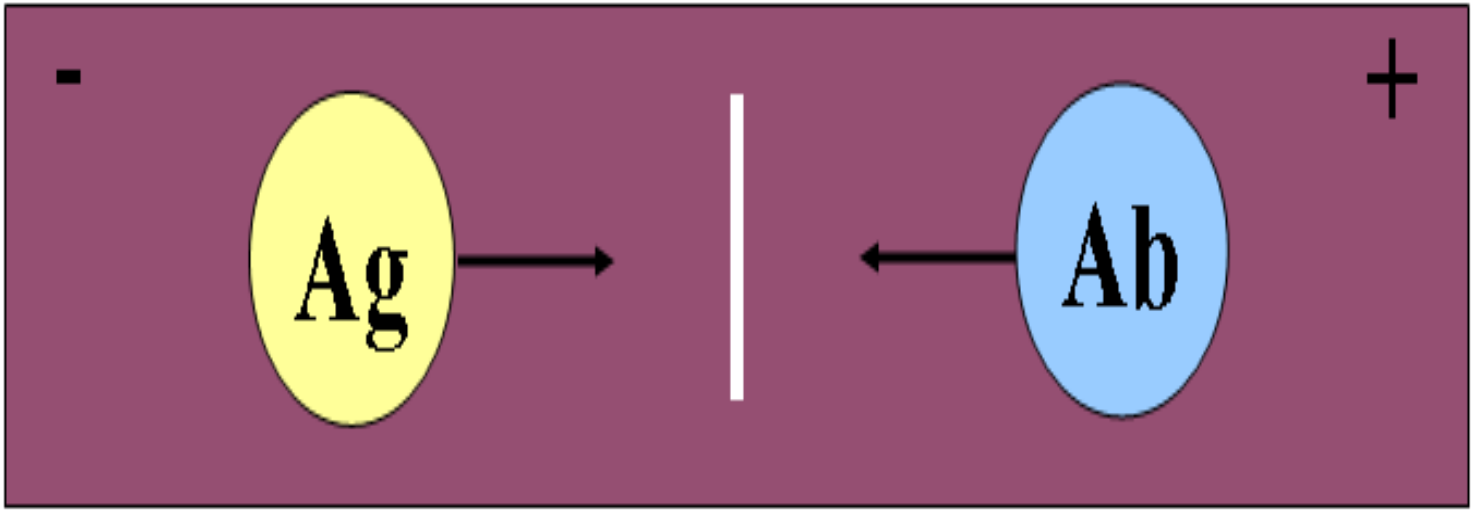
1. A drop of Ag is placed into a well in agar on glass slide.
2. Electric current is passed through agar
3. Ag move in the electric field according to their size & charge.
4. A trough is cut into agar & Ab is poured to it & diffusion is allowed to occur.
5. As the Ag & Ab diffuse they form series of lines

ADVANTAGE -> Number of Ag's can be identified in serum.



COUNTER-CURRENT IMMUNOELECROPHORESIS

It depends upon the movement of antigen towards the anode (positive) & antibody towards the cathode (negative) through agar in the electric field.



Procedure:

1.Performed on glass slide with agarose in which pair of wells are punched.

2. One is filled with Ag & the other with the Ab
3. Electric current is passed
4. Migration is visible in 30-60 mins.

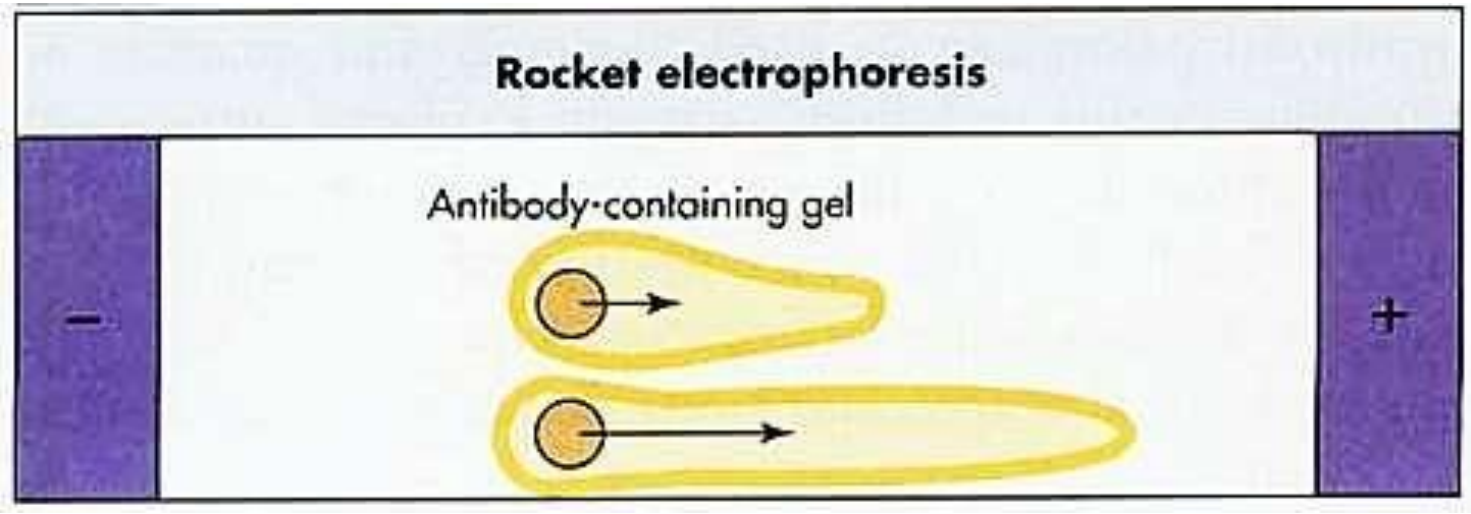
5.It is a rapid & highly specific method for the detection of Ag & Ab in serum, CSF, other body fluids for detection of diseases.

USE -> Commonly used for hepatitis-B surface antigen.

ROCKET ELECTROPHORESIS

It is an adaptation of radial immunodiffusion developed by 'Laurel'.

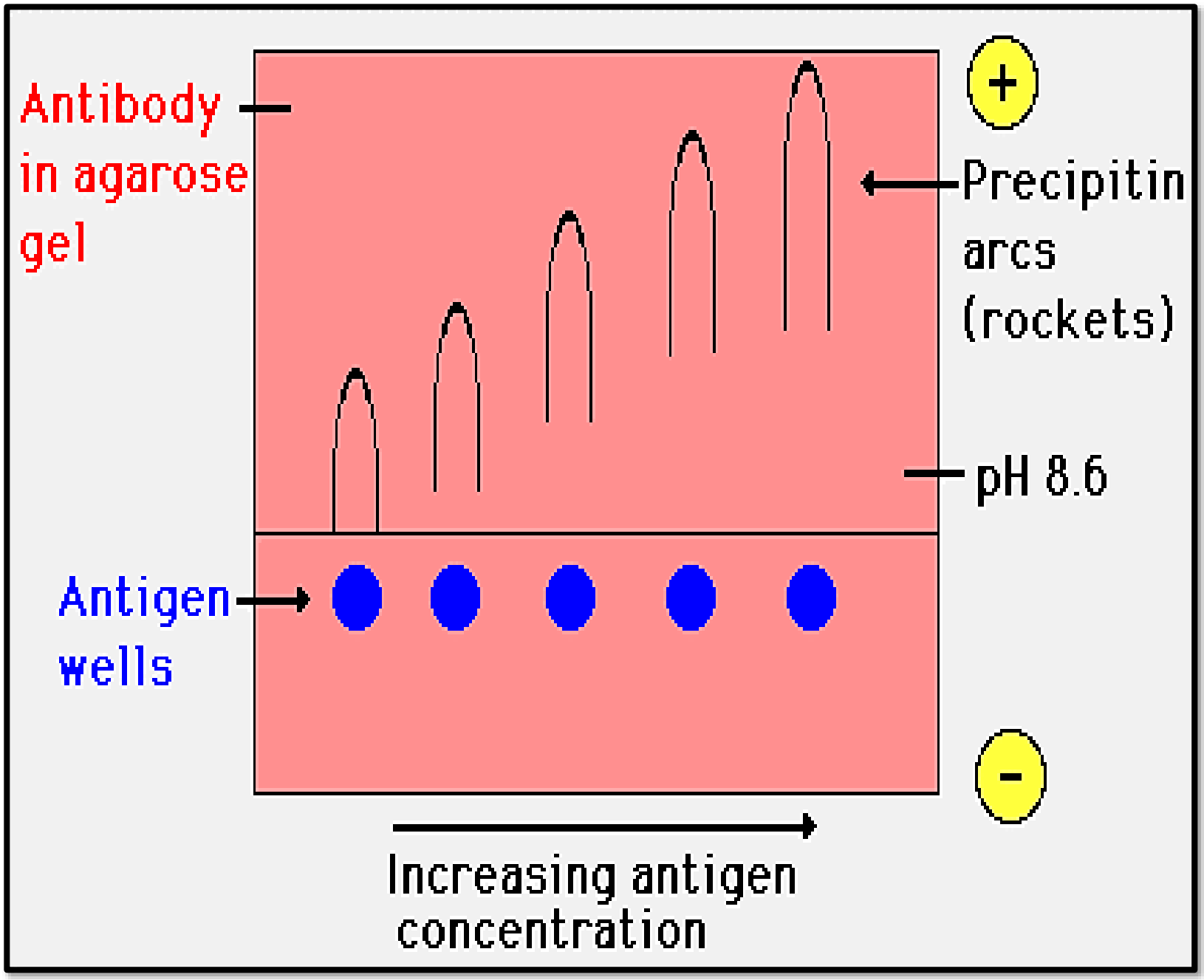
It is called so due to the appearance of precipitin bands in the shape of cone-like structures (rocket appearance) as the end result.



Procedure:

1.Ab is incorporated in the gel, Ag's are placed in wells cut in gel
2.Electric current is passed, which facilitated migration of Ag into agar
3.This results in formation of precipitin conical in shape, resembling rocket.
4.The height of rocket is directly proportional to concentration of antigen.

USE -> For quantitative estimation of antigen in serum


Antibody in agarose gel
+
Precipitin arcs (rockets)
pH 8.6
Antigen wells
-
Increasing antigen concentration

CONCLUSION

Thus we hereby conclude with the fact that antigen-antibody reactions are very important for serological testing of human beings, as they give you a complete picture of all the immune responses occurring the body & helps determining the immunological disorders by the antigen (either self or non-self).


THANK
YOU!