Immunoelectrophoresis

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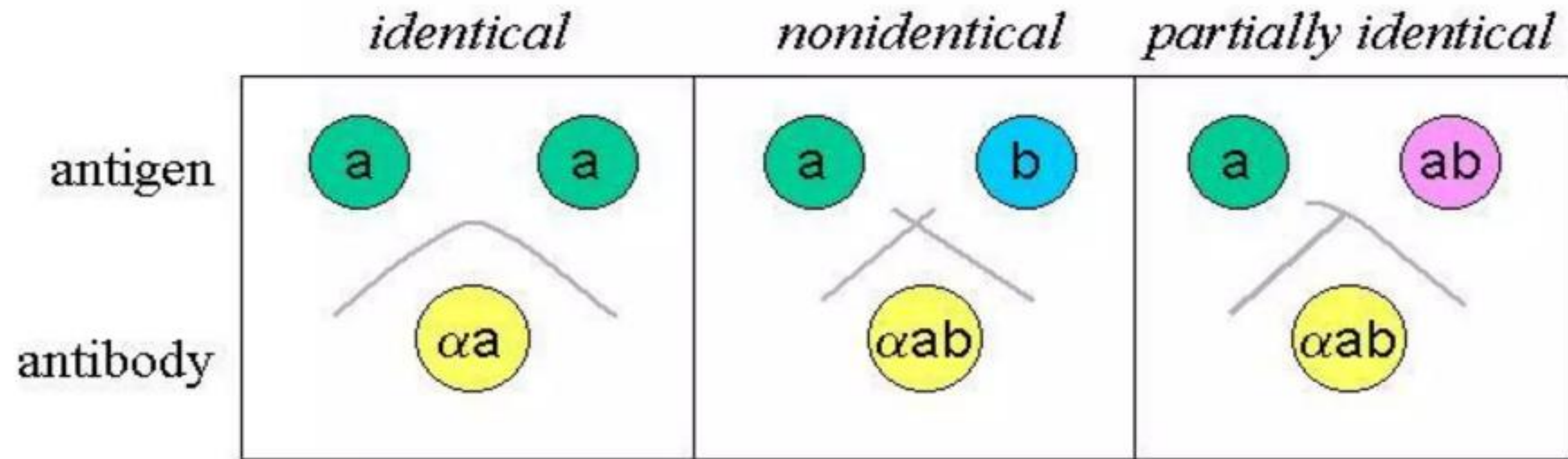
IMMUNOELECTROPHORESIS

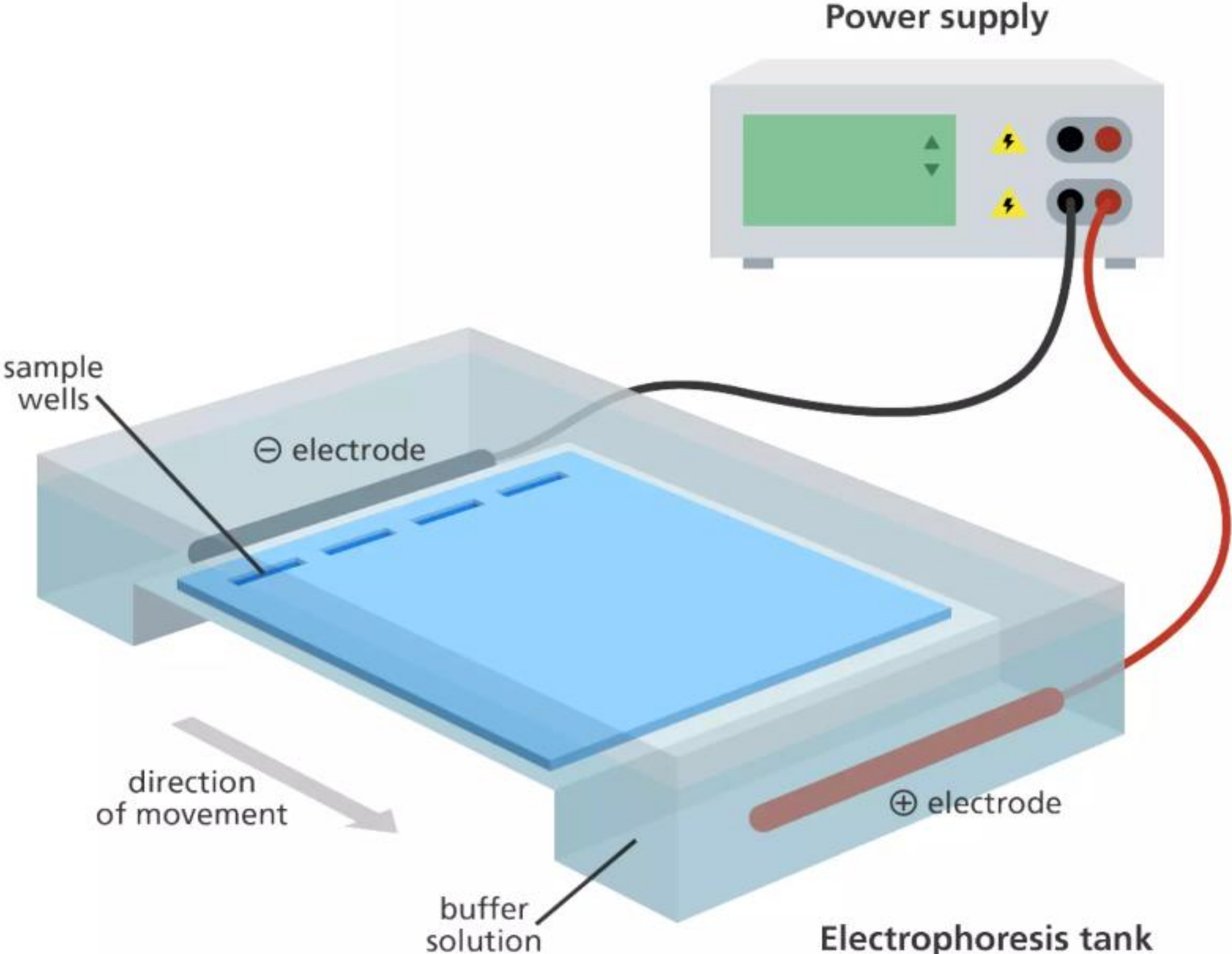
- Immunoelectrophoresis was first coined by **Grabar and Williams** in 1953.
- Immunoelectrophoresis is a general name for a number of biochemical methods for **separation and characterization of proteins based on electrophoresis and reaction with antibodies**.
- Immunoelectrophoresis is the combination of **Immunodiffusion** (Mancini's Single Radial Immunodiffusion and Ouchterlony Double Diffusion) and **Electrophoresis**.

Single Radial Immunodiffusion

Double Immunodiffusion

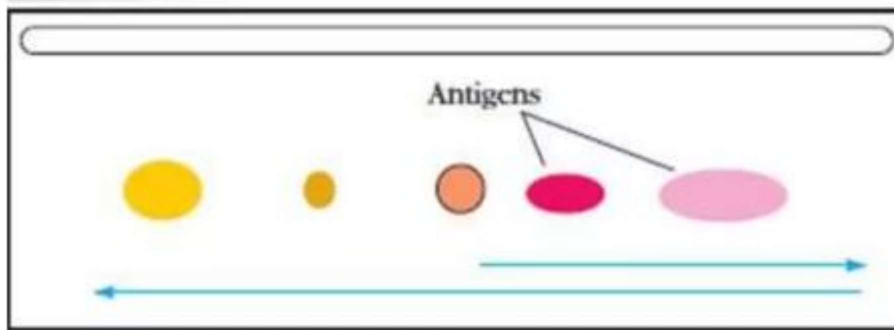


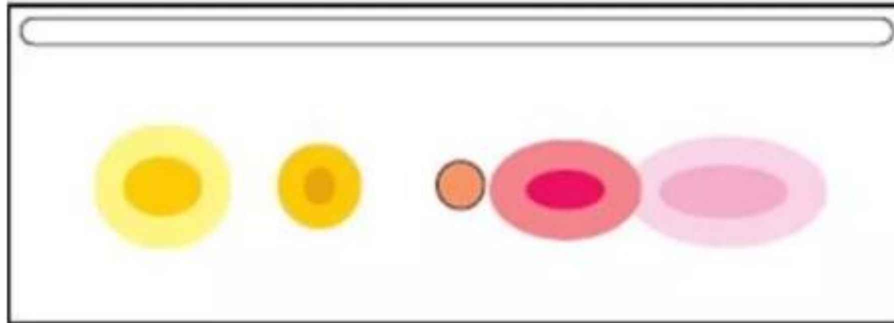
Electrophoresis

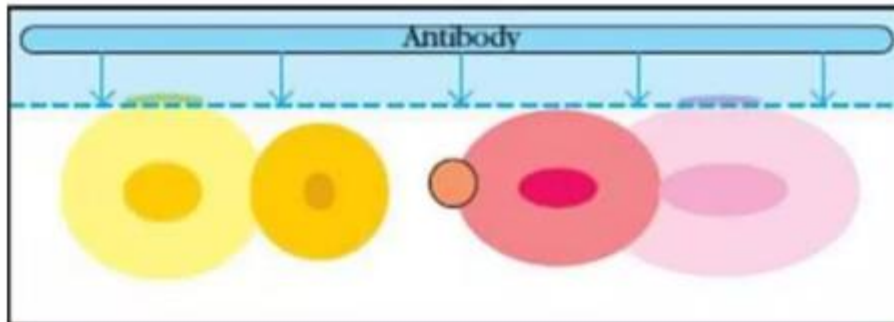


IMMUNOELECTROPHORESIS

- In Immunoelectrophoresis, the antigen mixture is first electrophoresed to separate its components by charge.
- Troughs are then cut into the agar gel parallel to the direction of the electric field, and antiserum is added to the troughs.
- Antibody and antigen then diffuse toward each other and produce lines of precipitation where they meet in appropriate proportions.
- Immunoelectrophoresis is a strictly Qualitative technique that only detects relatively high antibody concentrations (greater than 100 g/ml), it utility is limited to the detection of quantitative abnormalities only when the departure from normal is striking, as in immunodeficiency states.







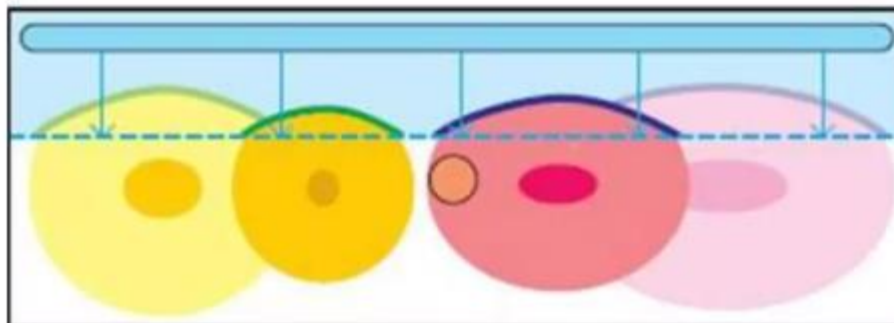


Figure:
Immunoelectrophoresis of an antigen mixture.

-An antigen preparation (orange) is first electrophoresed, which separates the component antigens on the basis of charge.

-Antiserum (blue) is then added to troughs on one or both sides of the separated antigens and allowed to diffuse.

-In time, lines of precipitation (colored arcs) form where specific antibody and antigen interact.

Procedure of Immunoelectrophoresis

1. Agarose gel is prepared on a glass slide put in a horizontal position.
2. Using the sample template, wells are borne on the application zone carefully.
3. The sample is diluted 2:3 with protein diluent solution (20µl antigen solution +10 µl diluent).
4. Using a 5 µl pipette, 5 µl of control and sample is applied across each corresponding slit (Control slit and Sample slit).
5. The gel is placed into the electrophoresis chamber with the samples on the cathodic side, and electrophoresis runs for 20 mins/ 100 volts.
6. After electrophoresis completes, 20 µl of the corresponding antiserum is added to troughs in a moist chamber and incubated for 18- 20 hours at room temperature in a horizontal position.
7. The agarose gel is placed on a horizontal position and dried with blotter sheets.
8. The gel in saline solution is soaked for 10 minutes and the drying and washing repeated twice again.
9. The gel is dried at a temperature less than 70°C and may be stained with protein staining solution for about 3 minutes followed by decolorizing the gel for 5 minutes in distaining solution baths.
10. The gel is dried and results evaluated.

Results of Immunoelectrophoresis

1. The presence of elliptical precipitin arcs represents antigen-antibody interaction.
2. The absence of the formation of precipitate suggests no reaction.
3. Different antigens (proteins) can be identified based on the intensity, shape, and position of the precipitation lines.

Applications of Immunoelectrophoresis

- Immunoelectrophoresis is used in clinical laboratories to detect the presence or absence of proteins in the serum.
- Detection of Immunodeficiency (In Immunodeficiency sample, no precipitin band is formed with particular antigen).
- Detection of Over production of Serum proteins (Albumin, Immunoglobulin and Transferrin).
- Detection of deficiency in Complement.
- Used to identify normal and abnormal proteins in Urine or Serum.
- Testing the purity of Antigen.