Immunoelectrophoresis

- Immunoelectrophoresis refers to precipitation in agar under an electric field.
- It is a process of combination of immuno-diffusion and electrophoresis.
- An antigen mixture is first separated into its component parts by electrophoresis and then tested by double immuno-diffusion.
- Antigens are placed into wells cut in a gel (without antibody) and electrophoresed.
- A trough is then cut in the gel into which antibodies are placed.
- The antibodies diffuse laterally to meet diffusing antigen, and lattice formation and precipitation occur permitting determination of the nature of the antigens.
- The term “immunoelectrophoresis” was first coined by Grabar and Williams in 1953.

Principle

- When electric current is applied to a slide layered with gel, antigen mixture placed in wells is separated into individual antigen components according to their charge and size.
- Following electrophoresis, the separated antigens are reacted with specific antisera placed in troughs parallel to the electrophoretic migration and diffusion is allowed to occur.
- Antiserum present in the trough moves toward the antigen components resulting in formation of separate precipitin lines in 18-24 hrs, each indicating reaction between individual proteins with its antibody.

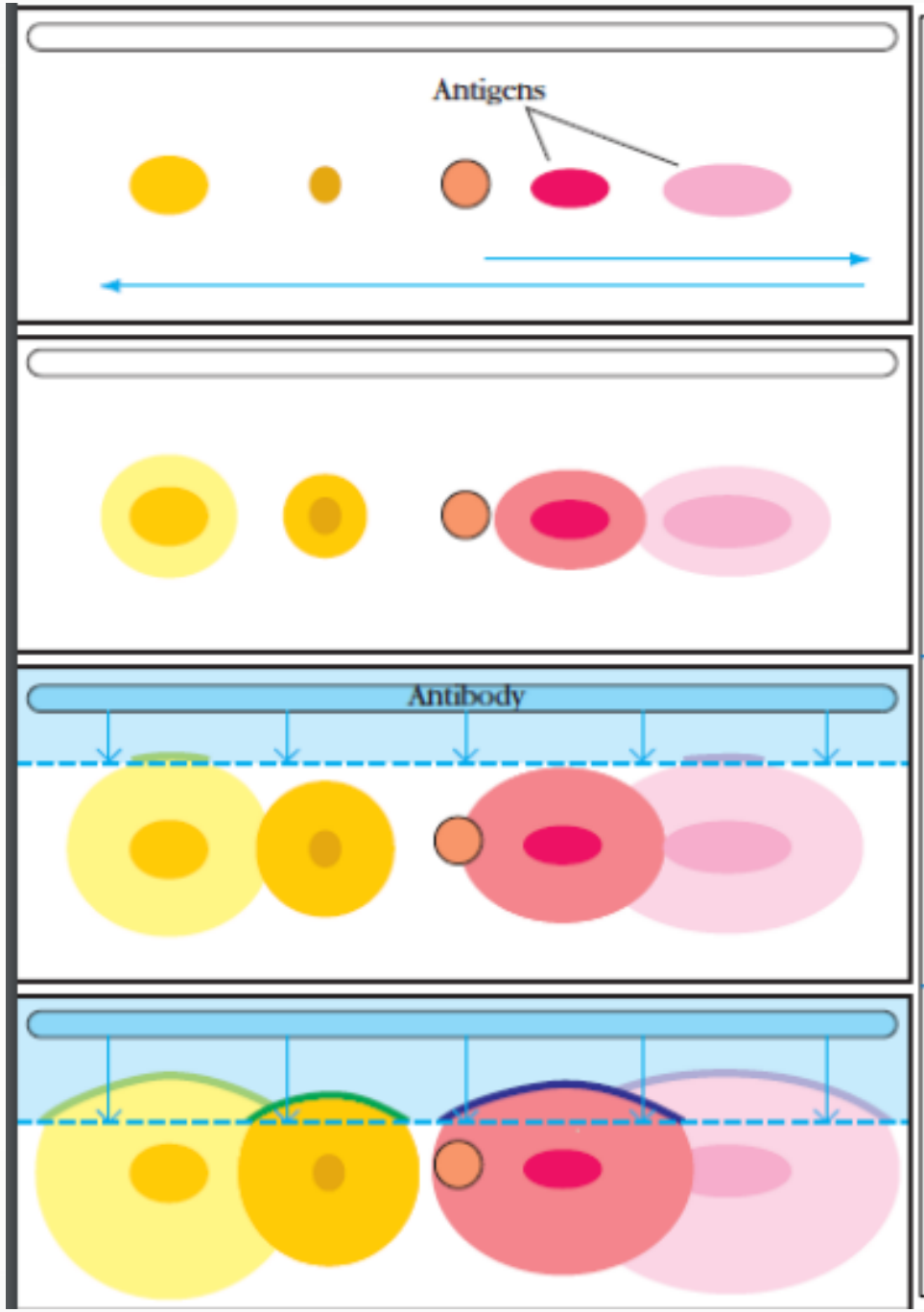

Figure:
Immunoelectrophoresis of an antigen mixture.

-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

- Agarose gel is prepared on a glass slide put in a horizontal position.
- Using sample template, wells are borne on the application zone carefully.
- The sample is diluted 2:3 with protein diluent solution (20µl antigen solution +10 µl diluent).
- Using a 5 µl pipette, 5 µl of control and sample is applied across each corresponding slit (Control slit and Sample slit).
- The gel is placed into the electrophoresis chamber with the samples on the cathode side, and electrophoresis run for 20 mins/ 100 volts

- After electrophoresis completes, 20 µl of the corresponding antiserum is added to troughs in moist chamber and incubated for 18- 20 hours at room temperature on a horizontal position.
- The agarose gel is placed on a horizontal position, and dried with blotter sheets.
- The gel in saline solution is soaked for 10 minutes and the drying and washing repeated twice again.
- 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.
- The gel is dried and results evaluated.

Results

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

Video

videoplayback.mp4

Applications of Immunoelectrophoresis

- The test helps in the identification and approximate quantization of various proteins present in the serum. Immunoelectrophoresis created a breakthrough in protein identification and in immunology.
- Immunoelectrophoresis is used in patients with suspected monoclonal and polyclonal gammopathies.
- The method is used to detect normal as well as abnormal proteins, such as myeloma proteins in human serum.
- Used to analyze complex protein mixtures containing different antigens.

- The medical diagnostic use is of value where certain proteins are suspected of being absent (e.g., hypogammaglobulinemia) or overproduced (e.g., multiple myeloma).
- This method is useful to monitor antigen and antigen-antibody purity and to identify a single antigen in a mixture of antigens.
- Immunoelectrophoresis is an older method for qualitative analysis of M-proteins in serum and urine.
- Immunoelectrophoresis aids in the diagnosis and evaluation of the therapeutic response in many disease states affecting the immune system.

Advantages of Immunoelectrophoresis

- Immunoelectrophoresis is a powerful analytical technique with high resolving power as it combines the separation of antigens by electrophoresis with immunodiffusion against an antiserum.
- The main advantage of immunoelectrophoresis is that a number of antigens can be identified in serum.

Limitations of Immunoelectrophoresis

- Immunoelectrophoresis is slower, less sensitive, and more difficult to interpret than Immunofixation electrophoresis.
- IEP fails to detect some small monoclonal M-proteins because the most rapidly migrating immunoglobulins present in the highest concentrations may obscure the presence of small M-proteins.
- The use of immunoelectrophoresis in food analysis is limited by the availability of specific antibodies.

References

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