IMMUNOELECTROPHORESIS TEST

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The term "immunoelectrophoresis" was first coined by Grabar and Williams in 1953. Immunoelectrophoresis refers to precipitation in agar under an electric field. It is a process of a 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 to meet diffusing antigens, and lattice formation and precipitation occur permitting determination of the nature of the antigens.

Principle:

When an electric current is applied to a slide layered with gel, the 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 the formation of separate precipitin lines in 18-24 hrs, each indicating reaction between individual proteins with its antibody.

Materials required:

Agarose gel, slides, well cutters, Immunoelectrophoresis machine, buffer, etc.

Procedure:

1. Agarose gel is prepared on a glass slide put in a horizontal position.
2. After well cutting, sample is added in well.
3. The gel is placed into the electrophoresis chamber with the samples on the cathodic side, and electrophoresis runs for 20 mins/ 100 volts.
4. After electrophoresis completes, the corresponding antiserum is added to troughs in a moist chamber and incubated for 18- 20 hours at room temperature in a horizontal position.

5. Washing and drying of the gel is done.
6. Then the gel may be stained and dried.

Result:

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:

1. 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.

2. This method is useful to monitor antigen and antigen-antibody purity and to identify a single antigen in a mixture of antigens.

Advantages of Technique:

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