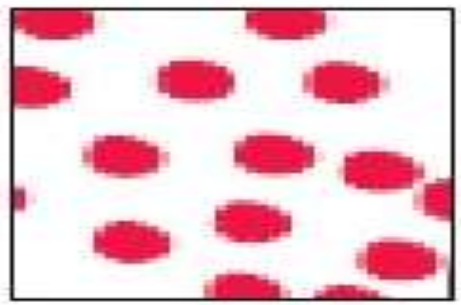
Immunoelectrophoresis

Separation of Immunogenic molecules

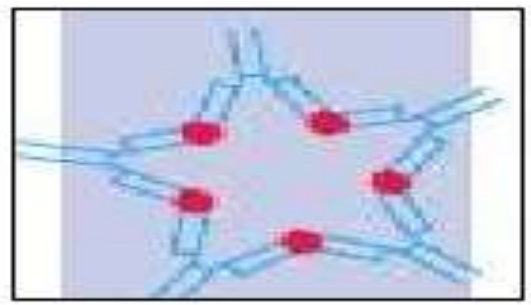
Precipitation Reaction:

When a soluble Ag combines with its Ab in the presence of an electrolyte (NaCl) at a particular temperature and pH, it forms an insoluble precipitate of Ag-Ab complex. The Ab causing precipitation is called Precipitin and the reaction is called as precipitation reaction.

Antibodies



Antigens



Ag-Ab complex

Immunoelectrophoresis

- The term "immunoelectrophoresis" was first coined by Grabar and Williams in 1953
- This refers to precipitation of antigen antibody complex 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
- A trough is then cut in the gel into which antibodies are placed
- The antibodies diffuse laterally to meet diffusing antigens, and lattice formation and precipitation occur permitting determination of the nature of the antigens

Principle of Immunoelectrophoresis

- 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
- 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 antigens with its antibody

Procedure of Immunoelectrophoresis

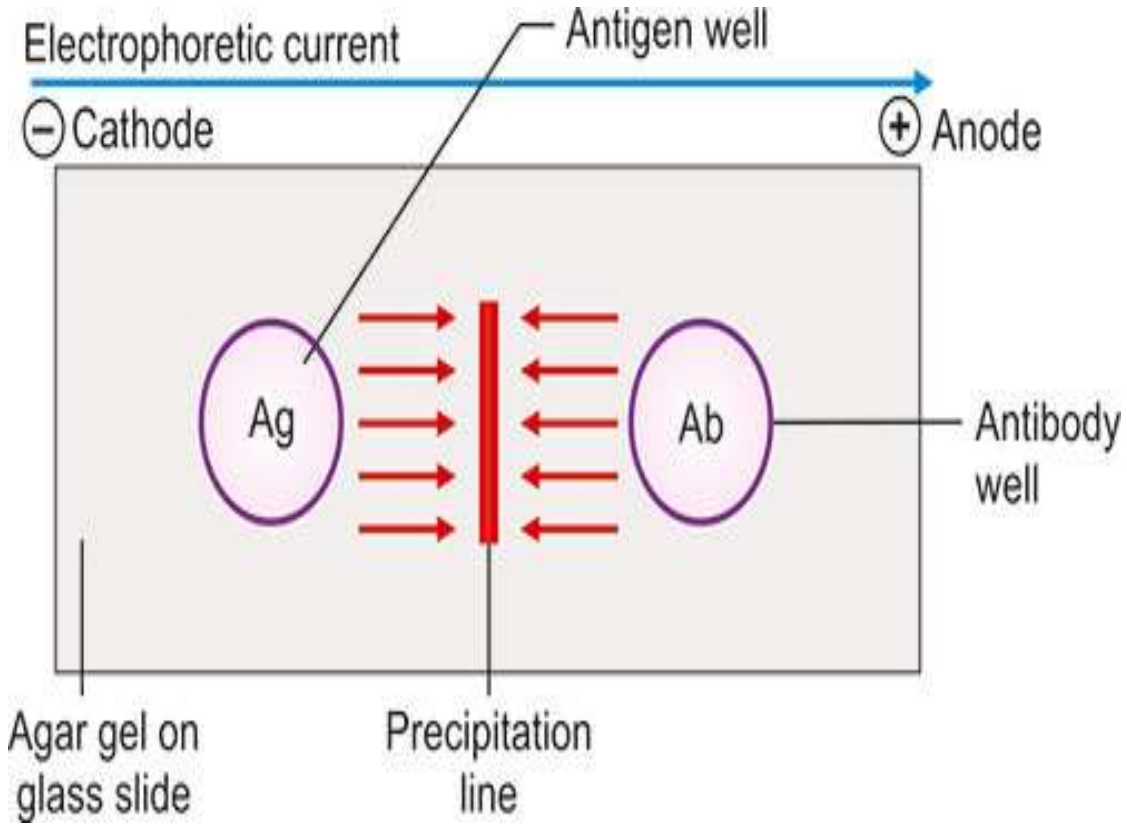
1. Agarose gel is prepared on a glass slide using the sample template, wells are borne on the application zone carefully
2. 5µl of control and sample is applied across each corresponding slit
3. The gel is placed into the electrophoresis chamber with the samples on the cathodic side, and electrophoresis runs for 20 mins/ 100 volts
4. After electrophoresis completes, 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
5. The gel is dried and results evaluated

Results of Immunoelectrophoresis

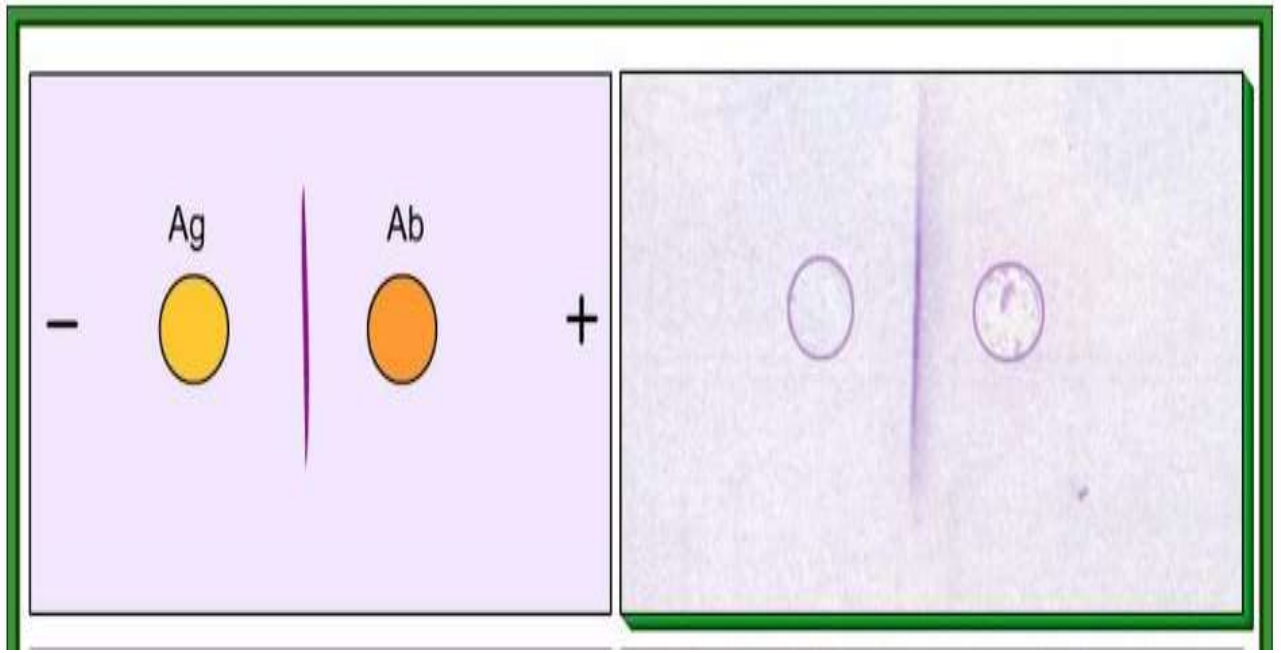
1. The presence of elliptical precipitin arcs represents antigen-antibody interaction.
2. The absence of the formation of precipitate suggests no reaction.
3. Different antigens (proteins) can be identified based on the intensity, shape, and position of the precipitation lines.

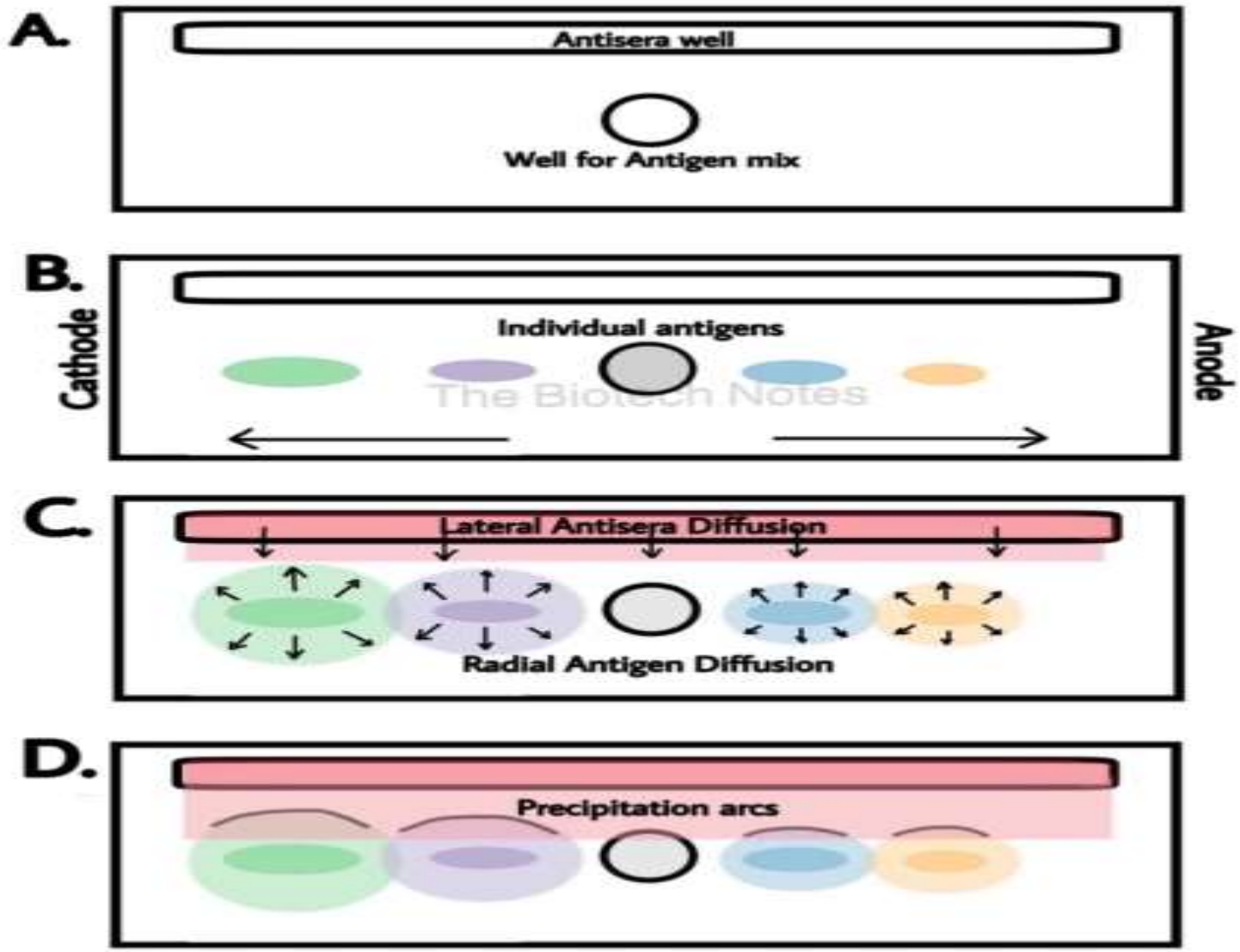
Immunoelectrophoresis instrument

Separation



Result




A.
Antisera well
Well for Antigen mix
B.
Cathode
Individual antigens
Anode
The Biotech Notes
C.
Lateral Antisera Diffusion
Radial Antigen Diffusion
D.
Precipitation arcs

Applications of Immunoelectrophoresis

- Quantization of various proteins present in the serum
- Diagnosis and evaluation of the therapeutic response in many immune disease
- Monitor antigen and antigen-antibody purity and to identify a single antigen in a mixture of antigens
- 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)
- The method is used to detect normal as well as abnormal proteins, such as myeloma proteins in human serum

Advantages of Immunoelectrophoresis

1. Immunoelectrophoresis is a analytical technique 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

Limitations of Immunoelectrophoresis

1. Immunoelectrophoresis is slower, less sensitive, and more difficult to interpret
2. IEP fails to detect some small monoclonal M-proteins
3. The use of immunoelectrophoresis in food analysis is limited by the availability of specific antibodies