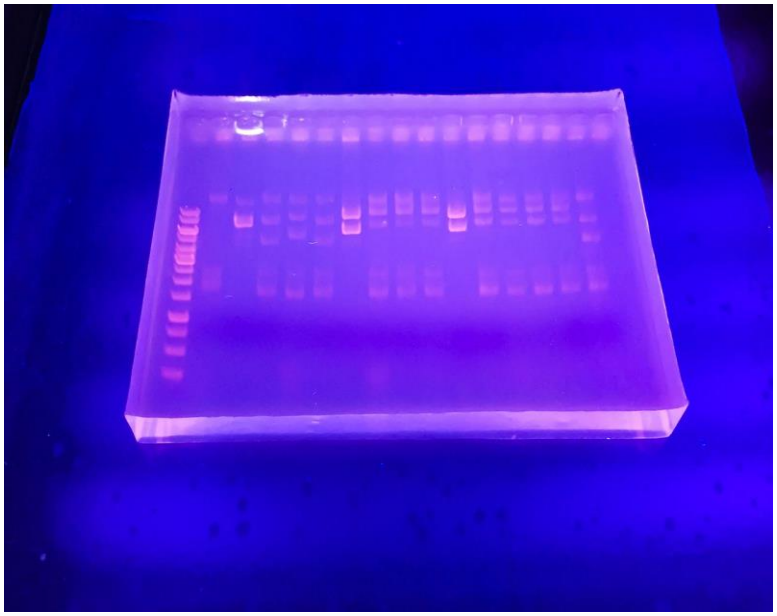
Electrophoresis



Dr. Kalsoom Tariq

Learning Objectives:

- What is electrophoresis?
- Purpose/Uses of electrophoresis
- Factors affecting the electrophoretic mobility
- Description of the technique
- Types of electrophoresis
- Paper electrophoresis
- Gel electrophoresis
- Iso electric focusing
- Immuno electrophoresis

ELECTROPHORESIS

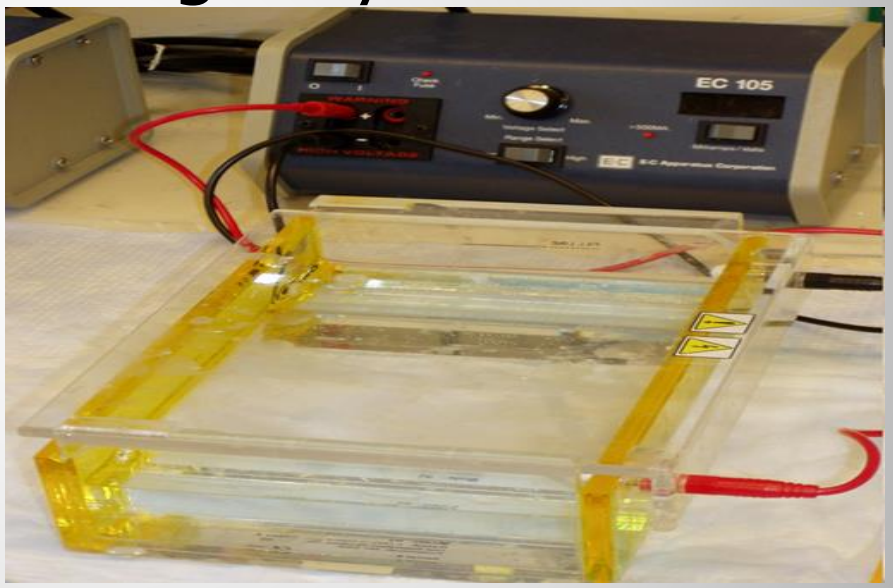
- **Definition**: It describes migration of charged particles or molecules under the influence of electric field.
- **Purpose for carrying out electrophoresis**
- 1. To determine the number, amount and mobility of components in given sample or to separate them.
- 2. Determination of molecular weight of proteins and DNA sequencing.
- 3. To obtain information about the electrical double layers surrounding the particles.
- **Factors affectng electrophoretic mobility**

1. Charge
2. Size
3. Shape

Description of technique:

The electrophoresis unit

- 1. Horizontal /vertical gel system
- 2. Power supply:
- 3. Current
- 4. voltage



Principle:

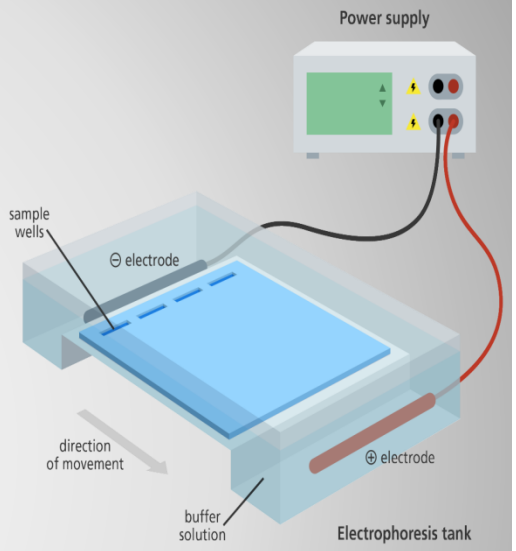
- Positive ions move towards cathode
  Negative ions move towards anode

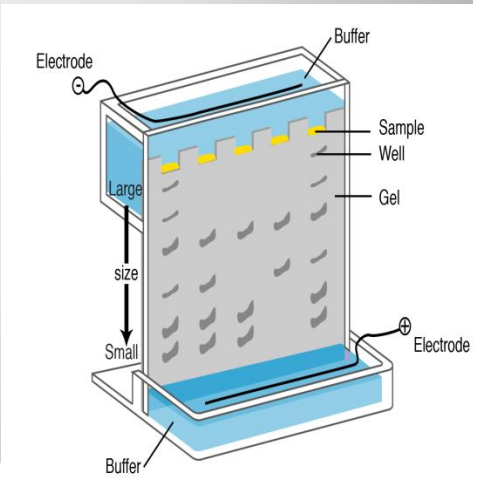
TYPES OF ELECTOPHORESIS

1. Zone electrophoresis
   a) Paper electrophoresis
   b) Gel electrophoresis

2. Isoelectric focusing

3. Immunoelectrophoresis





- **PAPER ELECTROPHORESIS :**

It is the form of electrophoresis that is carried out on filter paper. This technique is useful for separation of small charged molecules such as **amino acids** and **small proteins**. The serum proteins are separated into 5 distinct bands- albumin, α1-, α2-, β- and γ-globulins.

- **GEL ELECTROPHORESIS :**

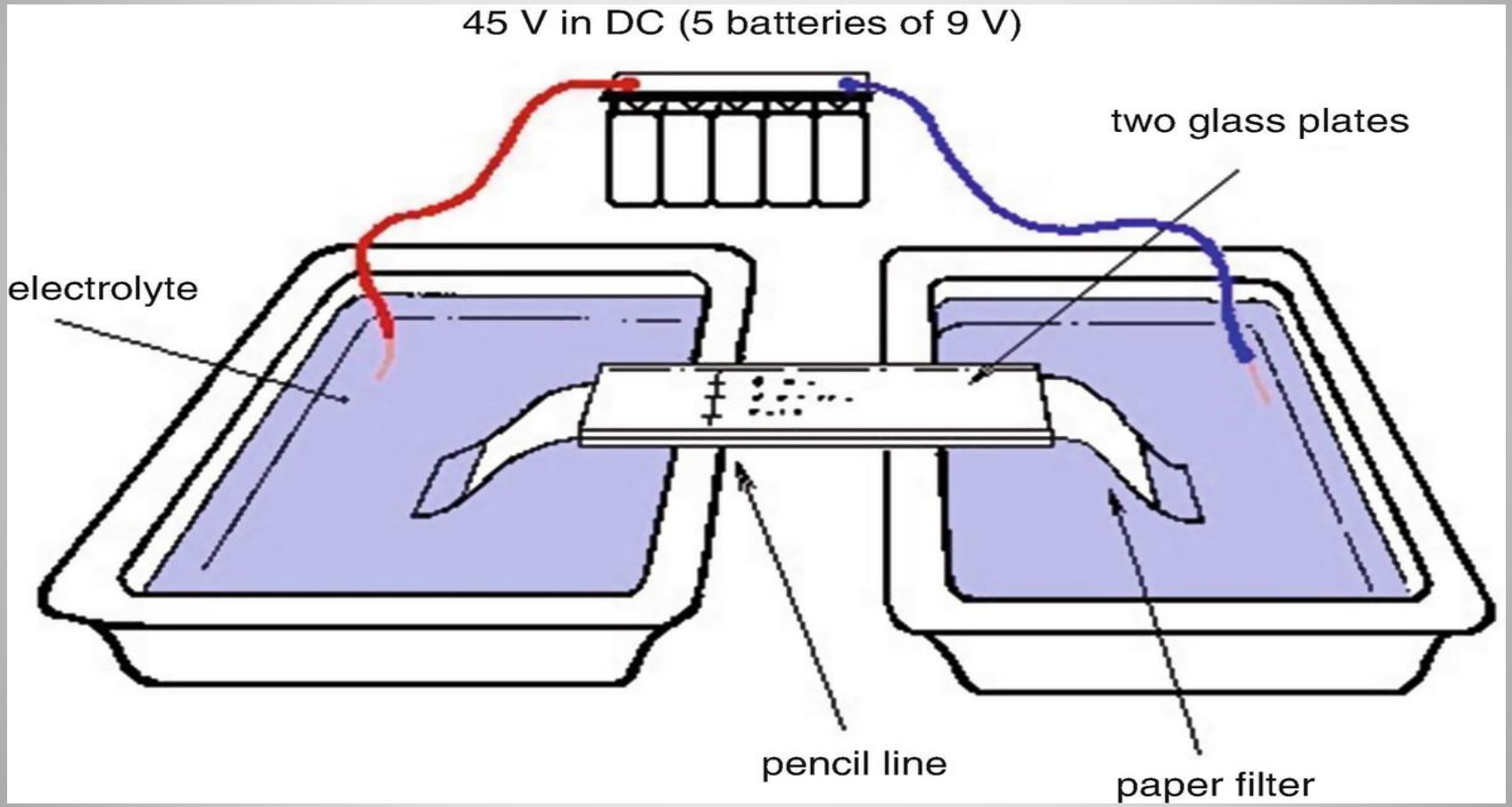
It is a technique used for the separation of **DNA, RNA**, or **protein molecules** according to their size and charge using an electric current applied to the gel matrix.

The serum proteins can be separated to about 15 distinct bands.

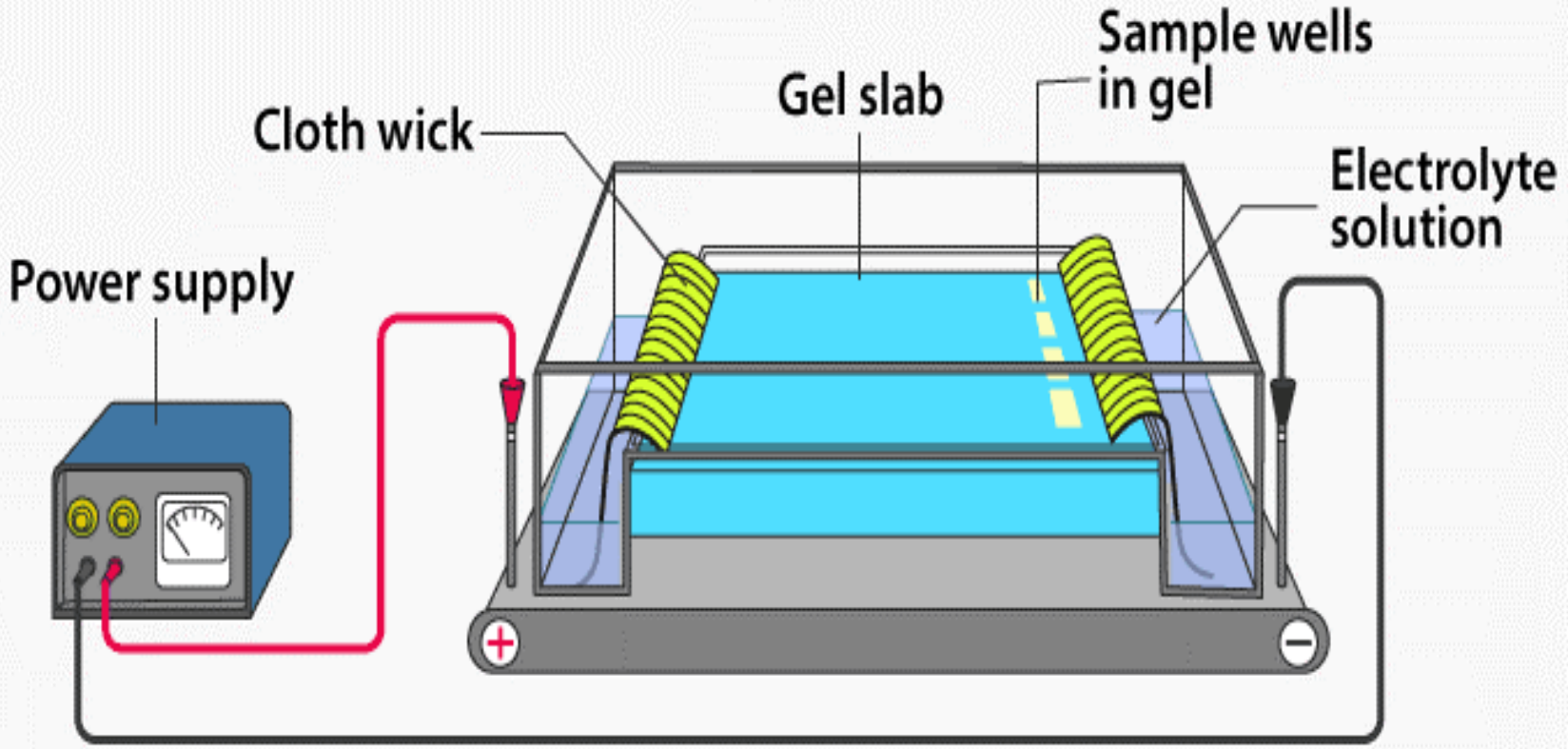
Types of the gel:

- 1. Agarose gel
- 2. Polyacrylamide gel
- 3. Sodium dodecyl sulphate (SDS)

Paper electrophoresis



GEL ELETROPHORESIS

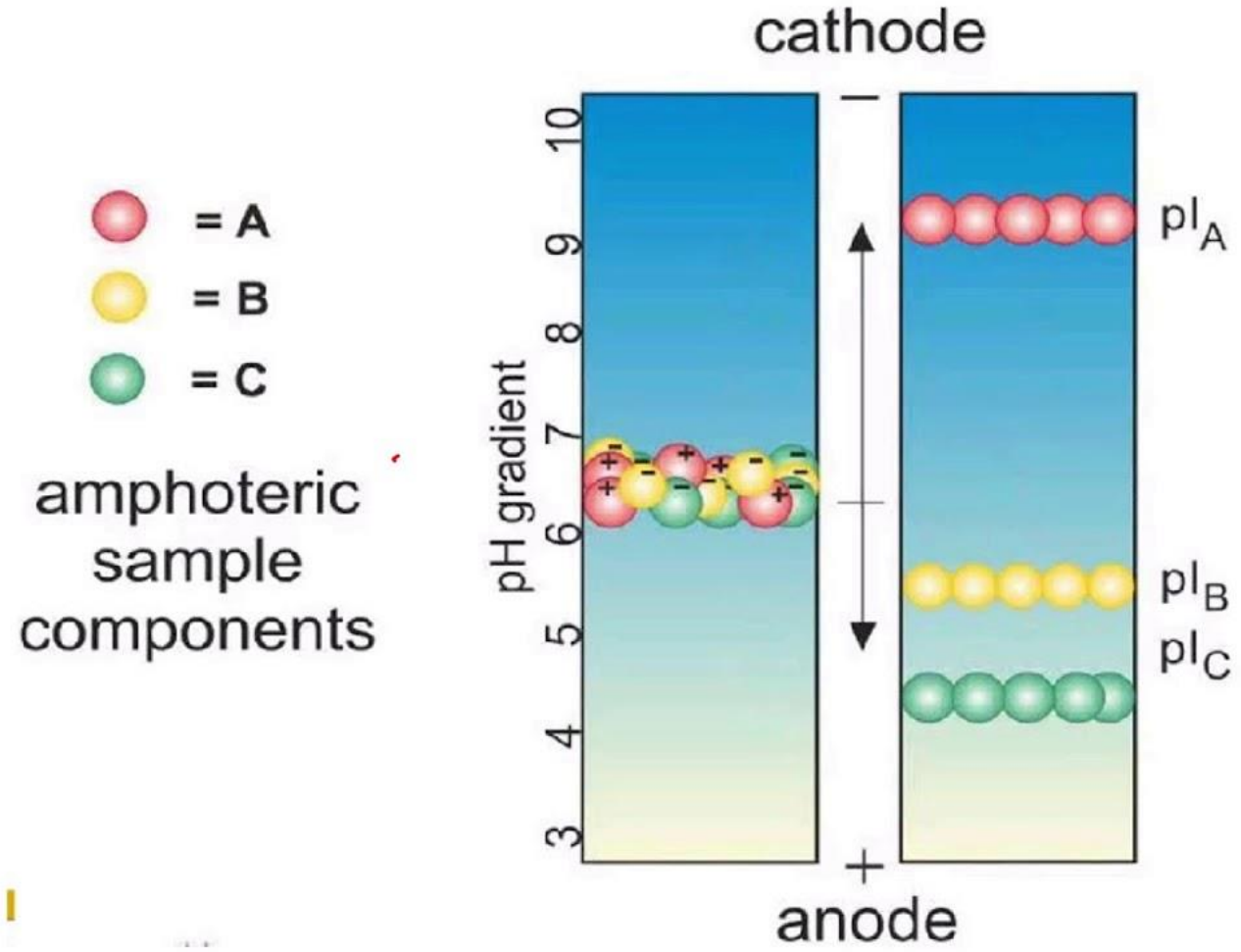


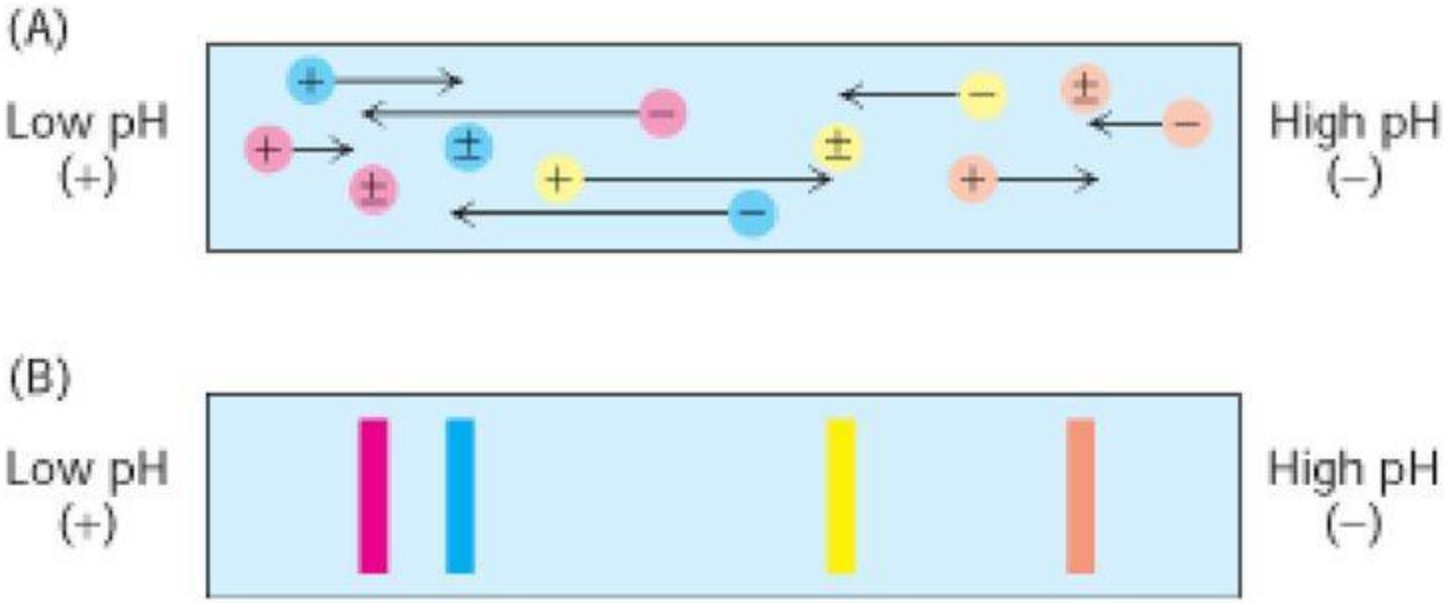
- Polyacrylamide is employed for the determination of molecular weights of proteins in a popularly known electrophoresis technique known as SDS-PAGE.

ISOELECTRIC FOCUSING

- This technique is based on the immobilization of the molecules at isoelectric pH during electrophoresis.
- It is ideal for separation of amphoteric substances.
- It's gels contain synthetic buffers called ampholytes that smooth the pH gradients.
- The serum proteins can be separated to as many as 40 bands.

ISOELECTRIC FOCUSING





The Principle of Isoelectric Focusing. A pH gradient is established in a gel before loading the sample. (A) The sample is loaded and voltage is applied. The proteins will migrate to their isoelectric pH, the location at which they have no net charge. (B) The proteins form bands that can be excised and used for further experimentation.

- **IMMUNOELECTROPHORESIS :**

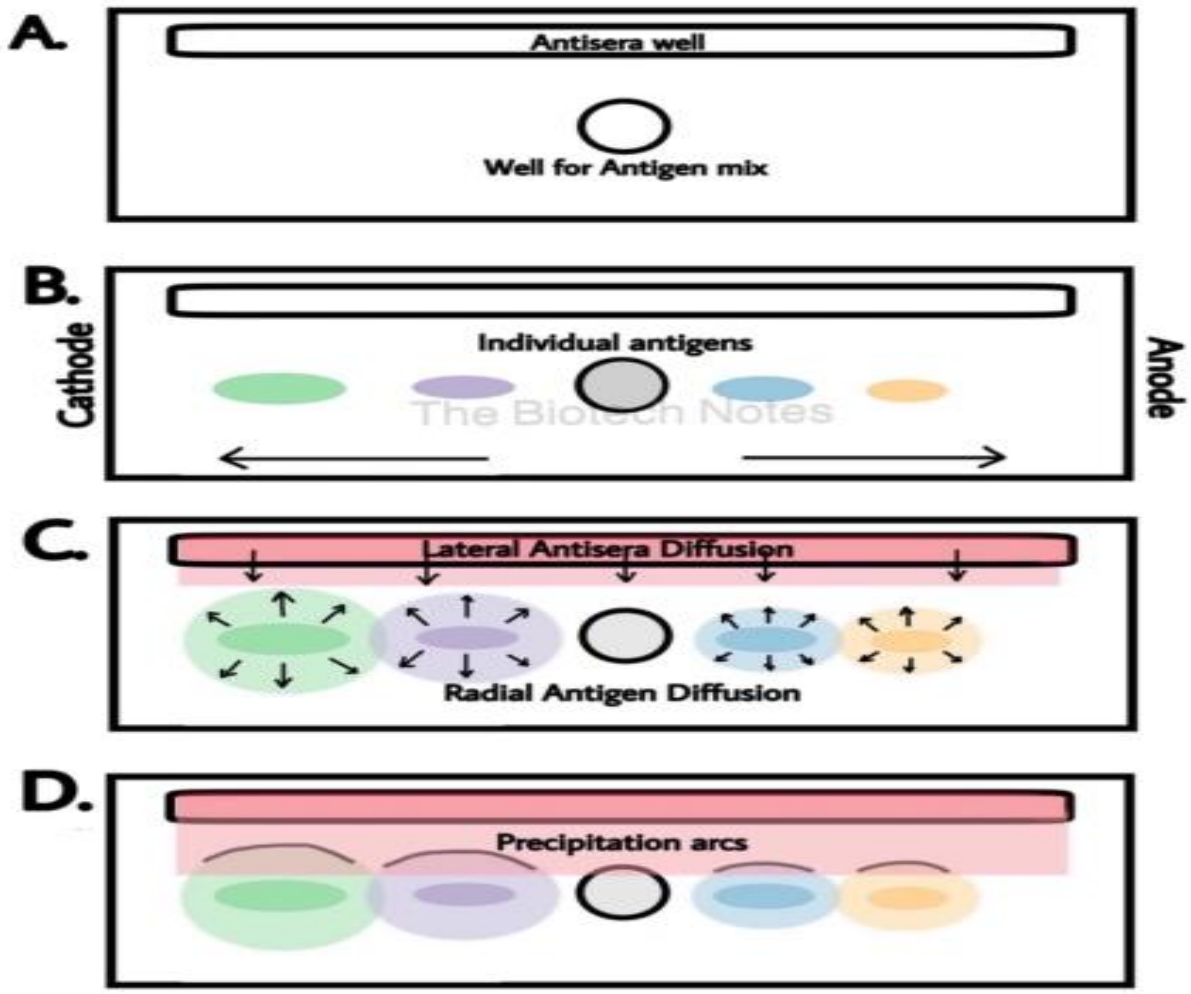
It is a two stage process,

Electrophoresis is conducted in first stage

and

immuno precipitation using antibodies against specific proteins in the second stage. The antibodies when come in contact with antigens, precipitation occurs, resulting in the formation of **precipitin bands**.

IMMUNOELECTROPHORESIS :



THANK YOU