ELECTROPHORESIS

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ELECTROPHORESIS

- GEL ELECTROPHORESIS
- IMMUNOELECTROPHORESIS

- Electrophoresis is a technique used to separate macromolecules in a fluid or gel based on their charge, binding affinity, and size under an electric field.
- In the year 1807, Ferdinand Frederic Reuss was the first person to observe electrophoresis.
- He was from Moscow State University. Anaphoresis is the electrophoresis of negative charge particles or anions whereas cataphoresis is electrophoresis of positive charge ions or cations.
- Electrophoresis has a wide application in separating and analysing biomolecules such as proteins, plasmids, RNA, DNA, nucleic acids.

- Electrophoresis Principle and its types:
- Charged macromolecules are placed in the electric field move towards the negative or positive pole based on their charge. Nucleic acid has a negative charge and therefore it migrates towards the anode.
- This technique is divided into two types viz slab electrophoresis and capillary electrophoresis.
- Types of Electrophoresis:

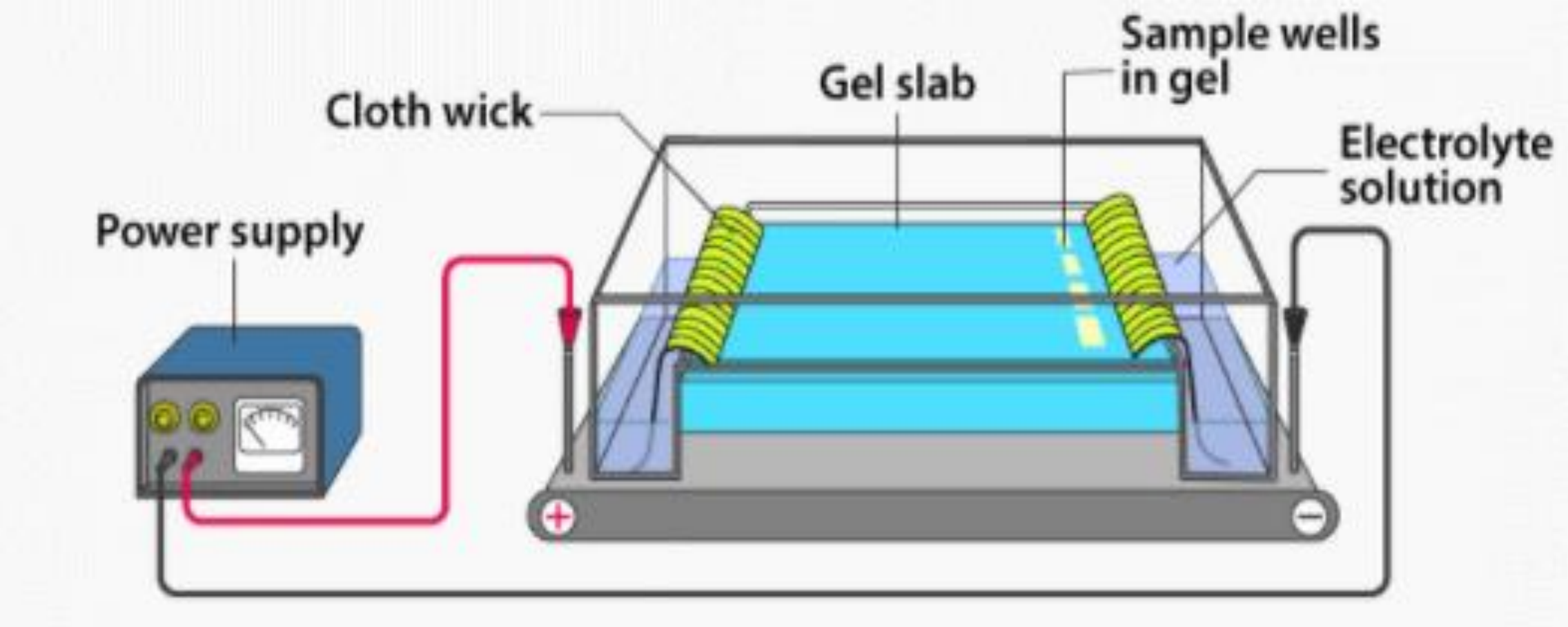
1.Capillary electrophoresis

1. **Gel electrophoresis**
2. Paper electrophoresis

2.Slab electrophoresis

1. Zone electrophoresis
2. **Immunoelectrophoresis**
3. Isoelectrofocusing

GEL ELECTROPHORESIS



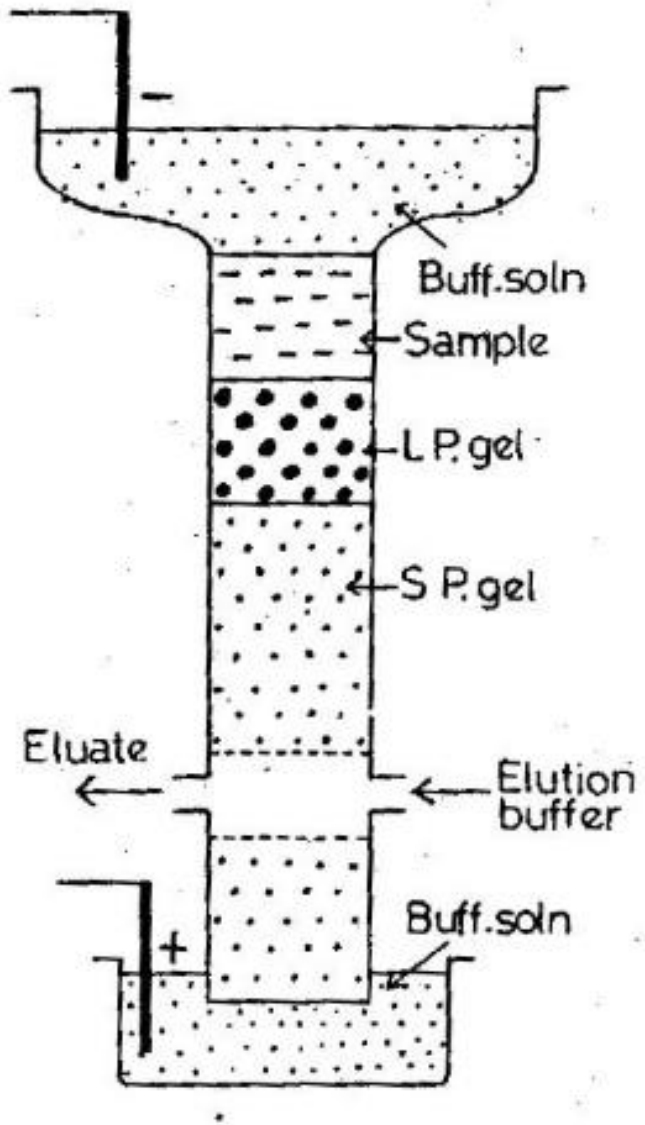
- Gel electrophoresis procedure:
- Below we have explained the steps conducted during DNA electrophoresis.
- **Step 1:** Prepare sample –
- Isolate the DNA and prepare the solution by adding blue dye so that it will be easy to observe the movement of the sample taking place in the gel.
- **Step 2:** Prepare an agarose TAE gel solution –
- TAE buffer solution helps to generate an electric field during the process of electrophoresis. To prepare the solution, for example, if there is a requirement of 1% agarose gel then add 100mL TAE to 1 g of agarose. The higher percentage of agarose will give a denser screen. Dissolve the agarose by heating the agarose TAE solution.

- **Step 3:** Gel casting –
- Pour the agarose TAE solution in a casting tray. Allow it to cool and solidify. A gel slab along with the wells is ready to use for the experiment.
- **Step 4**: How to set up the electrophoresis chamber?
- Fill a chamber with TAE buffer. Place the solid gel in the chamber. Place the gel in such a position such that it is near the negative electrode.
- **Step 5**: Gel loading –
- Load the wells with the DNA sample and DNA ladder (a reference for sizes).

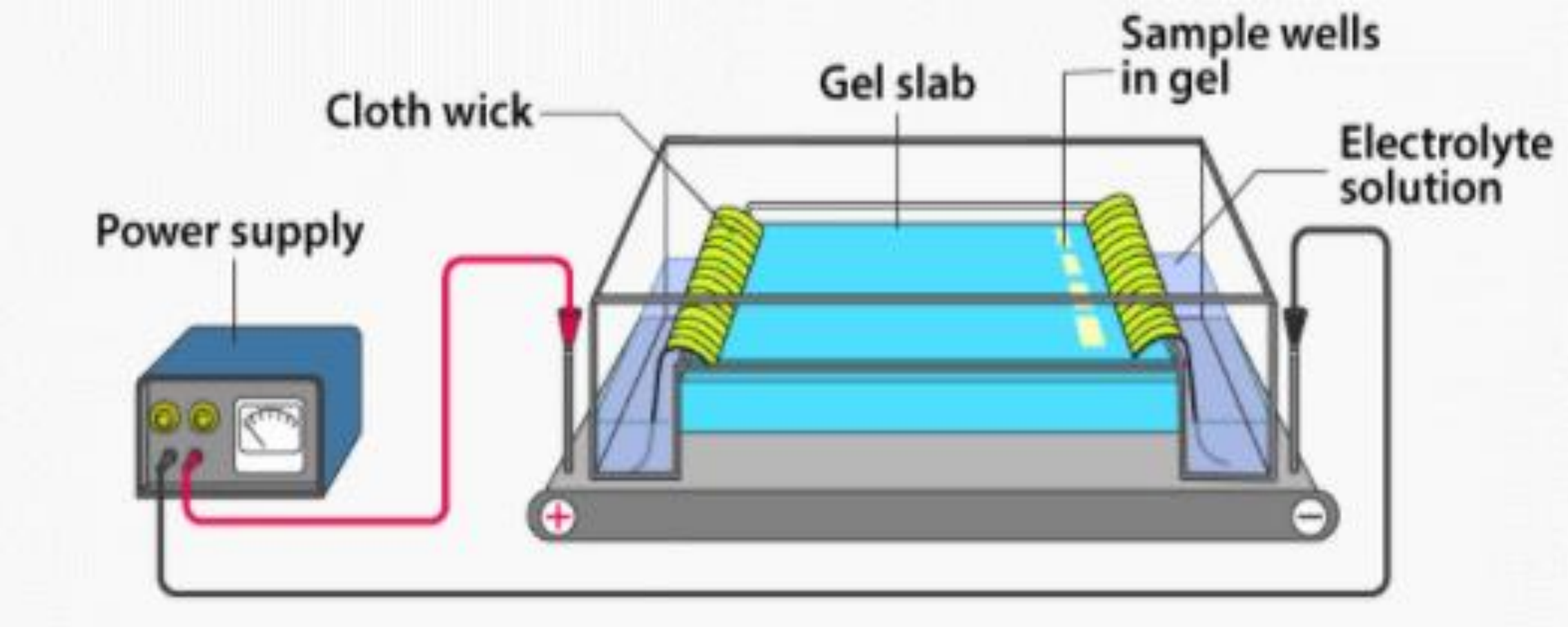
- **Step 6:** Process of electrophoresis –
- Connect the positive and negative points to the power supply and chamber.
- Switch on the power and migration in the DNA sample due to the electric field generated.
- The negatively charged sample will move towards the positive point and away from the negative electrode.
- **Step 7:** Observe the DNA –
- Once you see the migration of the blue colored DNA samples in the gel switch off the power supply.
- Remove the gel and place it in the ethidium bromide solution.

- **Step 8:** Expose the ethidium bromide stained gel under UV light and take a picture.
- DNA bands appear in the lane of respective well. Also, the DNA ladder is visible.
- Therefore, the length of DNA bands can be determined. Below is the image of the experiment conducted.

Gel Electrophoresis



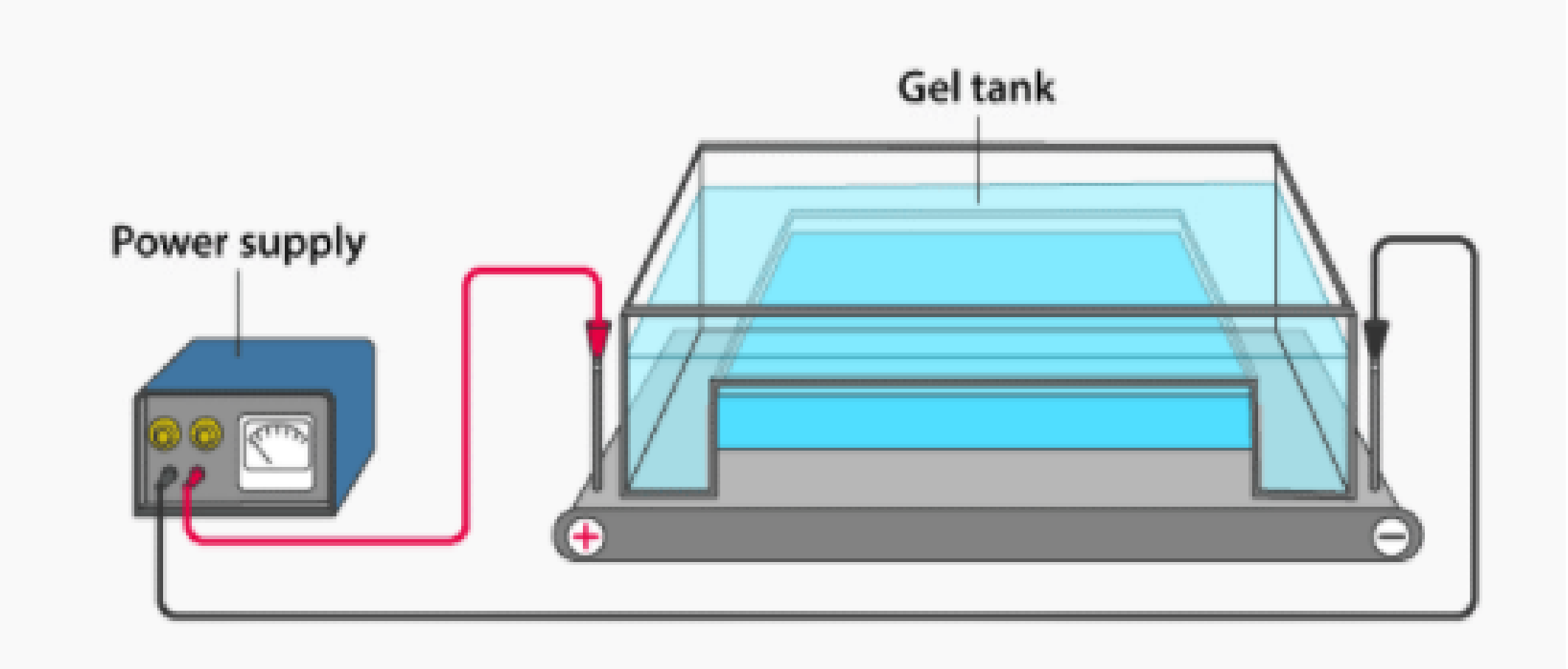
GEL ELECTROPHORESIS

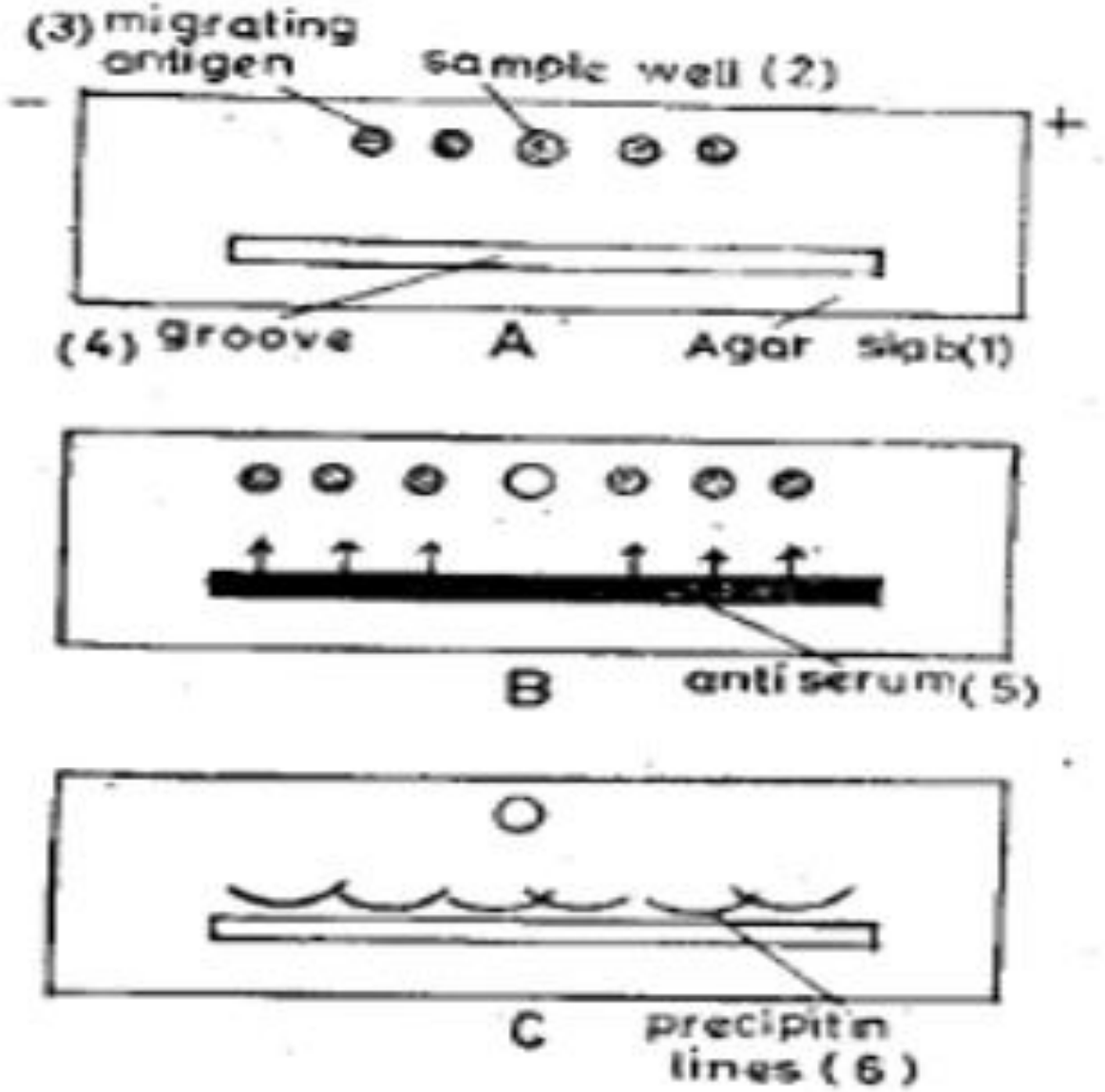


- **Immunoelectrophoresis procedure:**

1. Prepare agarose gel on a glass slide in a horizontal position.
2. Use sample template and carefully move the wells to the application zone.
3. Make the sample dilution in the ratio 2:3 with the diluent protein solution.
4. Take a 5 µl pipette and add 5 µl of sample and control across each slit.
5. Place the gel in the chamber for electrophoresis positioning the sample near the cathode side. Carry out the electrophoresis for 20 mins at 100 volts.
6. Take 20 µl of antiserum in a trough and incubate for 8- 20 hours at room temperature on competing the electrophoresis.
7. Soak the agarose gel for 10 minutes in saline solution, dry it and wash it twice.
8. Dry the gel below 70°C and stain it with protein stain solution for 3 minutes. Decolorize the gel in destaining solution for 5 minutes.
9. Determine the results once the gel is dried.

IMMUNOELECTROPHORESIS





படம் 109 – இம்யூனோ எலக்ட்ரோபோரிஸிஸ்

Immuno Electrophoresis