ELECTROPHORESIS

GENERAL PRINCIPLE

- The term electrophoresis describes the **migration of a charged particle** under the influence of an **electric field.**

- Many important biological molecules, such as amino acids, peptides, proteins, nucleotides and nucleic acids, possess ionisable groups and, therefore, at any given pH, exist in solution as electrically charged species, either cations or anions .

- Under the influence of an electric field these **charged particles will migrate either to the cathode or to the anode, depending on the nature of their net charge**

- The equipment required for electrophoresis consist basically of two items, **a power pack** and an **electrophoresis unit.**
- Electrophoresis requires supporting media such as **agarose gel , polyacrylamide gel, cellulose acetate paper etc.**
- Different buffers can be used and are selected depending on the samples to be analysed.
- Buffers like **tris borate, barbitone, tris-acetate** can be used for performing electrophoresis.

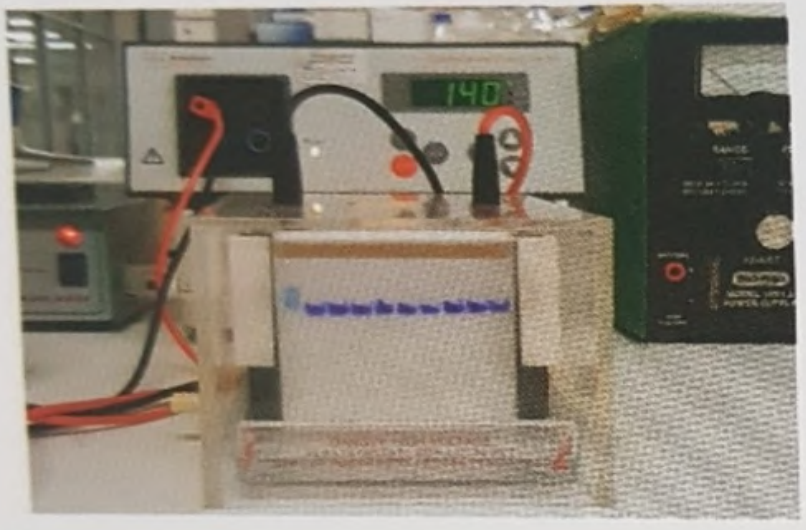


Figure 6.1 Photograph showing an assembled SDS-PAGE minigel. Nine wells that have been loaded can be identified by the blue dye (bromophenol blue) that is incorporated into the loading buffer. The outermost left lane was loaded with a protein marker.

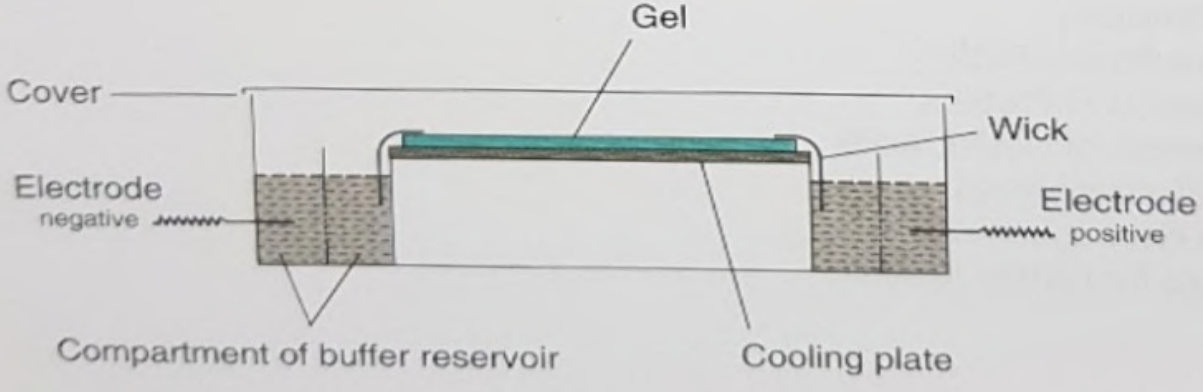


Figure 6.2 A typical horizontal apparatus, such as that used for immunoelectrophoresis, isoelectric focussing and the electrophoresis of DNA and RNA in agarose gels.

SEPARATION OF SERUM PROTEINS BY AGAROSE GEL ELECTROPHORESIS

Reagents

1. Tris- Borate buffer

- Ionic strength- 0.1 M, pH- 8.6

2. Dye solution (Amido Schwarz)

3. Destaining Solution (3% acetic acid)

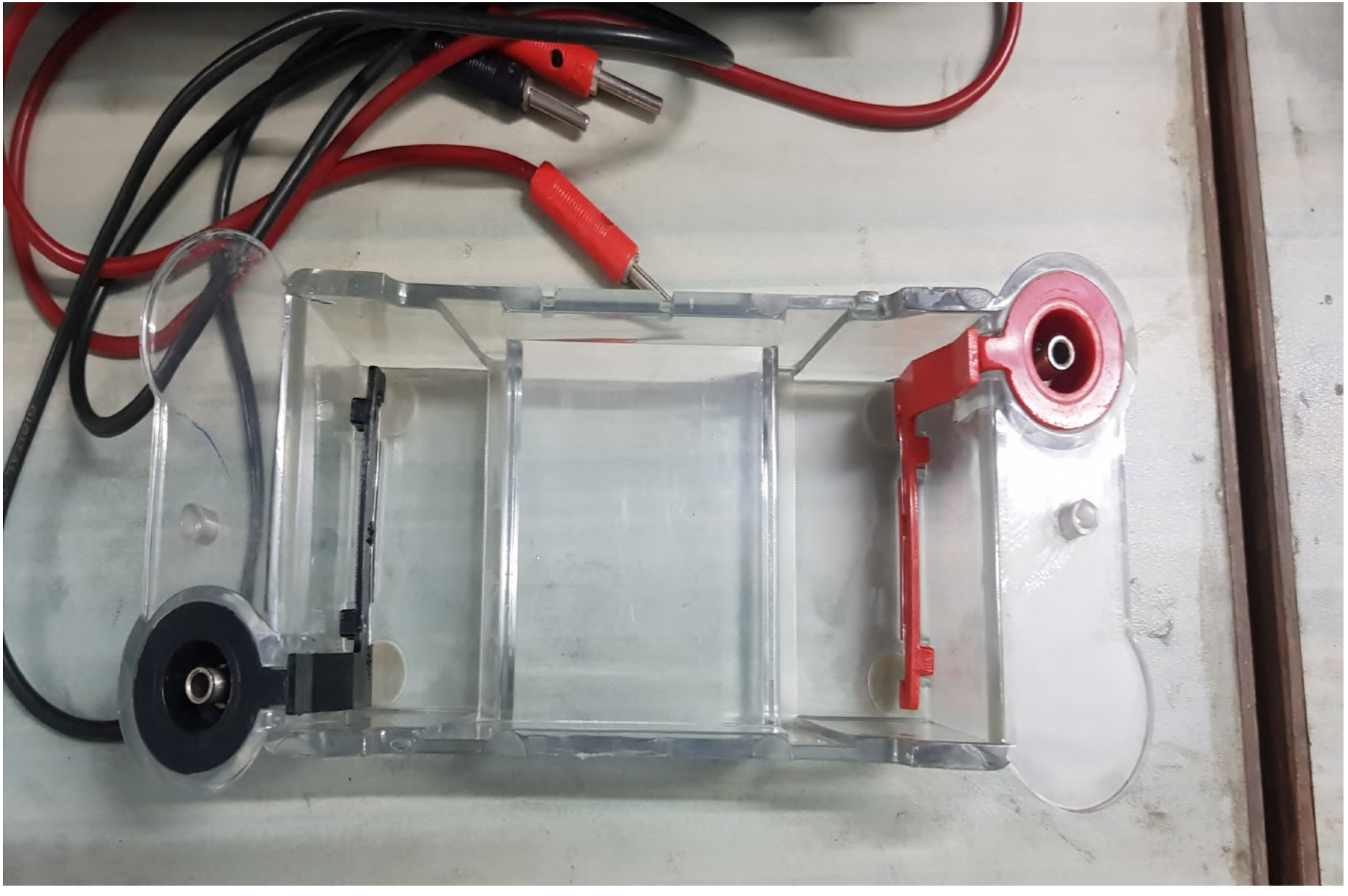
4. Agarose gel solution (1%)

5. Alcohol (95%)

6. Equipments

PROCEDURE

- 1. Fill the electrode vessel of the cabinet with enough buffer.
- 2. Spread 1.3-1.4 ml of hot agarose gel solution on a clean microscopic slide uniformly and allow it to stand for 2-5 minutes.
- 3. Now apply serum on one end of the slide by soaking a small piece of filter paper in serum mixed with a tracking dye.
- 4. Transfer the slide to the electrophoresis cabinet and establish contact of slides with buffer by means of a filter paper at both ends.
- 5. Close the lid and adjust the current at 3 milli ampere or 200 mv using power pack and leave it for 2 hours

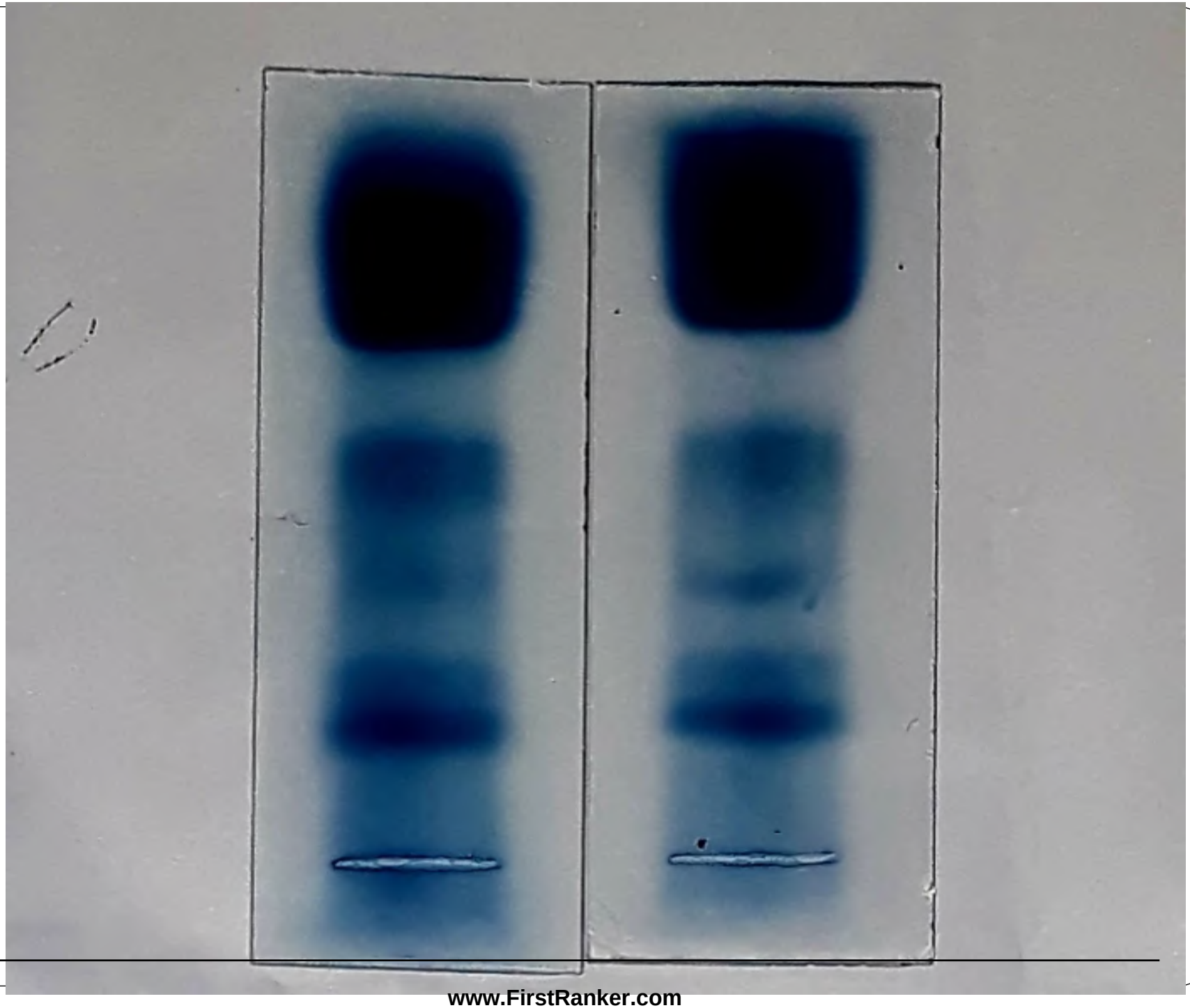


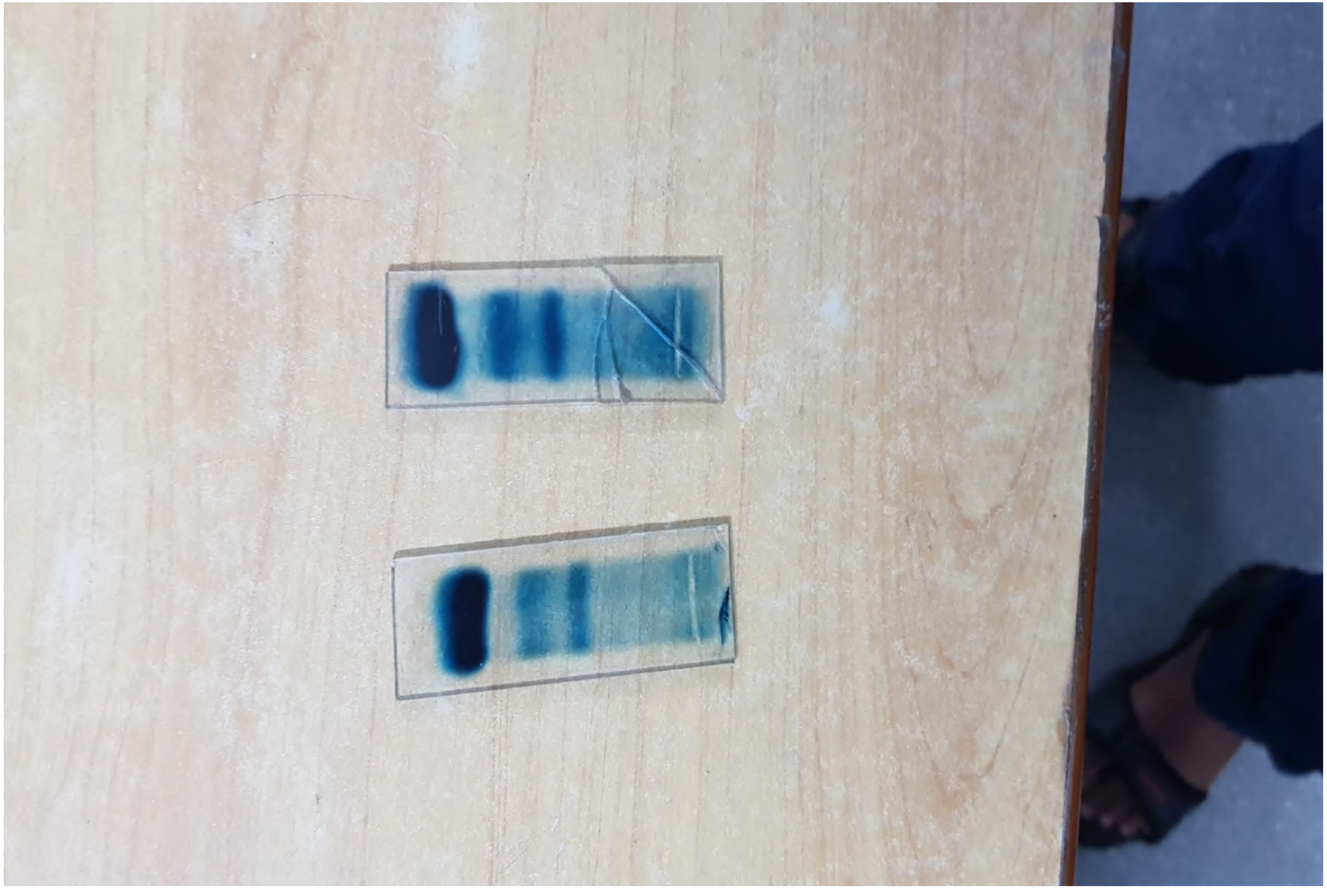
- 6. Slides are taken out and alcohol is gently poured. Let it stand for 30 minutes at room temperature.
- 7. Dry the slide in hot air oven. Avoid overheating as the gel may crack.
- 8. Stain the slide using the pipette. Allow it to stand for about 5 minutes.
- 9. Destain the slide by keeping it in 3% acetic acid and observe the pattern.

Normal Patterns and Interpretations

- In agarose gel electrophoresis, normal serum is separated in to 5 bands. Their normal relative concentrations are given below:
- **Albumin- 55-65%**
- **Aplha-1-globulin- 2-4%**
- **Alpha-2-globulin- 6-12%**
- **Beta globulin- 8-12%**
- **Gamma globulin- 12-22%**

Protein fraction	Concentration (g/dL)
Albumin	3.2–5.6
α_1-Globulins	0.1–0.4
α_2-Globulins	0.4–1.2
β-Globulins	0.5–1.1
γ-Globulins	0.5–1.6





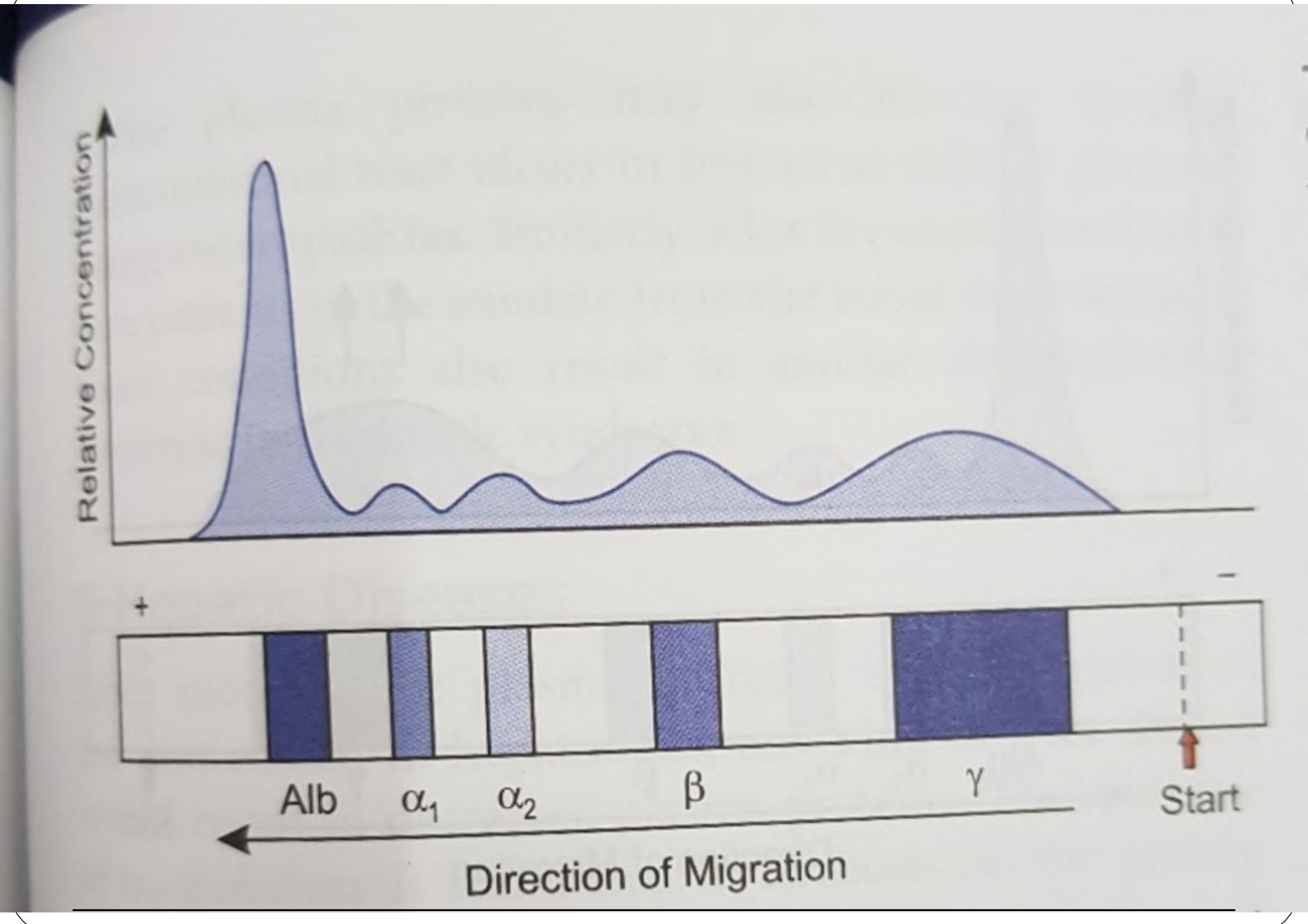


Table 25.5 Serum acute-phase proteins and their relative changes

Protein	Position	Change
α_1-Acid glycoprotein	α_1	Increased (++)
α_1-Antitrypsin	α_1	Increased (+)
Ceruloplasmin	α_2	Normal or increased (N/+)
C-reactive protein	β/γ	Increased (+++)
Albumin	Alb	Decreased (–)
α_2-Macroglobin	α_2	Decreased (–)

...e γ-globulin peak (Fig. 25.20).

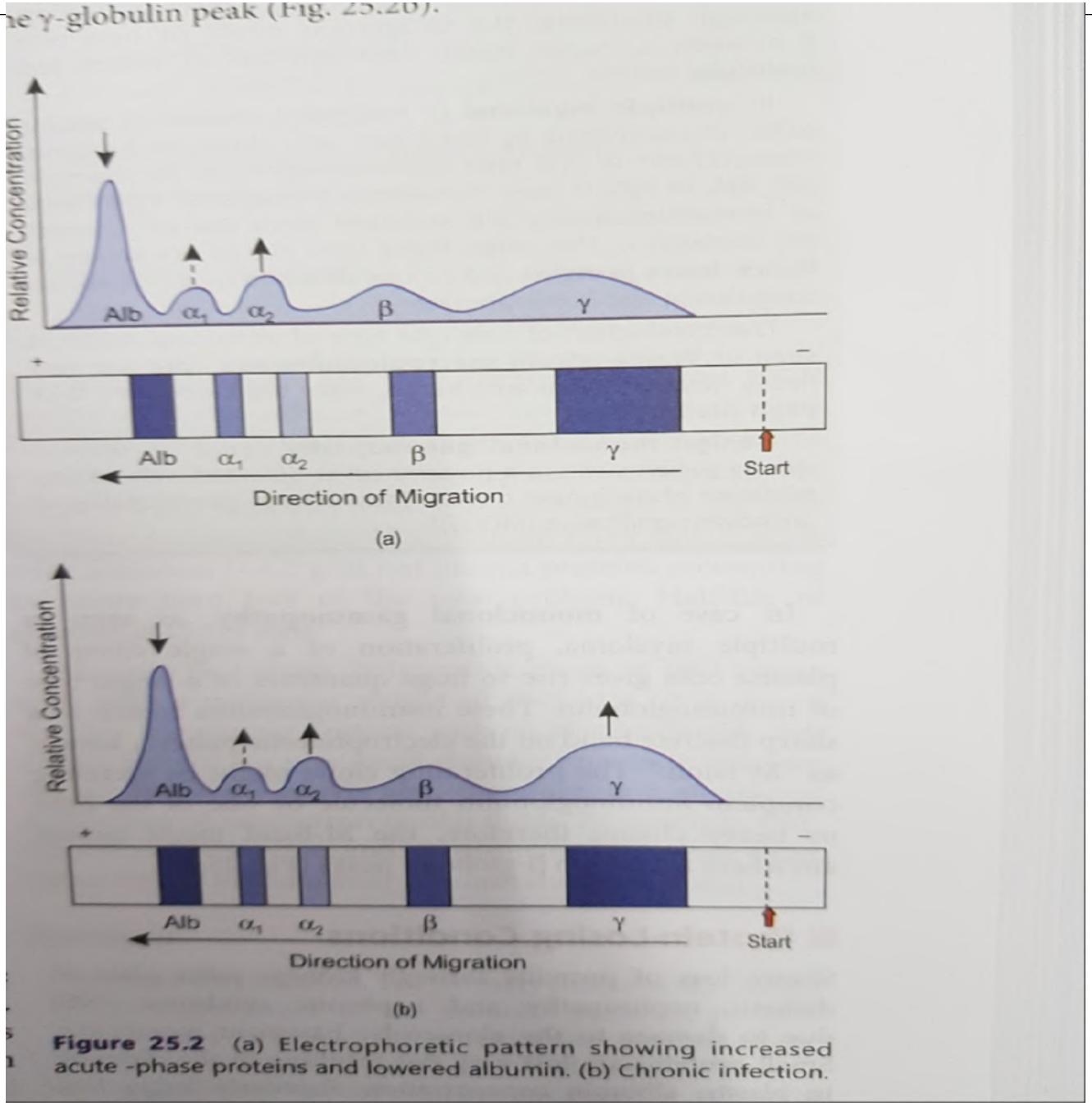


Figure 25.2 (a) Electrophoretic pattern showing increased acute -phase proteins and lowered albumin. (b) Chronic infection.

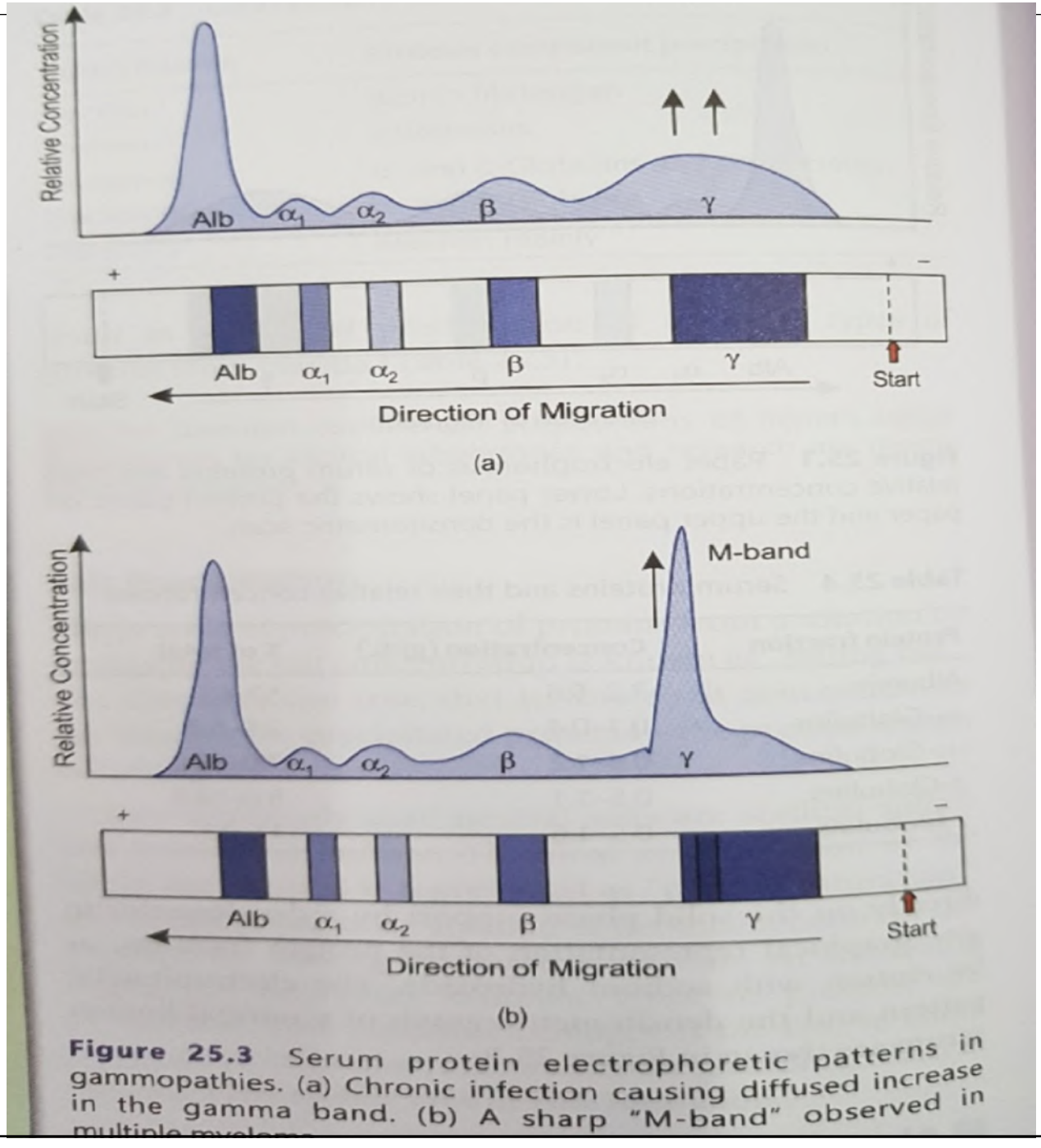


Figure 25.3 Serum protein electrophoretic patterns in gammopathies. (a) Chronic infection causing diffused increase in the gamma band. (b) A sharp "M-band" observed in multiple myeloma.

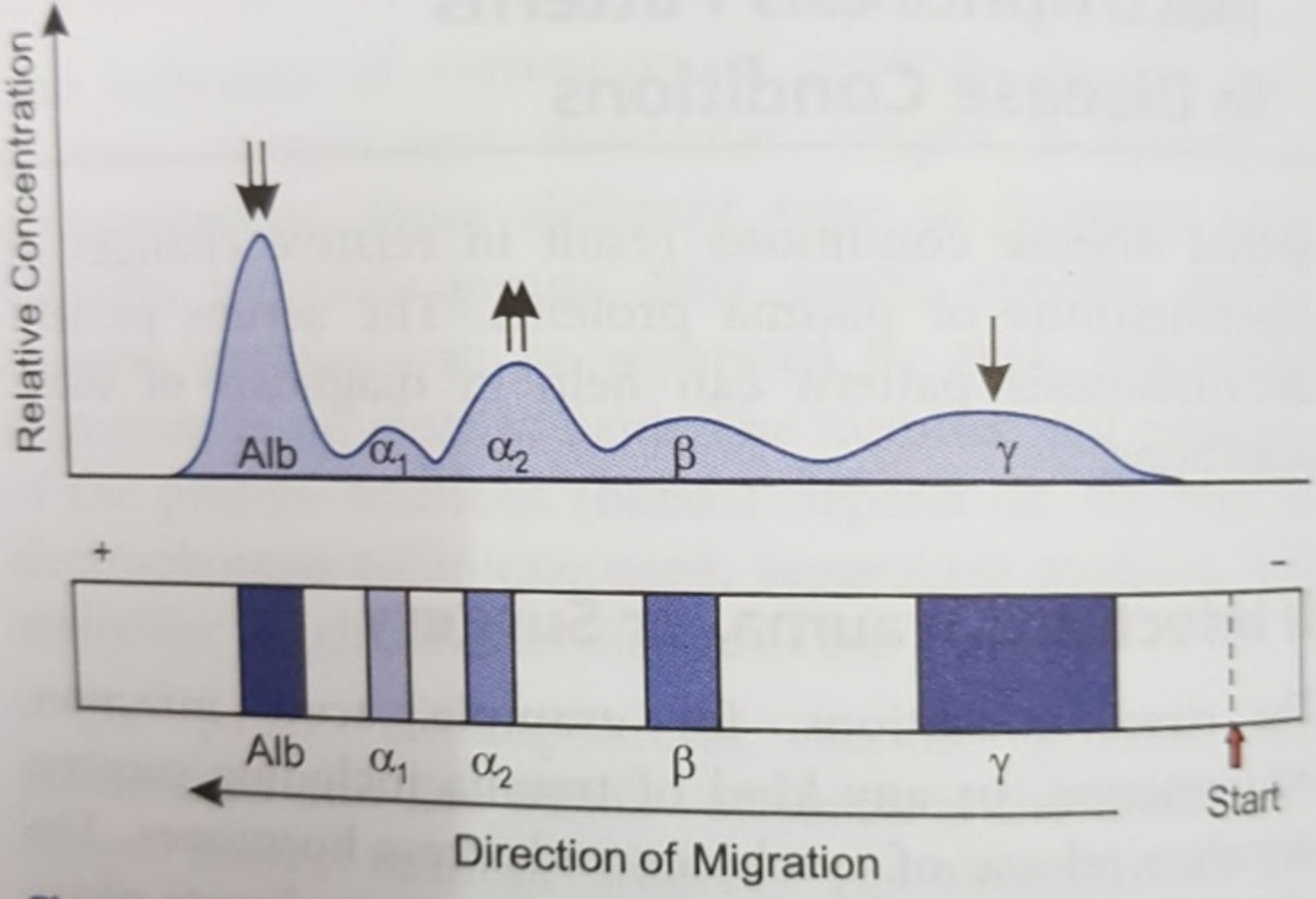


Figure 25.4 Electrophoretic pattern showing decreased albumin and γ- globulins in protein-losing conditions.

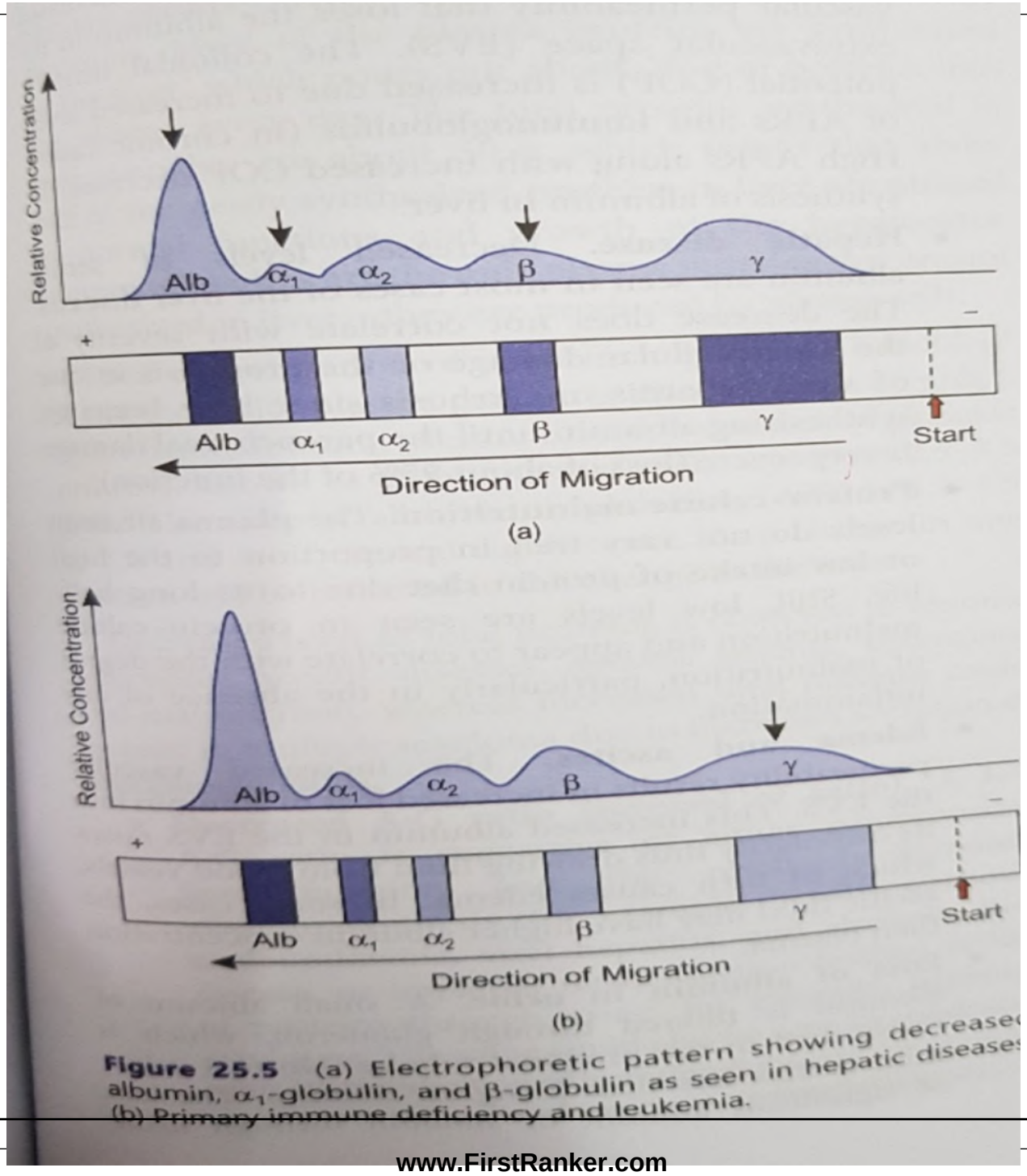


Figure 25.5 (a) Electrophoretic pattern showing decreased albumin, α_1-globulin, and β-globulin as seen in hepatic diseases (b) Primary immune deficiency and leukemia.

Contd...

Type of electrophoresis	Supporting media used	Property used to separate the proteins
Cellulose acetate electrophoresis	Cellulose acetate membrane	
Polyacrylamide gel electrophoresis (PAGE)	Polymer of acrylamide	Based on charge and molecular weight (size)
Sodium dodecyl sulfate (SDS)—PAGE	Sodium dodecyl sulfate and polyacrylamide SDS imparts equal negative charge so that it masks the inherent charge of the protein. Now proteins separate based on size only	Based on molecular weight (size)
Capillary electrophoresis	Separation done in a capillary tube	Based on charge
Isoelectric focusing	Supporting media with pH gradient	Based on isoelectric pH