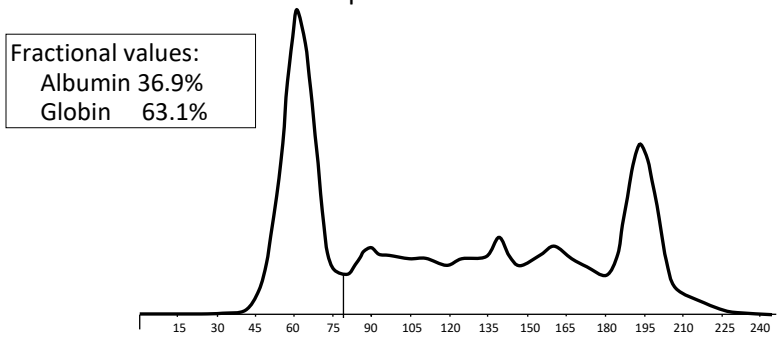
Chapter 8

Electrophoresis

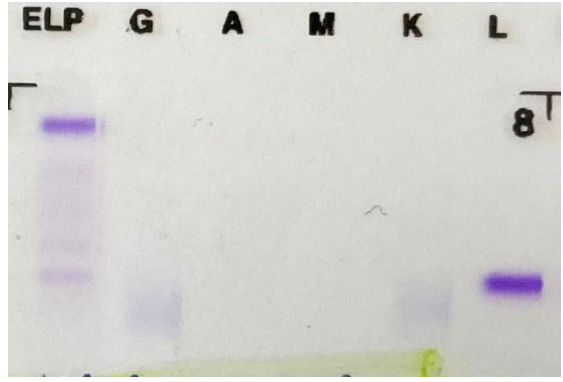
Anu S. Maharajan

1. A 74-year-old male with a medical history for CKD Stage III and diabetes had labs ordered for urine protein electrophoresis and urine immunofixation electrophoresis. How should you interpret the results below?

i. Urine Protein Electrophoresis

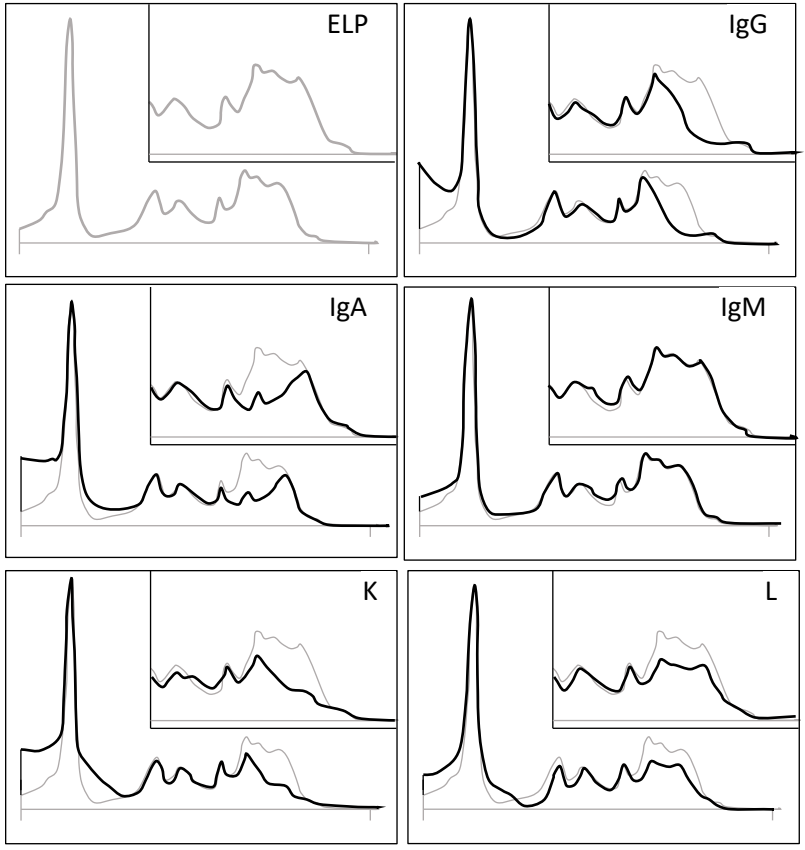


ii. Urine Immunofixation Electrophoresis (uIFE)



A) Bence Jones protein with lambda chain specificity
B) Bence Jones protein with kappa chain specificity
C) No monoclonal or Bence Jones protein
D) Multiple monoclonal proteins

2. A 67-year-old male patient with anemia, weakness, and weight loss for six months. His physical examination was normal with no fever, no bone lesions on imaging studies. Laboratory results showed low hemoglobin and albumin concentrations with increased total protein. Capillary electrophoresis with immunosubtraction is shown below.



How should these immunosubtraction results be interpreted?

A) Biclonal gammopathy with IgA kappa and IgG lambda
B) Triclonal paraprotein with 2 IgG kappa, and 1 IgG lambda
C) IgG lambda monoclonal gammopathy
D) No monoclonal proteins identified

3. A sample of serum protein is applied to a neutral-coated capillary with a total length of 25.0 cm and 22.0 cm to the detector. The migration times of two proteins are 13.3 minutes and 14.2 minutes respectively when using an applied voltage of 20.0 kV. What is the electrophoretic mobility of the protein that migrated at 14.2 minutes?

A) 1.94 $cm^2/kV \cdot min$
B) 2.08 $cm^2/kV \cdot min$
C) 1.80 $cm^2/kV \cdot min$
D) 1.65 cm/min

4. Two isoforms of a protein are found to elute with migration times of 16.6 minutes and 20.3 minutes from a capillary electrophoresis. The measured areas of protein peaks are 3430 and 1235 units, respectively. What is the relative amount of each protein in the sample?

A) 202, 62
B) 207, 61
C) 343, 124
D) 169, 50.4

5. The electrophoretic migration rate of a molecule is most dependent on the _____ of the molecule:

A) Size
B) Shape
C) Magnitude of the charge and size
D) Solubility

6. Which of the following is most accurate:

A) When the pH is above the isoelectric point, proteins that have a net negative charge migrate toward the anode.
B) At the isoelectric point, proteins that have a net negative charge migrate toward the anode.
C) When the pH is above the isoelectric point, proteins that have a net positive charge migrate toward the cathode.
D) At the isoelectric point, proteins that have a net positive charge migrate toward the cathode.

7. On protein electrophoresis, gamma globulins migrate to the cathode because:

A) The charge on gamma globulins is positive.
B) The isoelectric point of gamma globulins is less than that of the other proteins
C) Endosmosis
D) The size of the immunoglobulins is the most important factor in determining their migration.

8. Increasing the ionic strength of the buffer used for electrophoresis would result in:

A) Increased rate of migration due to increased current being carried by buffer ions
B) Decreased rate of migration due to increased current being carried by buffer ions
C) The rate of migration would not change
D) Increased charge of proteins

9. The equation for Ohm's law is (I = current; R = resistance; V = difference in potential):

A) I = VR
B) R = IV
C) V = I/R
D) V = IR

10. When heat dissipation becomes a problem using gel electrophoresis, then:

A) The voltage should be kept constant
B) The current should be kept constant
C) The voltage and the current should be kept constant
D) Neither the voltage nor the current will impact heat dissipation

11. The usual pH for protein electrophoresis is:

A) 7.0
B) 7.6
C) 8.0
D) 8.6

12. During agar gel electrophoresis at pH 8.6, the gel has _____.

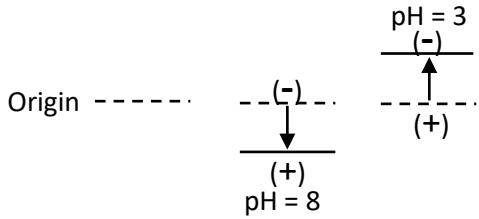
A) No charge
B) Positive charge
C) Negative charge
D) The charge depends on the strength of the buffer

13. Serum protein migration during electrophoresis at pH 8.6:

A) All proteins migrate toward the anode
B) All proteins migrate toward the anode except gamma globulin which migrates toward the cathode because of endosmosis.
C) All proteins migrate toward the cathode
D) All proteins migrate toward the cathode except gamma globulin which migrates toward the anode because of endosmosis.

14. The electrophoretic migration of a protein at two different pH's is shown in the next Figure:

Electrophoretic Migration of a Protein at Two Different pH's



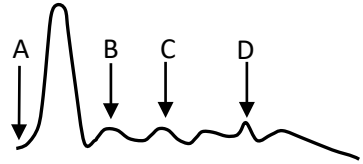
The isoelectric point (pI) of the protein is:

A) Greater than 8
B) Between 3 and 8
C) Less than 3
D) Cannot be determined based on the information given

15. Migration of immunoglobulins toward the cathode during serum protein electrophoresis at pH 7.0 compared to electrophoresis at pH 8.6:

A) Is slower
B) Is faster
C) Is not altered
D) Will have a similar migration speed of other proteins

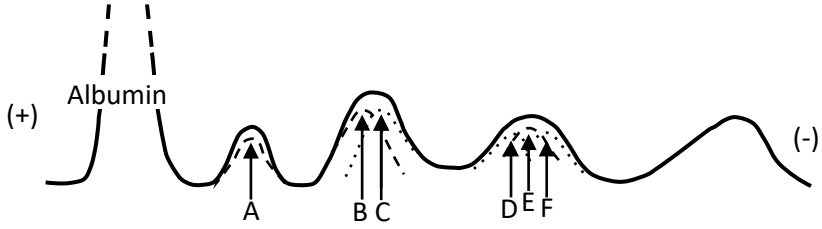
16. A protein electrophoretic pattern is given in the next Figure:



Indicate the location of fibrinogen:

A) A
B) B
C) C
D) D

17. A protein electrophoresis pattern is given in the Figure below. The major components of the alpha-1, alpha-2 and beta globulins are indicated by letters. Which of the following is accurate:



A) C could be Beta lipoprotein
B) D could be Haptoglobin
C) F could be Alpha-1 antitrypsin
D) E could be Transferrin

18. On serum protein electrophoresis, fibrinogen migrates in the _____ globulin region.

A) Alpha-1
B) Alpha-2
C) Beta-1
D) Beta-2

19. The serum protein electrophoretic abnormality in alpha-1 antitrypsin deficiency is:

A) Increased alpha-1 globulin
B) Decreased alpha-1 globulin
C) Increased alpha-2 globulin
D) Decreased alpha-2 globulin

20. Decreased serum alpha-1 antitrypsin in a child might be correlated with disease in:

A) Liver
B) Cerebrum
C) Kidney
D) Small intestine

21. Which of the following is correct?

A) Decreases in the beta-1 band can be seen in iron deficiency anemia
B) Haptoglobin, which is in the beta-2 band binds circulating hemoglobin
C) Increases in the alpha-2 band can be seen in nephrotic syndrome
D) Increases in the alpha-1 band can be seen in hypercholesterolemia

22. The characteristic protein electrophoretic pattern changes seen in nephrotic syndrome are:

A) Increased A_2 and decreased gamma globulin
B) Increased albumin and decreased gamma globulin
C) Decreased A_1 and decreased albumin
D) Increased A_1 and increased gamma globulin

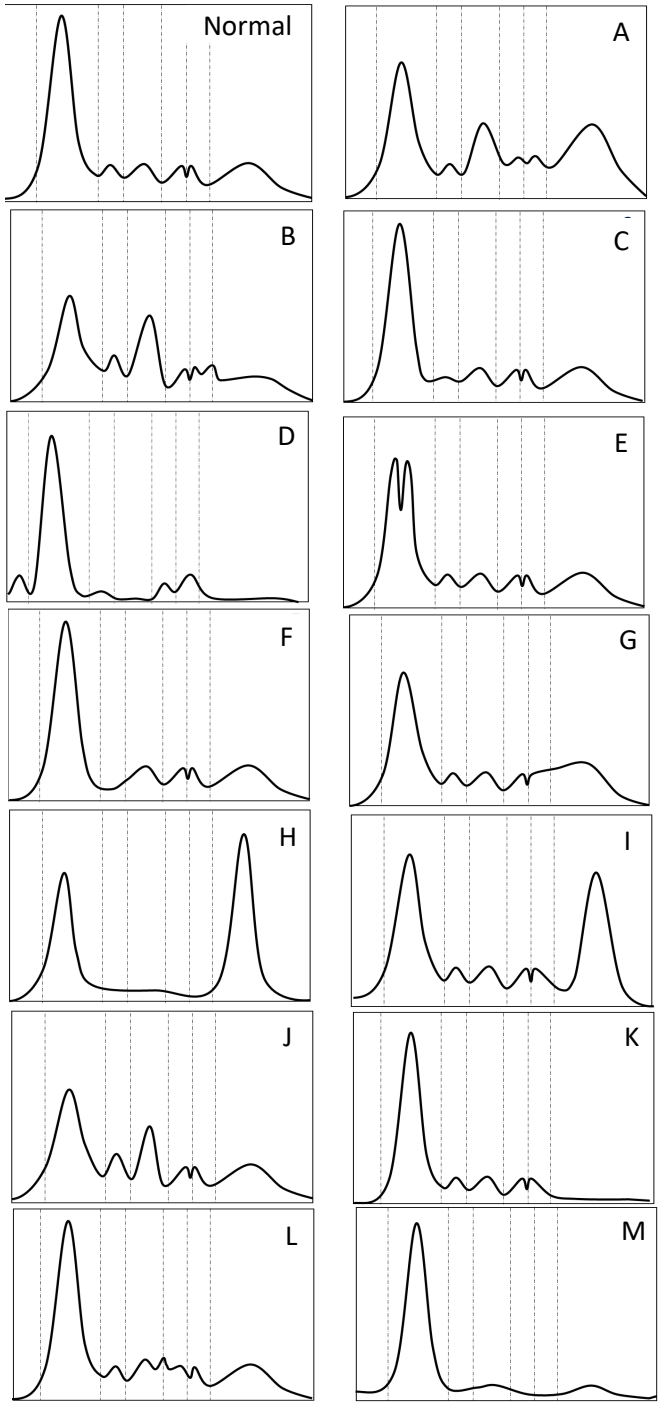
23. One of the following serum proteins has the greatest effect on the sedimentation rate:

A) Albumin
B) Fibrinogen
C) Globulin
D) Transferrin

24. A young woman on oral contraceptive pills has green plasma and increased alpha globulin; the most likely etiology is:

A) Increased anti-trypsin
B) Increased beta lipoprotein
C) Increased haptoglobin
D) Increased ceruloplasmin

Questions 25-30: Refer to the following electrophoretic patterns:



25. Panel E is characteristic of which of the following?

A) Acute inflammation
B) Hemolysis
C) Bisalbuminemia

26. Which of these electrophoretic patterns is seen in hypogammaglobulinemia?

A) Panel D
B) Panel G
C) Panel I
D) Panel K

27. Which of these electrophoretic patterns is seen in monoclonal gammopathy?

A) Panel A
B) Panel H
C) Panel I
D) Panel K

28. Which of these electrophoretic patterns is seen in alpha 1 antitrypsin deficiency?

A) Panel C
B) Panel D
C) Panel F
D) Panel M

29. Which of these electrophoretic patterns is seen in chronic inflammation?

A) Panel A
B) Panel B
C) Panel C
D) Panel L

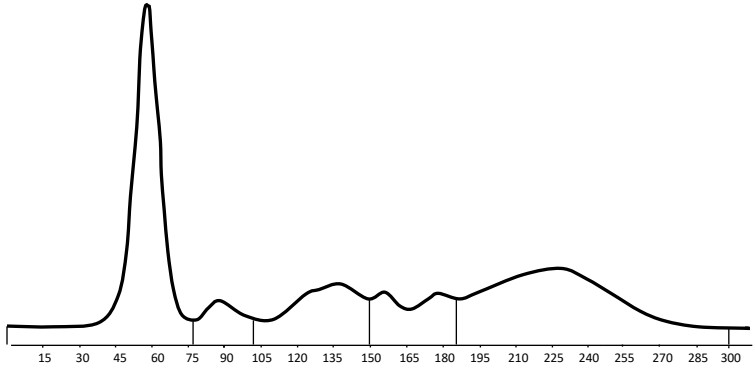
30. Which of the following electrophoretic patterns seen in nephrotic syndrome?

A) Panel B
B) Panel J
C) Panel G
D) Panel H

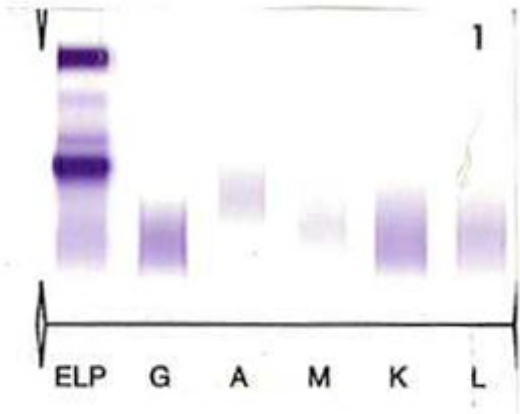
31. A 54-year-old female with no recurrent multiple myeloma for the past 3 years was seen at the Cancer Center. It was a difficult blood draw for the phlebotomist as the patient had thin veins and the phlebotomist ended up using a butterfly with a 23-gauze needle. The provider ordered capillary electrophoresis along with quantitative kappa/lambda and immunofixation electrophoresis. What is the best interpretation for this finding?

Component	Reference Interval	Results
Albumin	3.75-5.01 g/dL	4.18 g/dL
Alpha 1	0.19-0.46 g/dL	0.29 g/dL
Alpha 2	0.48-1.05 g/dL	1.30 g/dL
Beta	0.48-1.10 g/dL	0.63 g/dL
Gamma	0.62-1.51 g/dL	0.72 g/dL
Total Protein	6.3-8.2 g/dL	7.1 g/dL
IgG	768-1632 mg/dL	904 mg/dL
IgA	68-408 mg/dL	196 mg/dL
IgM	35-263 mg/dL	45 mg/dL
Kappa Free Light Chains (Quantitative)	3.30-19.40 mg/L	16.20 mg/L
Lambda Free Light Chains (Quantitative)	5.71-26.30 mg/L	11.36 mg/L
Kappa/Lambda Free Light Chain Ratio	0.26-1.65	1.43

i. Capillary Electrophoresis (CE)



ii. Serum Immunofixation Electrophoresis (sIFE)



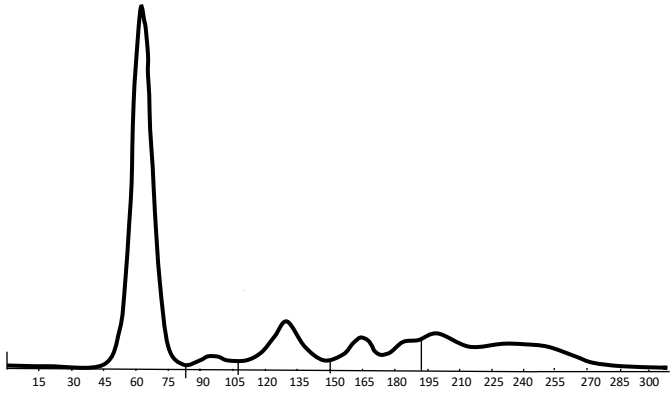
A) Indeterminate study
B) Likely positive for IgD or IgE monoclonal gammopathy
C) Interference due to hemolysis
D) Possibly repeat IFE with reducing agents such as beta-mercaptoethanol (2-ME) or dithiothreitol (DTT)

32. A protein has an electrophoretic mobility of 1.80 $cm^2/kV.min$ when migrating 22.0 cm to the detector in a capillary of a total length of 25.0 cm. This protein's diffusion coefficient is approximately 2.0 x 10^{-7} cm^2/s in its running buffer. During the separation by capillary electrophoresis, the longitudinal diffusion is the only significant band broadening process present. If 25.0 kV is applied, what is the maximum number of theoretical plates that would be expected of this protein's peak?

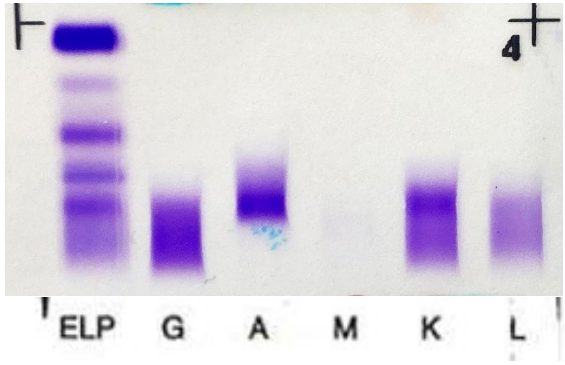
A) 1.3 x 10^6 theoretical plates
B) 1.9 x 10^6 theoretical plates
C) 2.0 x 10^6 theoretical plates
D) 1.7 x 10^6 theoretical plates

33. A 72-years-old female who had never seen a doctor since last childbirth was recently seen at internal medicine, and her lab work showed a serum creatinine of 3.39 and eGFR of less than 20. Her case was shared with providers at nephrology and hematology. How should you interpret her results below?

i. Serum Protein Electrophoresis (SPEP)



ii. Serum Immunofixation Electrophoresis (sIFE)



A) Monoclonal protein with IgG lambda chain specificity
B) Multiple monoclonal protein bands present
C) Polyclonal protein present
D) Monoclonal protein with IgA kappa chain specificity

Chapter 8

Electrophoresis: Answers

1. **Answer A** UPEP displays the increased globulin and an M-spike. Urine immunofixation electrophoresis shows the presence of a lambda light chain with no corresponding heavy chain. This shows Bence Jones protein with lambda chain specificity.

2. **Answer B** Immunosubtraction shows the absence of 3 peaks from IgG, 2 peaks from kappa, and 1 peak from lambda. The presence of polyclonal IgA complicates the interpretation, but the removal in the IgG is more substantial than the A frame for the distortion in the beta-gamma region. Immunosubtraction method consists of incubating serum in the presence of antisera for specific heavy chains and light chains. Specific antisera remove the M-protein targeted by the antisera by precipitation, so detection is based on the absence of that targeted M-protein.

   Reference: Aksungar FB, Ayer M, Serteser M, Coskun A, Unsal I. A triclonal gammopathy in a relapsing multiple myeloma patient, detected by immunosubtraction method. Ann Clin Biochem. 2014 Sep;51(Pt 5):606-10. doi: 10.1177/0004563213512801. Epub 2013 Dec 20. PMID: 24361990.

3. **Answer A** Electrophoretic mobility of the protein is based on the velocity of protein migration over the electrical field used on the length of the electrophoretic method.

   The electrophoretic mobility for an analyte in capillary electrophoresis can be expressed as $\mu = v/E \rightarrow (L_d/t_m)/(V/L)$.
   μ = electrophoretic mobility in $m^2/V{\cdot}s$ or $cm^2/kV{\cdot}min$
   $v = L_d/t_m \rightarrow L_d$ is the distance the protein traveled from the point of application to the detector, t_m is the time for this migration to the detector. $E = V/L \rightarrow V$ is the applied electric field, and L is the length of the electrophoretic media.

   $\mu = v/E$

   $\mu = (L_d/t_m)/(V/L) = (22.0\ cm/14.2\ minutes)/(20.0\ kV/25.0\ cm)$

   $= 1.94\ cm^2/kV.min$

   Reference: William Clarke. Contemporary Practice in Clinical Chemistry, 3rd edition; 2. David S. Hage and James D. Carr. Analytical Chemistry and Quantitative Analysis, 2011. Prentice Hall.

4. **Answer B** If both proteins have a similar response to the detector, we can assume that the corrected areas are the approximate amount of proteins in the sample, so you can calculate protein by dividing protein area with its specific migration times.

   Protein 1: (3430)/(16.6 minutes) = 207
   Protein 2: (1235)/(20.3 minutes) = 61

   Reference: 1. David S. Hage and James D. Carr. Analytical Chemistry and Quantitative Analysis, 2011. Prentice Hall.

5. **Answer C** The electrophoretic migration is directly proportional to the net charge and inversely proportional to the size of the molecule.

   Reference: Bazydlo LAL and Landers JP. Electrophoresis. In: Rifai N, Horvath AR, Wittwer CT, eds. Tietz Textbook of Laboratory Medicine. 6th ed. Philadelphia, PA: Elsevier; 2017:226.e1.

6. **Answer A** The pH at which protein net charge is zero and its mobility is zero is known as the isoelectric point (pI). At a pH below pI, proteins have a positive net charge and will migrate towards the cathode which is negatively charged. At a pH above pI, proteins have a negative net charge and will migrate towards the anode which is positively charged.

   Reference: Bazydlo LAL and Landers JP. Electrophoresis. In: Rifai N, Horvath AR, Wittwer CT, eds. Tietz Textbook of Laboratory Medicine. 6th ed. Philadelphia, PA: Elsevier; 2017:234-235.

7. **Answer C** Gamma globulins migrate to the cathode due to endosmosis, a phenomenon induced by the application of an electric potential. When current is applied, ions in solution move towards the electrode of opposite polarity, dragging the solvent with them as these ions are highly hydrated. In systems with a pH of 8.0-9.0, proteins become negatively charged, forming a negative ion cloud that migrates towards the anode.

   Conversely, gels used in protein electrophoresis attract positive ions from the buffer, forming a positive ion cloud moving towards the cathode. The tension between these oppositely moving ion clouds affects the movement of sample macromolecules. Since the net negative charge of the gamma

globulins can be small it can travel backward and appear behind the point of sample application.

Reference: Bazydlo LAL and Landers JP. Electrophoresis. In: Rifai N, Horvath AR, Wittwer CT, eds. Tietz Textbook of Laboratory Medicine. 6th ed. Philadelphia, PA: Elsevier; 2017:226-227.

8. **Answer A** The buffer ions carry the applied current and therefore, the buffers' ionic strength determines the thickness of the ionic cloud surrounding a charged molecule, the rate of its migration, and the sharpness of the electrophoretic zones.

   Reference: Bazydlo LAL and Landers JP. Electrophoresis. In: Rifai N, Horvath AR, Wittwer CT, eds. Tietz Textbook of Laboratory Medicine. 6th ed. Philadelphia, PA: Elsevier; 2017:229.

9. **Answer D** Ohm's law is described by the following relationship: Voltage (V) = Current (I) x Resistance (R)

10. **Answer B** Heat is generated as a result of resistance encountered by a current passing through a medium. In constant-power supplies, direct effects of heat generation (Watts = Current x Voltage) are decreased resistance and rise in current to satisfy V= IR (voltage = Current x Resistance), which ultimately increases protein migration and water evaporation. If a constant current setting is used, as resistance decreases during the run, voltage will also decrease. This in turn decreases the heat effect and stabilizes the migration rate.

    Reference: Bazydlo LAL and Landers JP. Electrophoresis. In: Rifai N, Horvath AR, Wittwer CT, eds. Tietz Textbook of Laboratory Medicine. 6th ed. Philadelphia, PA: Elsevier; 2017:229.

11. **Answer D** Agarose gel electrophoresis is typically run at pH 8.6.

12. **Answer C** At pH 8.6, the charge of the gel in agar gel electrophoresis is negative. This is because agarose gel has negatively charged sulfate groups, which become deprotonated at higher pH values resulting in a net negative charge on the gel.

    Reference: Bazydlo LAL and Landers JP. Electrophoresis. In: Rifai N, Horvath AR, Wittwer CT, eds. Tietz Textbook of Laboratory Medicine. 6th ed. Philadelphia, PA: Elsevier; 2017:229.

13. **Answer B** Gamma globulins migrate to the cathode due to endosmosis, a phenomenon induced by the application of an electric potential. When current is applied, ions in solution move towards the electrode of opposite polarity, dragging the solvent with them as these ions are highly hydrated. In systems with a pH of 8.0-9.0, proteins become negatively charged, forming a negative ion cloud that migrates towards the anode.

    Conversely, gels used in protein electrophoresis attract positive ions from the buffer, forming a positive ion cloud moving towards the cathode. The tension between these oppositely moving ion clouds affects the movement of sample macromolecules. Since the net negative charge of the gamma globulins can be small it can travel backward and appear behind the point of sample application.

    Reference: Bazydlo LAL and Landers JP. Electrophoresis. In: Rifai N, Horvath AR, Wittwer CT, eds. Tietz Textbook of Laboratory Medicine. 6th ed. Philadelphia, PA: Elsevier; 2017:226-227.

14. **Answer B** In the figure it can be observed that the protein acquires a positive charge at pH 3 and a negative charge at pH 8. Therefore, the isoelectric point where the protein has a net charge of zero is between 3 and 8.

15. **Answer A** At pH 7.0, the proteins will have a lower net negative charge due to protonation of ionizable groups, resulting in slower migration compared to higher pH (pH 8.6), where proteins are more negatively charged and migrate faster under the influence of the electric field.

16. **Answer D** The proteins are separated into five main fractions: albumin, alpha-1 (α1), alpha-2 (α2), beta (β) and gamma (γ). The β region is sometimes divided into β1 and β2, which would account for a sixth fraction. Alpha-2 macroglobulin and haptoglobin migrate in the alpha-2 fraction with the haptoglobin being slightly more cathodic than the alpha-2 macroglobulin. The beta fraction contains the C3 complement, Transferrin, and beta lipoprotein in which the C3 complement is the most cathodic and the beta lipoprotein is the most anodic from the three.

Reference: Bazydlo LAL and Landers JP. Electrophoresis. In: Rifai N, Horvath AR, Wittwer CT, eds. Tietz Textbook of Laboratory Medicine. 6th ed. Philadelphia, PA: Elsevier; 2017:230.

17. **Answer D** Fibrinogen migrates in the beta 2 fraction

18. **Answer D** Specimens containing fibrinogen will have an abnormal band that migrates to the β2/γ region.

19. **Answer B** Alpha-1 antitrypsin migrates in the alpha-1 fraction, therefore, a decrease in the production of this protein will show a decrease in the alpha-a globulin band.

20. **Answer A** Alpha-1-antitrypsin deficiency is an important cause of pediatric liver disease that can develop at any age. Hepatocellular injury occurs as a result of an abnormally folded Z form of AAT which cannot be excreted from the liver thereby inducing mitochondrial dysfunction, caspase activation and autophagy.

    Reference: Feldman A, Sokol RJ. Alpha-1-Antitrypsin Deficiency: An Important Cause of Pediatric Liver Disease. Lung Health Prof Mag. 2013;4(2):8-11. PMID: 27019872; PMCID: PMC4805363.

21. **Answer C** Alpha-2 macroglobulin is increased in nephrotic syndrome and is observed in the alpha-2 band. Haptoglobin forms a complex with hemoglobin and is observed in the alpha-2 band. Transferrin is increased in iron deficiency anemia and is observed in the beta-1 band. LDL-cholesterol is increased in traditionally hypercholesteremia and is found in the beta-2 band.

22. **Answer A** In nephrotic syndrome, there is an increased urinary excretion of proteins with lower molecular weights. In contrast, the leakage of alpha-2 macroglobulin is impeded because of its larger molecular size. Therefore, there is a heightened prominence of the alpha-2 band, attributed to the high concentration of alpha-2-macroglobulin, which serves to sustain oncotic pressure.

    Reference: de Sain-van der Velden MG, Rabelink TJ, Reijngoud DJ, Gadellaa MM, Voorbij HA, Stellaard F, Kaysen GA. Plasma alpha 2 macroglobulin is increased in nephrotic patients as a result of increased synthesis alone. Kidney Int. 1998 Aug;54(2):530-5. doi: 10.1046/j.1523-1755.1998.00018.x. PMID: 9690220.

23. **Answer B** Large-molecular-weight proteins like fibrinogen have the greatest effect in sedimentation rate, followed by β-globulins and distantly by α2-globulins, γ-globulins, and albumin.

    Reference: Steinvil A, Shapira I, Arbel Y, Justo D, Berliner S, Rogowski O. Determinants of the erythrocyte sedimentation rate in the era of microinflammation: excluding subjects with elevated C-reactive protein levels. Am J Clin Pathol. 2008 Mar;129(3):486-91. doi: 10.1309/U04E2YFJRR6JQQTK. PMID: 18285274.

24. **Answer D** In normal plasma, the yellow color arises from the combination of bilirubin, carotenoids, hemoglobin, and iron transferrin. Estrogen components found in oral contraceptives stimulate the production of ceruloplasmin (Cp), a blue plasma protein containing copper. Ceruloplasmin migrates in the alpha-2 band, and an increase in this band's intensity can be detected as a result of increased Cp. The green plasma is a result of the combination of blue (ceruloplasmin) and yellow (normal serum), becoming more prominent with a concurrent decrease in yellow or red tones.

    Reference: Tovey LA, Lathe GH. Caeruloplasmin and green plasma in women taking oral contraceptives, in pregnant women, and in patients with rheumatoid arthritis. Lancet. 1968 Sep 14;2(7568):596-600. PMID: 4175158.

25. **Answer C** Panel E shows a case of bisalbuminemia characterized by the occurrence of bicuspid electrophoretic pattern in the albumin region. This phenomenon is rarely encountered and incidentally discovered while screening serum samples for protein electrophoresis.

    Reference: Chhabra S, Bansal F, Saikia B, Minz RW. Bisalbuminemia: a rarely encountered protein anomaly. J Lab Physicians. 2013 Jul;5(2):145-6. doi: 10.4103/0974-2727.119869. PMID: 24701116; PMCID: PMC3968632.

26. **Answer D** Panel K shows a pattern of hypogammaglobulinemia where the gamma region is dramatically decreased because of low production of immunoglobulins. Other plasma proteins are normal.

27. **Answer C** Panel I shows a pattern consistent with a monoclonal gammopathy where a narrow and large peak (M spike) is observed in the gamma region. Panel H is incorrect because other plasma proteins should be normal.

28. **Answer C** Panel F shows a pattern compatible with homozygous alpha-1 antitrypsin deficiency, where the alpha 1 region is dramatically suppressed. Ninety-five percent of severe cases of AAT deficiency result from the homozygous substitution of a single amino acid, Glu342Lys (the Z allele).

    Reference: Strnad P, McElvaney NG, Lomas DA. Alpha1-Antitrypsin Deficiency. N Engl J Med. 2020 Apr 9;382(15):1443-1455. doi: 10.1056/NEJMra1910234. PMID: 32268028.

29. **Answer A** Panel A shows a pattern of chronic inflammation, where the alpha-2 and gamma bands are increased, and albumin band is decreased.

30. **Answer A** Panel B shows a pattern of nephrotic syndrome characterized by the increase of alpha-2 fraction and the decrease of albumin and gamma globulins. A fibrinogen peak is observed which migrates in the beta-gamma region and can be confused with a monoclonal peak. Monoclonal gammopathy can be excluded by performing immunofixation electrophoresis or by adding thrombin to assess for clot formation.

    Reference: McCudden CR, Jacobs JFM, Keren D, Caillon H, Dejoie T, Andersen K. Recognition and management of common, rare, and novel serum protein electrophoresis and immunofixation interferences. Clin Biochem. 2018 Jan;51:72-79. doi: 10.1016/j.clinbiochem.2017.08.013. Epub 2017 Aug 24. PMID: 28843491.

31. **Answer C** Capillary electrophoresis (CE) displays the probable M-spike peak on alpha-2 region. Serum immunofixation electrophoresis shows no signs of monoclonal protein. False positive serum CE such as this can occur due to hemolyzed samples. Other forms of interference in CE include any analytes that can be detected at 200 nm. Some examples include contrast dyes and antibiotics.

Reference: Recognition and management of common, rare, and novel serum protein electrophoresis and immunofixation interferences. Clin Biochem. 2018 Jan;51:72-79. doi: 10.1016/j.clinbiochem.2017.08.013. Epub 2017 Aug 24. PMID: 28843491.

32. Answer D The source of band-broadening in CE is the result of longitudinal diffusion. Under these conditions, the number of theoretical plates can be given by the expression:

$$N = \mu EL_d/2D \text{ or } N = \mu VL_d/2DL$$

where D is the diffusion coefficient of the analyte, μ is the electrophoretic mobility of the analyte, E is the electric field strength, L is the total length of the capillary, Ld is the distance from the point of application to the detector, and V is the applied voltage.

$N = \mu EL_d/2D$

$= [(1.80\ cm^2/kV{\cdot}min)(25\ kV)(22.0cm)]/[2(2.0 \times 10^{-7}\ cm^2/s) \times (60s/min)(25.0\ cm)]$

$= 1.7 \times 10^6$ theoretical plates

Reference: David S. Hage and James D. Carr. Analytical Chemistry and Quantitative Analysis, 2011. Prentice Hall.

33. Answer D SPEP displays the M-spike peak starting from the end of beta and beginning of gamma region. Serum immunofixation electrophoresis shows the presence of associated IgA and kappa chain specificity. This shows monoclonal protein with IgA kappa chain specificity.