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Interferences in Protein Electrophoresis

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Learning Objectives

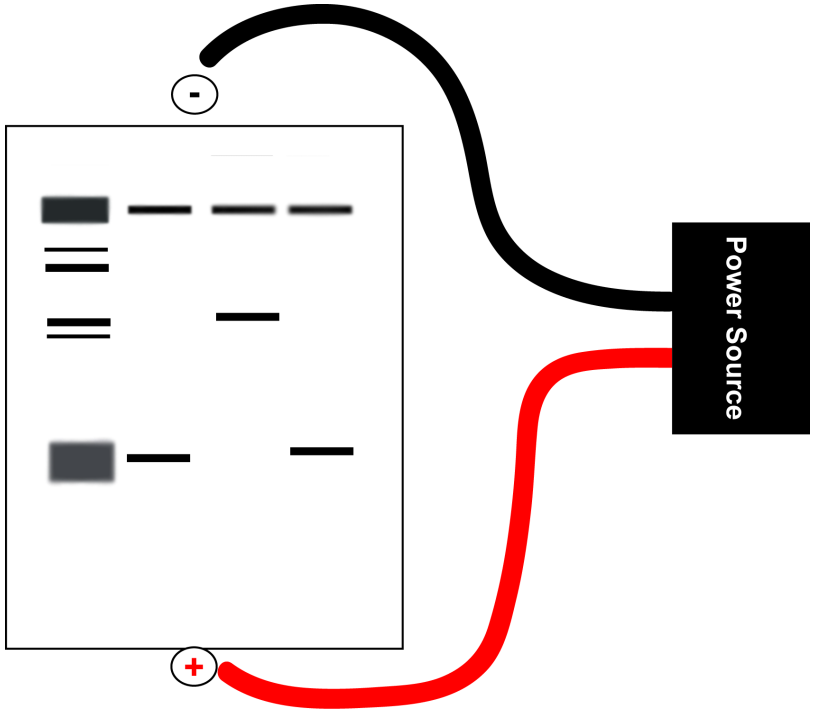
- Identify a variety of electrophoresis formats
- Differentiate various analytical interferences in protein electrophoresis
- Summarize approaches for minimizing analytical interferences in protein electrophoresis

Gel Electrophoresis

Migration of charged particles in a gel relative to a fluid under the influence of an electric field

Features of electrophoresis

- Support Media (various pore sizes)
  - Agarose
- Buffer: pH ~8.6
- Detection System
  - Visualizing Stains - Densitometry

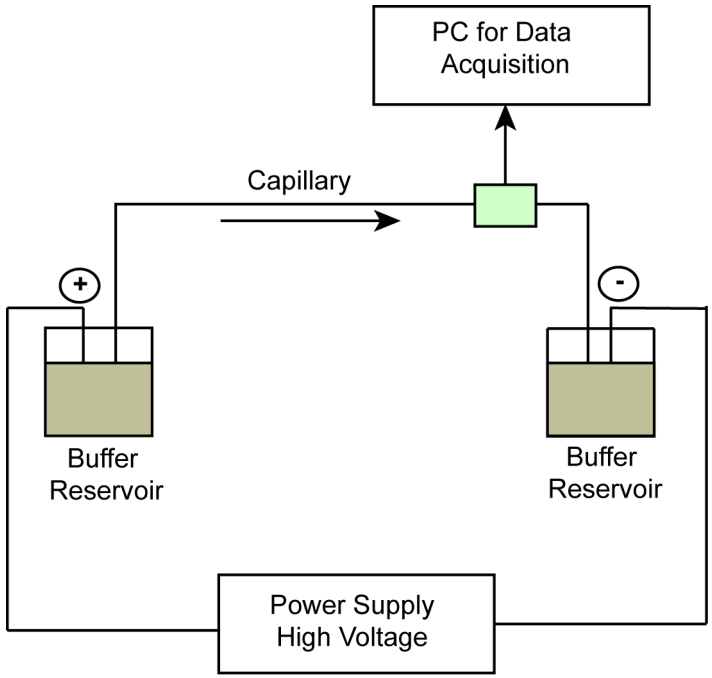


Capillary Electrophoresis (CE)

Electrophoretic separation in a capillary tube. Movement of proteins with the flow of the buffer, which is due to electroosmotic force.

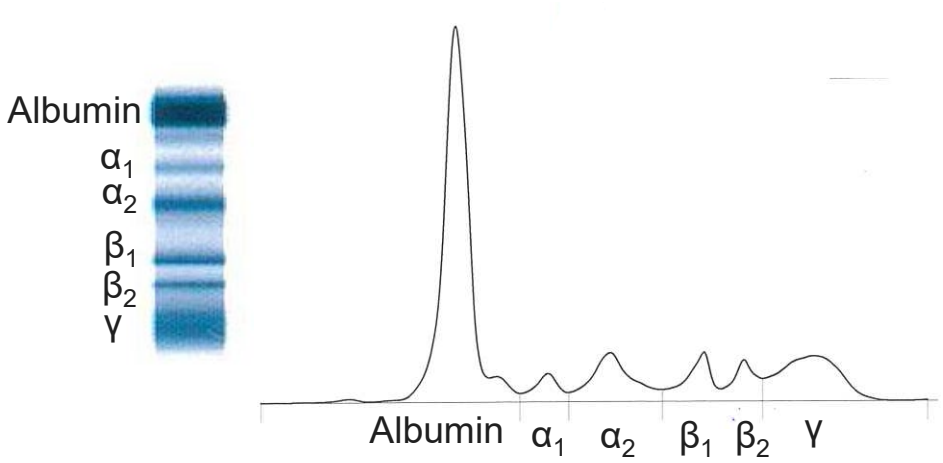
Features of CE

- Alkaline buffer
- Faster separation due to higher voltages (25–30 kV)
- UV detection (200 nm)



Protein Electrophoresis

Protein pattern separated into albumin or globulins (α_1,α_2,β, and γ)

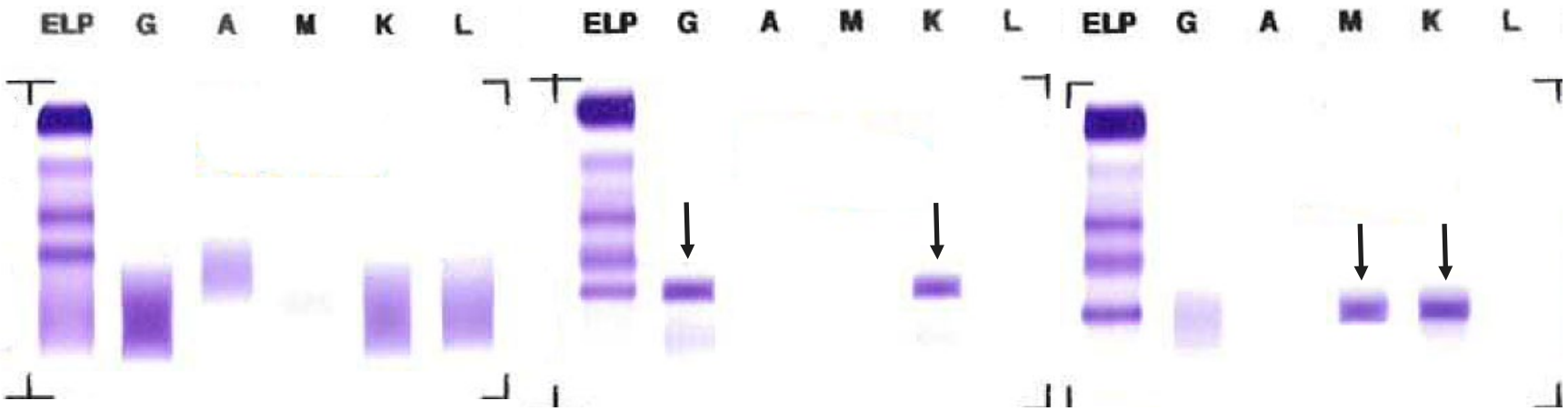


Images: Courtesy of Protein Immunology Laboratory, ARUP Laboratories

- Albumin: Most abundant protein component of human serum
- α_1: α_1-antitrypsin, α_1-acid glycoprotein, α_1-antichymotrypsin, lipoprotein
- α_2: α_2-macroglobulin, haptoglobulin, ceruloplasmin
- β: β_1 – Transferrin, β-lipoprotein, C4; β_2 – C3, fibrinogen (plasma)
- γ: Immunoglobulins, C-reactive protein

Immunofixation (IFE)

Following electrophoresis, specific anti-immunoglobulin antisera are used to characterize the specific monoclonal protein.



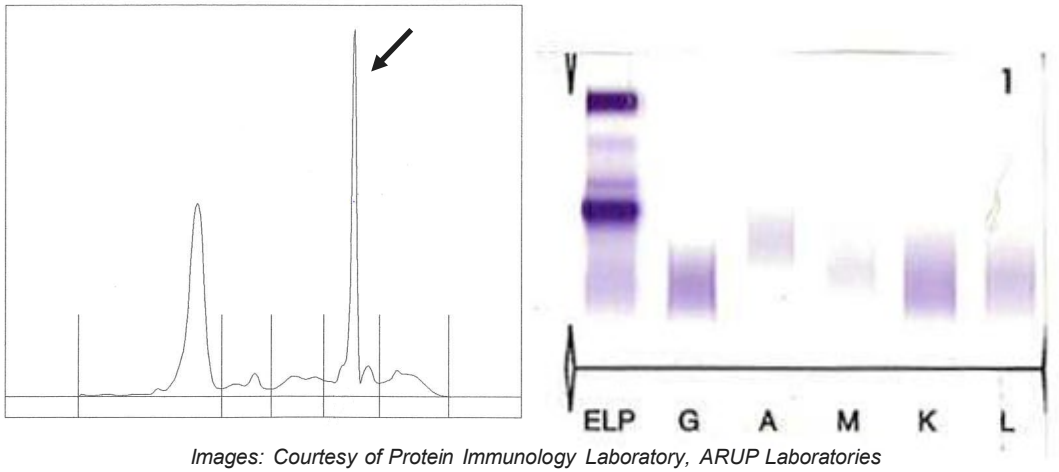
Normal IFE | IgG Kappa | IgM Kappa

Images: Courtesy of Protein Immunology Laboratory, ARUP Laboratories

- Used to diagnose and monitor multiple myeloma or Waldenstrom's macroglobulinemia
- Better analytical sensitivity than protein electrophoresis

Interference – Hemolysis

Hemolysis – Additional discrete bands on α_2/β regions

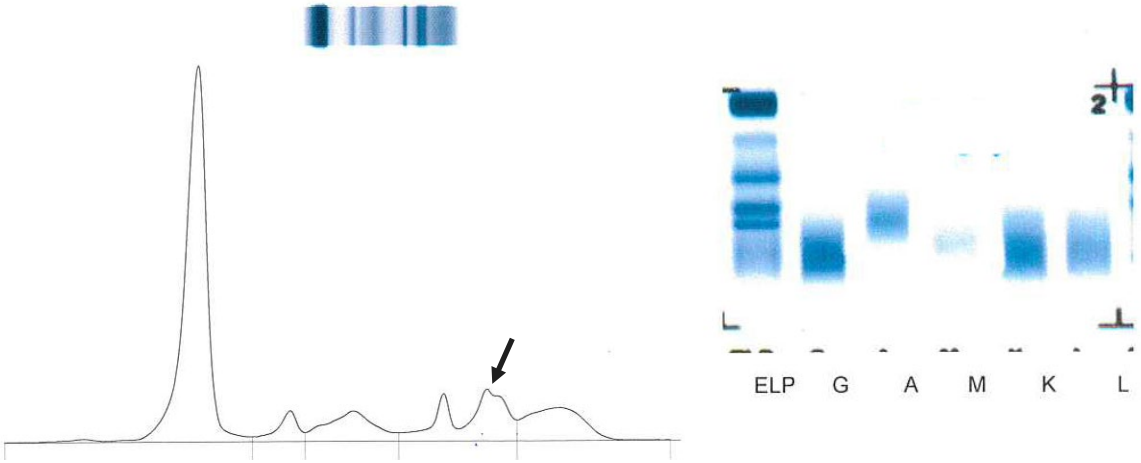


Images: Courtesy of Protein Immunology Laboratory, ARUP Laboratories

- Improper sample collection – mechanical rupture or prolonged storage
- *In vivo* hemolysis may occur due to pre-eclampsia, hemolytic anemia, or sickle cell disease
- Additional bands may be misinterpreted as monoclonal proteins

Interference – Fibrinogen

Fibrinogen – migrates in the β/γ region

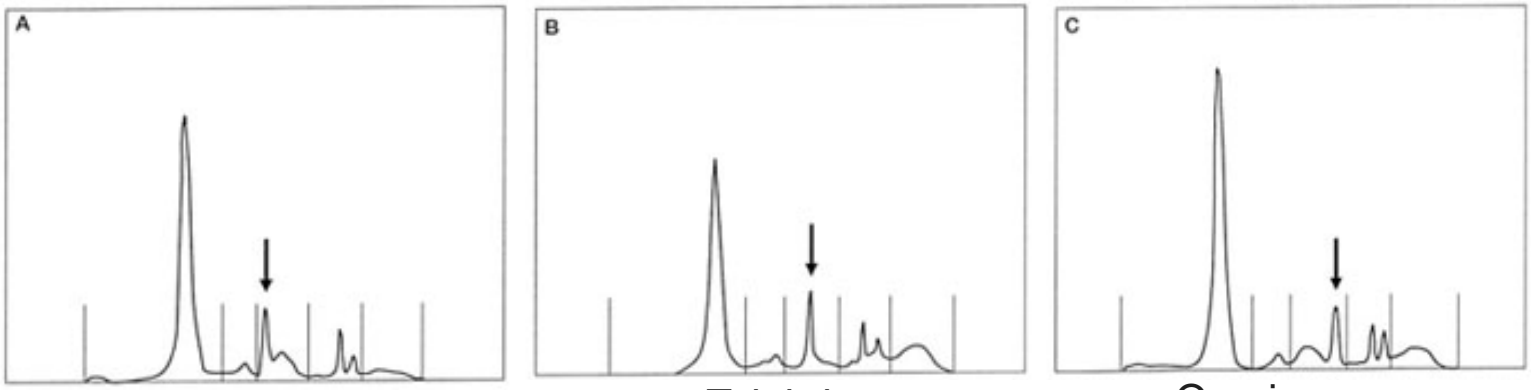


Images: Courtesy of Protein Immunology Laboratory, ARUP Laboratories

- Erroneous sample collection of plasma instead of serum
- IFE can rule out monoclonal protein from fibrinogen interference
- Treatment with thrombin or ethanol removes the fibrinogen peak
- Quantofix EDTA to identify EDTA samples

Interference – Contrast Dyes

Contrast Dyes – Interference in α_2 region

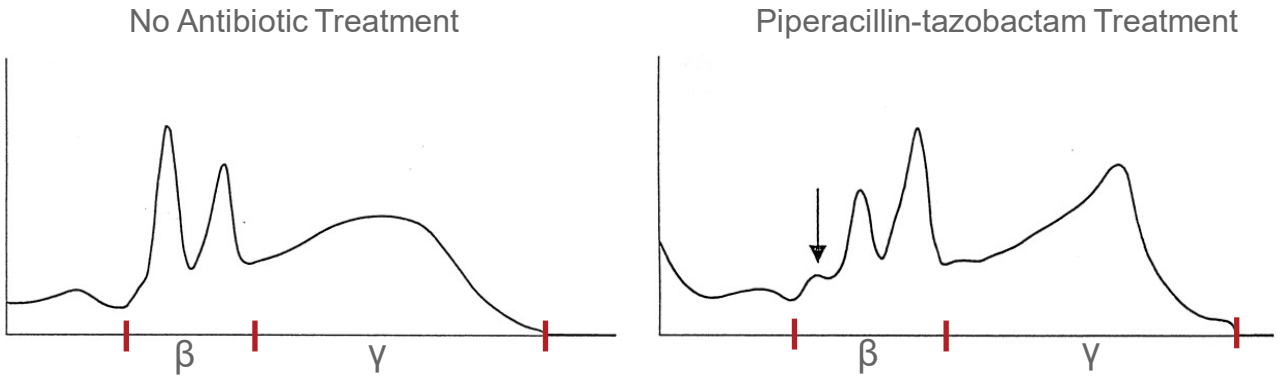


- Contrast dyes absorb light at ~200 nm, which is the same wavelength used to quantify proteins in CE
- Contrast dyes have no effect on protein gel electrophoresis or immunofixation

Bossuyt, X. Clin Chem, 1999;45: 129-131.

Interference – Antibiotics

Antibiotics – Interference in α or β region



- Antibiotics, such as piperacillin-tazobactam may produce an additional spike between α_2 and β_1 region
- Other antibiotics cause interference in CE: ceftriaxone, 5-flurocystosine (5-FC), and sulfamethoxazole
- Draw samples at trough to minimize interference

Modified from Bossuyt, X. Clin Chem, 2002;48: 204-205.

Monoclonal Antibody (mAb) Therapy

- Monoclonal antibody (mAb) therapy, such as daratumumab (Dara) and elotuzumab, are used for the treatment of relapsed or refractory multiple myeloma (MM)

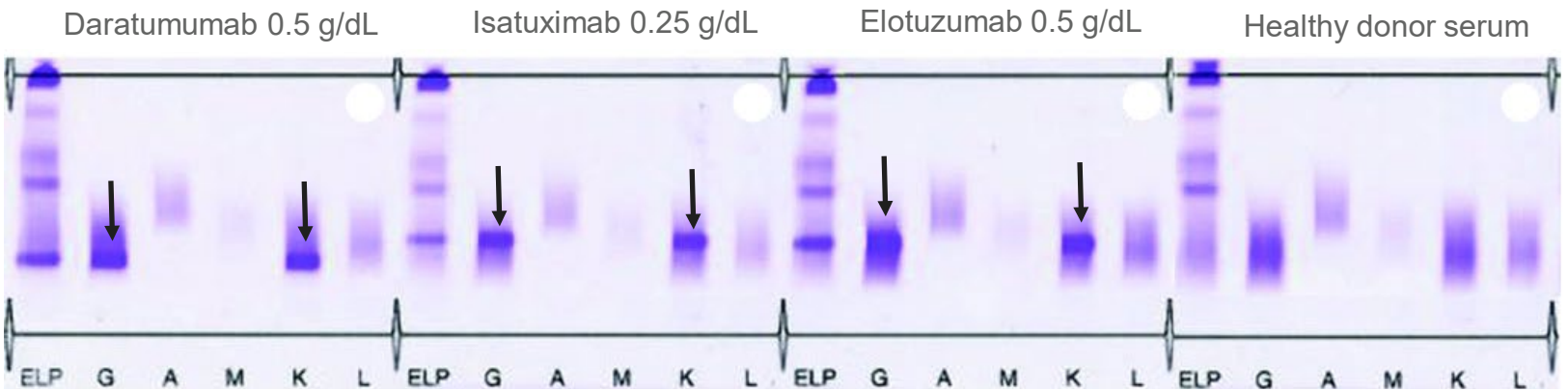
- Isatuximab is under FDA review for MM treatment

- Many mAb therapies are IgG κ antibody

Lazar-Molnar, E and Delgado, J. Clin Chem 2019; 65, doi:10.1373

Interference – Monoclonal Antibody Therapy

Monoclonal Therapy – Appears as monoclonal IgG κ in IFE

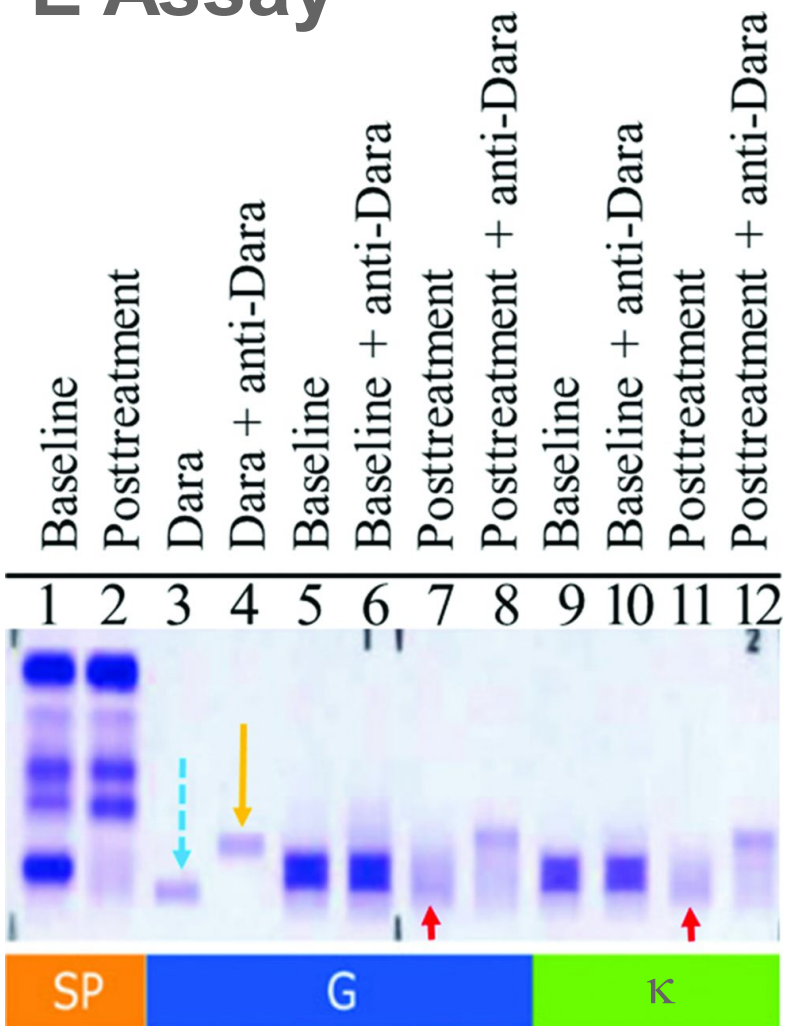


- Interfere with protein electrophoresis and IFE even weeks after treatment
- May lead to unnecessary additional investigation and disease misclassification

Modified from Mills, JR and Murray, DL. JALM 2017; 421-431.

Daratumumab-specific IFE Assay

- IFE assay with daratumumab-specific antibody (DIRA) that shifts the migration of Dara (control lanes 3 and 4)
- IFE is performed using both IgG and κ antisera



McCudden, C. et al. Clin Chem Lab Med 2016; 1095-1104.

Mass Spectrometry Methods to Detect mAb Therapy

MASS-FIX (MALDI-TOF Mass Spectrometry)

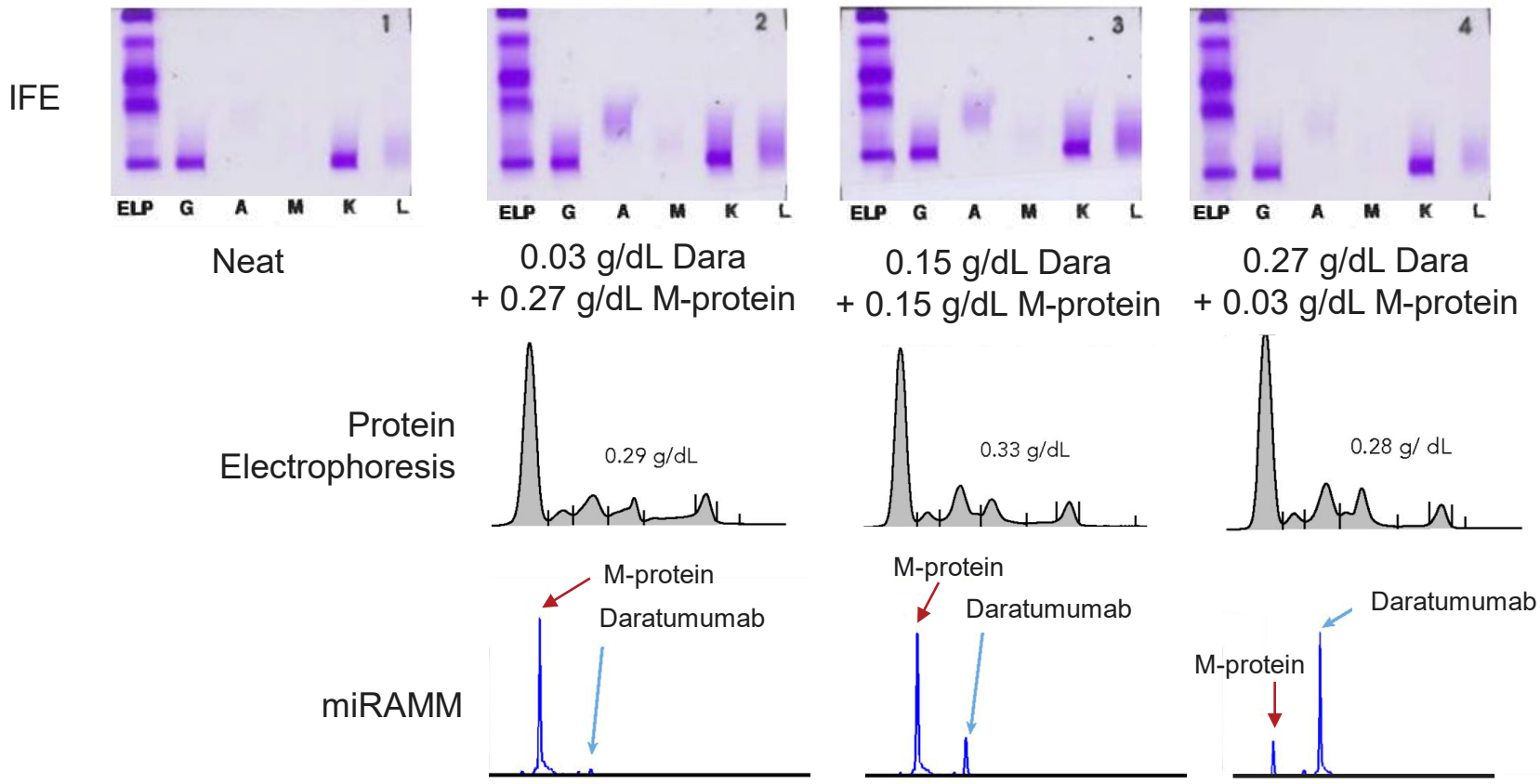
- Uses the mass of Dara to differentiate from an actual M-spike
- Distinguishes Dara from IgG κ M-protein in 84% of samples
- Not all samples are distinguishable

miRAMM (Monoclonal-immunoglobulin-rapid-accurate mass measurement)

- Uses microflow liquid chromatography-ESI-TOF MS to measure accurate molecular mass of the light chain portion of mAbs
- Still impractical for routine use due to long run time
- Not used in clinical laboratory yet

Moore, LM., et al. Clinica Chimica Acta 2019; 492:91-94.
Mills, JR et al. Blood 2018; 132: 670-672.

Performance of miRAMM compared to IFE



- Accurately separates endogenous M-protein from the signal produced by Dara

Modified from Mills, JR et al. Blood 2018; 132: 670-672..

Antigen Specific therapeutic monoclonal Antibody Depletion Assay (ASADA)

- Use of cognate antigen of the therapeutic antibodies
  - Magnetic beads coated with antigen to therapeutic antibodies (daratumumab or elotuzumab) to deplete the therapeutic antibodies
  - Dynabead coated with His-tagged CD38 or SLAMF7

- ASADA is highly specific and bypasses the development of new anti-sera for each new therapeutic antibodies
  - ASADA treatment specifically depleted daratumumab in 12 patient samples who had daratumumab therapy
  - Only 1 patient sample confirmed with daratumumab therapy did not show daratumumab depletion after ASADA treatment. The high concentration of endogenous IgG/κ co-migrated with daratumumab, causing persistent cathodal IgG/κ even after ASADA treatment.

Liu, L et al. Clin. Bioc. 2020; 75: 40-47.

Interferences in Protein Electrophoresis

Interfering Agent	Methods Affected	Resolution
Hemolysis	Gel electrophoresis and CE	IFE, awareness in the result interpretation
Fibrinogen	Gel electrophoresis and CE	IFE, thrombin treatment, rule out EDTA sample, awareness in the result interpretation
Contrast Dyes	CE	IFE
Antibiotics	CE	IFE
Monoclonal Antibody Therapy	Gel electrophoresis, CE, and IFE	Specific-mAb shift assay (eg. DIRA), mass spectrometry based identification, or Antigen Specific therapeutic monoclonal Antibody Depletion Assay (ASADA)

CE: capillary electrophoresis, IFE: immunofixation

Summary

- Recognizing various interferences will help determine the right method for resolution
- Monoclonal antibody therapy interference may be resolved by DIRA, mass spectrometry based assays, or ASADA
- Identification of interferences minimizes unnecessary follow-up tests on patients

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Disclosures/Potential Conflicts of Interest

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