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 (α₁, α₂, β, and γ)

- Albumin: Most abundant protein component of human serum
- α₁: α₁-antitrypsin, α₁-acid glycoprotein, α₁-antichymotrypsin, lipoprotein
- α₂: α₂-macroglobulin, haptoglobin, ceruloplasmin
- β: β₁ – Transferrin, β-lipoprotein, C4; β₂ – C3, fibrinogen (plasma)
- γ: Immunoglobulins, C-reactive protein

Images: Courtesy of Protein Immunology Laboratory, ARUP Laboratories
Immunofixation (IFE)

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

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

Images: Courtesy of Protein Immunology Laboratory, ARUP Laboratories
Interference – Hemolysis

Hemolysis – Additional discrete bands on $\alpha_2/\beta$ regions

- 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

Images: Courtesy of Protein Immunology Laboratory, ARUP Laboratories
Interference – Fibrinogen

Fibrinogen – migrates in the $\beta/\gamma$ 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 $\alpha_2$ 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

Interference – Antibiotics

Antibiotics – Interference in $\alpha$ or $\beta$ region

- Antibiotics, such as piperacillin-tazobactam may produce an additional spike between $\alpha_2$ and $\beta_1$ region
- Other antibiotics cause interference in CE: ceftriaxone, 5-flurocystosine (5-FC), and sulfamethoxazole
- Draw samples at trough to minimize interference

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
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

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

Performance of miRAMM compared to IFE

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

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.

# Interferences in Protein Electrophoresis

<table>
<thead>
<tr>
<th>Interfering Agent</th>
<th>Methods Affected</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolysis</td>
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</tr>
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</tr>
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<td>Specific-mAb shift assay (eg. DIRA), mass spectrometry based identification, or Antigen Specific therapeutic monoclonal Antibody Depletion Assay (ASADA)</td>
</tr>
</tbody>
</table>

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
References

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