Serology's Role in Diagnosing Myeloma

Clinical Context For Testing, Diagnosis, and Management

Taylor Jackson, DO, MLS (ASCP)^{CM}

University of Utah Pathology

JULY 19, 2024

Objectives

- Contrast SPEP/IFE test methods and their combined use in generating an interpretation
- Identify the clinical context when SPEP/IFE testing is indicated and what follow-up testing may be needed
- Understand potential test interferences caused by treatment of myeloma
- Review treatment and management following diagnosis in our clinical cases

Disclosures

- None

Helpful Acronyms

- SPEP: Serum protein electrophoresis
- UPEP: Urine protein electrophoresis
- IFE: Immunofixation Electrophoresis
- FLC: Free light chains

Plasma Cell Dyscrasias

- Plasma cell proliferative disorders:
  - » Plasma Cell Myeloma: aka multiple myeloma
  - » MGUS: Monoclonal gammopathy of undetermined significance
  - » Smoldering multiple myeloma (SMM)
  - » Waldenström's Macroglobulinemia
  - » Solitary plasmacytoma
  - » Primary Amyloidosis

Clinical Case #1

- 51yo M
- Presented May 2023 to the Emergency Department after worsening cognitive changes
  - » Forgetfulness, slowing of daily activities (dressing, speaking, doing errands), changes in balance
- No significant past medical history, no recent changes in medications
- Works as a mechanical engineer. His wife initially attributed fatigue and changes to demanding job, but symptoms persisted and worsened during a vacation.
- No pertinent family history
- Lost significant amount of weight over the last year (15lbs in 2 months)

Clinical Case #1, cont.

- Normal physical exam
- Tests ordered in the ED: CBC, CMP, Troponin, TSH, UA, 12-lead EKG, CT Head without contrast, CT Chest without contrast,

Comprehensive Metabolic Panel

Result	Value	Ref Range
Sodium S/P	109 (LL)	136 - 144 mmol/L
Potassium	3.4	3.3 - 5.0 mmol/L
Chloride	93 (L)	102 - 110 mmol/L
Carbon Dioxide	23	20 - 26 mmol/L
BUN	30 (H)	8 - 24 mg/dL
Creatinine S/P	2.35 (H)	0.72 - 1.25 mg/dL
Glucose	83	64 - 128 mg/dL
Anion Gap	-7 (L)	8 - 14 mmol/L
Calcium	10.4	8.4 - 10.5 mg/dL
Protein, Total, S/P	>12.3 (H)	6.5 - 8.4 g/dL
Albumin	2.0 (L)	3.5 - 5.0 g/dL
Bilirubin, Total	0.6	0.2 - 1.4 mg/dL
Alkaline Phosphatase	43	38 - 126 U/L
AST	45 (H)	16 - 40 U/L
ALT	20	5 - 60 U/L
eGFR, CKD-EPI CRT 2021	32	mL/min/1.73m2

Result	Value	Ref Range
WBC	6.54	4.30 - 11.30 k/uL
Hemoglobin	7.6 (L)	14.8 - 17.8 g/dL
Hematocrit	22.9 (L)	44.2 - 53.0 %
Platelet	103 (L)	159 - 439 k/uL
Mean Corpuscular Volume	103.6 (H)	81.2 - 96.6 fL
Red Blood Cell	2.21 (L)	4.70 - 6.14 M/uL
Mean Corpuscular Hemoglobin	34.4 (H)	25.8 - 33.1 pg
Mean Corpuscular HGB Concentration	33.2	31.9 - 35.2 g/dL
Red Cell Distribution Width	17.6 (H)	11.5 - 15.3 %
Mean Platelet Volume	9.8	8.6 - 12.3 fL
Neutrophil %		
NRBC %	7.0 (H)	0.0 - 0.0 %
NRBC #	0.46 (H)	0.00 - 0.01 k/uL

Clinical Case #1, cont.

- Additional Laboratory Tests and CT Imaging findings
- Mild elevation in Troponin
- All other tests within normal range
- Admitted to the hospital with additional workup

Differential Confirmation, Manual

Result	Value	Ref Range
Neutrophil %	52	39 - 73 %
Neutrophil # (ANC)	3.4	k/uL
Lymphocyte %	16 (L)	17 - 50 %
Monocyte %	10	4 - 13 %
Myelocyte %	1 (H)	0 - 0 %
NRBC %	3 (H)	0 - 0 %
Plasma cell %	21	%
Lymphocytes Abnormal	Not Applicable	Not Applicable
Lymphocytes Reactive	Not Applicable	Not Applicable, See Note
Platelet Estimate	Decreased (A)	Adequate
Path Review Status	Pending	

Impression:

1. No acute intracranial abnormality. No intracranial hemorrhage or mass.
2. Numerous lytic osseous lesions throughout the calvarium, skull base, and upper cervical spine. The differential includes lytic metastasis, multiple myeloma, and lymphoma. Correlate with any history of malignancy and consider MRI with contrast if clinically indicated.

Clinical Suspicion for MM

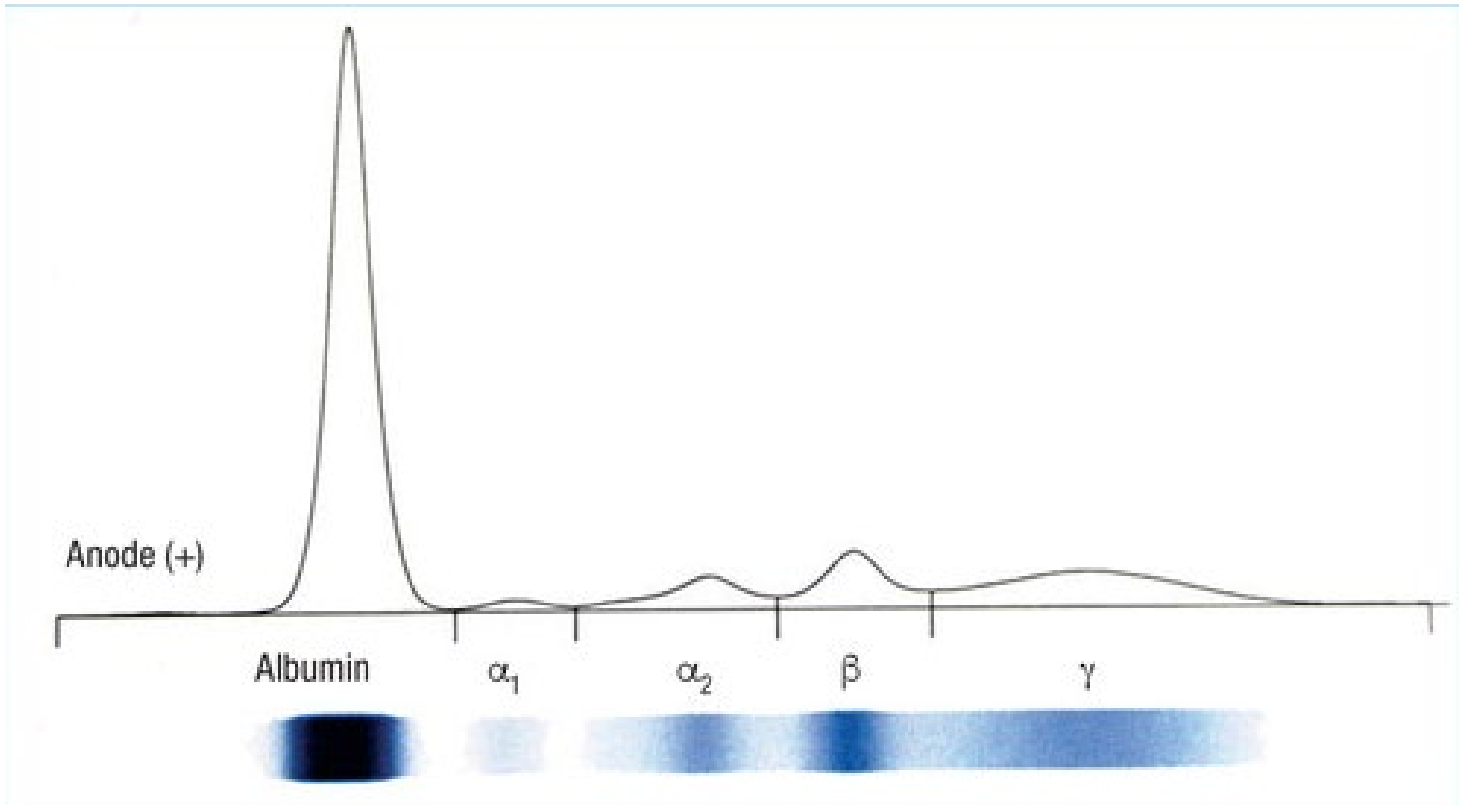
S. VINCENT RAJKUMAR

TABLE 1. International Myeloma Working Group Diagnostic Criteria for Multiple Myeloma and Related Plasma Cell Disorders

Disorder	Disease Definition
Non-IgM MGUS	All three criteria must be met:
	Serum monoclonal protein (non-IgM type) < 3 g/dL
	Clonal bone marrow plasma cells $< 10\%$*
	Absence of end-organ damage such as CRAB features that can be attributed to the plasma cell proliferative disorder
Smoldering MM	Both criteria must be met:
	Serum monoclonal protein (IgG or IgA) ≥ 3 gm/dL, or urinary monoclonal protein ≥ 500 mg per 24 h and/or clonal bone marrow plasma cells 10%–60%
	Absence of myeloma-defining events or amyloidosis
MM	Both criteria must be met:
	Clonal bone marrow plasma cells $\geq 10\%$ or biopsy-proven bony or extramedullary plasmacytoma
	Any one or more of the following myeloma defining events:
	Evidence of end organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically:
	Hypercalcemia: serum calcium > 0.25 mmol/L (> 1 mg/dL) higher than the upper limit of normal or > 2.75 mmol/L (> 11 mg/dL)
	Renal insufficiency: creatinine clearance < 40 mL/min or serum creatinine > 177 µmol/L (> 2 mg/dL)
	Anemia: hemoglobin value of > 2 g/dL below the lower limit of normal, or a hemoglobin value < 10 g/dL
	Bone lesions: one or more osteolytic lesions on skeletal radiography, CT, or PET-CT
	Clonal bone marrow plasma cell percentage $\geq 60\%$
	Involved: uninvolved serum FLC ratio ≥ 100 (involved FLC level must be ≥ 100 mg/L)
	> 1 focal lesion on MRI studies (at least 5 mm in size)

Protein
Ele c tro p h o re sis

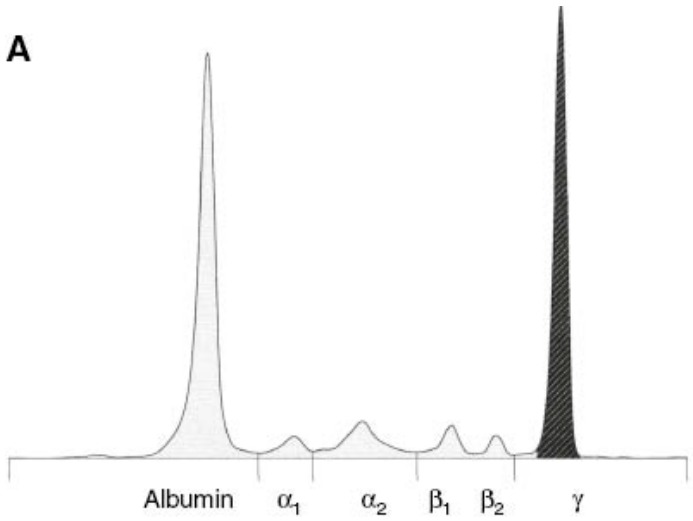
- Visual representation of proteins present in serum/urine
- Originally performed on acetate or agarose gel
- Electrical current applied and the proteins separate by charge, negative charges moving towards the anode
- Albumin most abundant protein in serum and most negatively charged (moves farthest towards the anode).
- Now mostly performed by Capillary electrophoresis



https://www.thebloodproject.com/ufaq/what-is-an-spep/

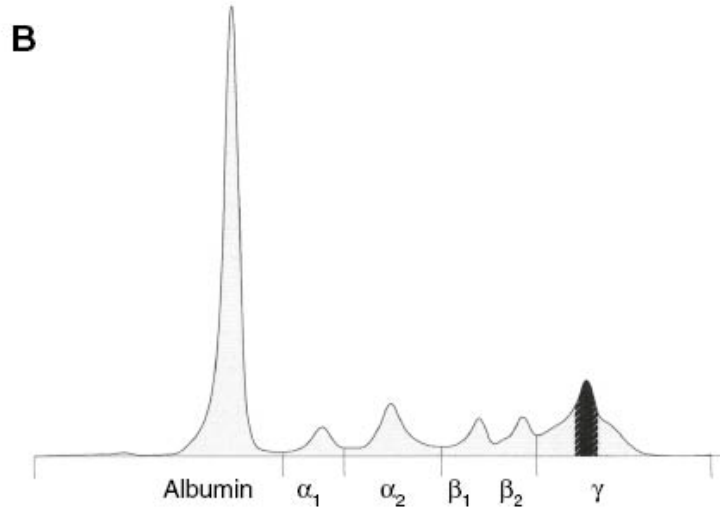
Monoclonal protein spike

- Normal gamma region should be rounded – polyclonal production of many different immunoglobulins
- Neoplasms that produce monoclonal proteins with produce a characteristic monoclonal protein spike, “M-spike”.
- M-spikes may be seen in plasma cell myeloma, Waldenström macroglobulinemia, smoldering myeloma, or MGUS.
- Most frequently seen in the gamma region, but may be seen in the other regions (beta, alpha-2, rarely alpha-1).



Total protein: 9.7 g/dL (6.0–8.3)
A/G: 0.83

Fractions	%	g/dL		Ref. g/dL
Albumin	45.4	4.40		3.43–4.84
α_1	3.4	0.33		0.21–0.44
α_2	8.4	0.81		0.54–0.97
$\beta_{1\,\&\,2}$	6.2	0.60	L	0.65–1.03
γ	36.6	3.55	H	0.70–1.47
M-Spike	35.7	3.46		

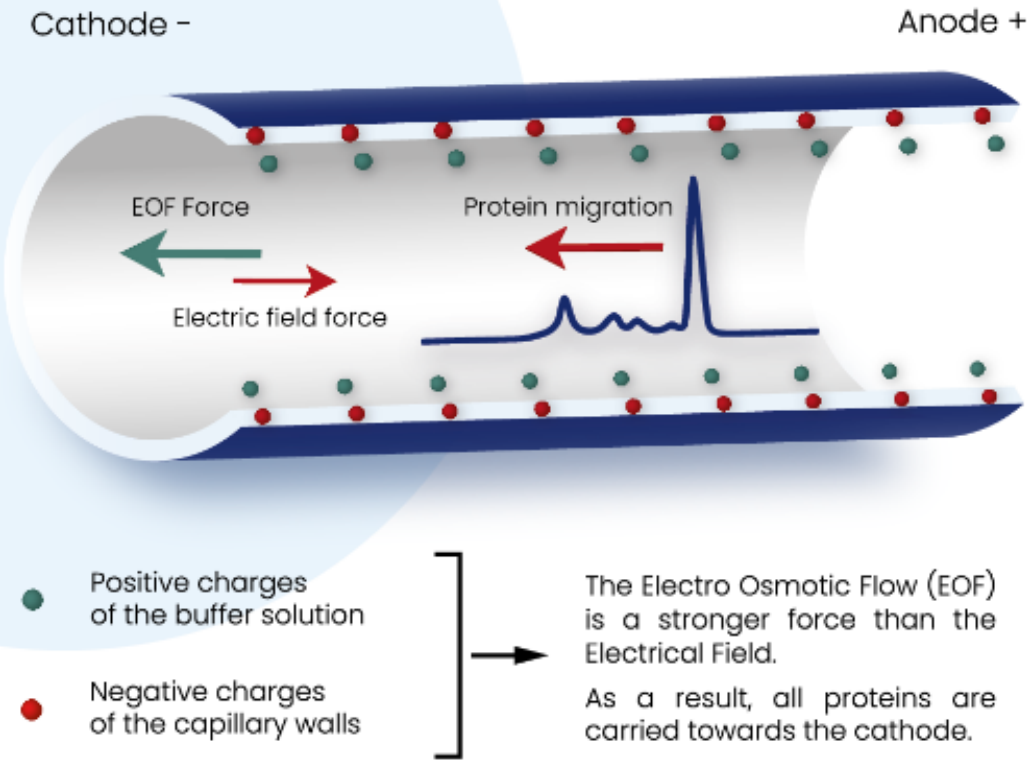


Total protein: 7.1 g/dL (6.0–8.3)
A/G: 1.12

Fractions	%	g/dL	Ref. g/dL
Albumin	52.9	3.76	3.43–4.84
α_1	4.8	0.34	0.21–0.44
α_2	11.2	0.80	0.54–0.97
$\beta_{1\,\&\,2}$	11.5	0.82	0.65–1.03
γ	19.6	1.39	0.70–1.47
M-Spike	8.3	0.59	

Capillary
Ele c tro p h o re sis

- Instead of being applied to and moving through a solid matrix (agarose gel), the sample is added to a buffer and moved through a silica capillary with an electric field generating electroosmotic flow.
- Proteins are separated based on electrical charge and electroosmotic flow.
- A detector monitors flow through the capillary and records the separating bands.



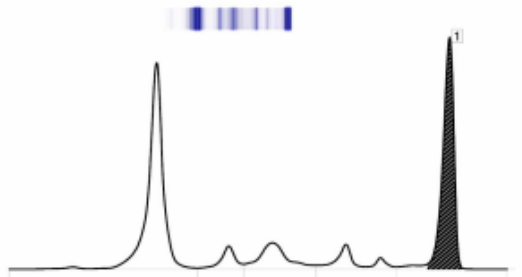
https://www.sebia.com/en-us/technologies/capillary-electrophoresis/

Clinical Case #1, cont.

- SPEP/IFE, June 2023
- Quantitative Immunoglobulins
  - » IgG – 3005 mg/dL (768-1632 mg/dL)
  - » IgA – 24 mg/dL (68-408 mg/dL)
  - » IgM – 17 mg/dL (35-263 mg/dL)
- Serum Free Light Chains
  - » Kappa LC – 260.57 mg/L (3.30-19.40 mg/dL)
  - » Lambda LC – 7.81 mg/dL (5.71-26.30 mg/dL)
  - » K/L Ratio – 33.36 (0.26-1.65)

Total Protein, Serum	7.5 g/dL		(Ref Interval: 6.3-8.2)
Albumin	**3.14 g/dL**	**L**	(Ref Interval: 3.75-5.01)
Alpha 1 Globulin	0.35 g/dL		(Ref Interval: 0.19-0.46)
Alpha 2 Globulin	0.76 g/dL		(Ref Interval: 0.48-1.05)
Beta Globulin	0.53 g/dL		(Ref Interval: 0.48-1.10)
Gamma	**2.72 g/dL**	**H**	(Ref Interval: 0.62-1.51)
Monoclonal Protein	2.62 g/dL		
Immunofixation	IFE Done		

Electrophoresis Image



SPEP/IFE Interpretation	See Note Monoclonal spike in the gamma region. IFE gel pattern shows an IgG type kappa monoclonal protein. This test has been reviewed and approved by Abdulrahman Saadalla, M.B., B.Ch.

Clinical Case #1, cont.

- SPEP/IFE, June 2023
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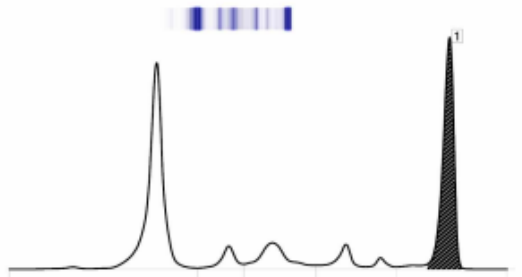

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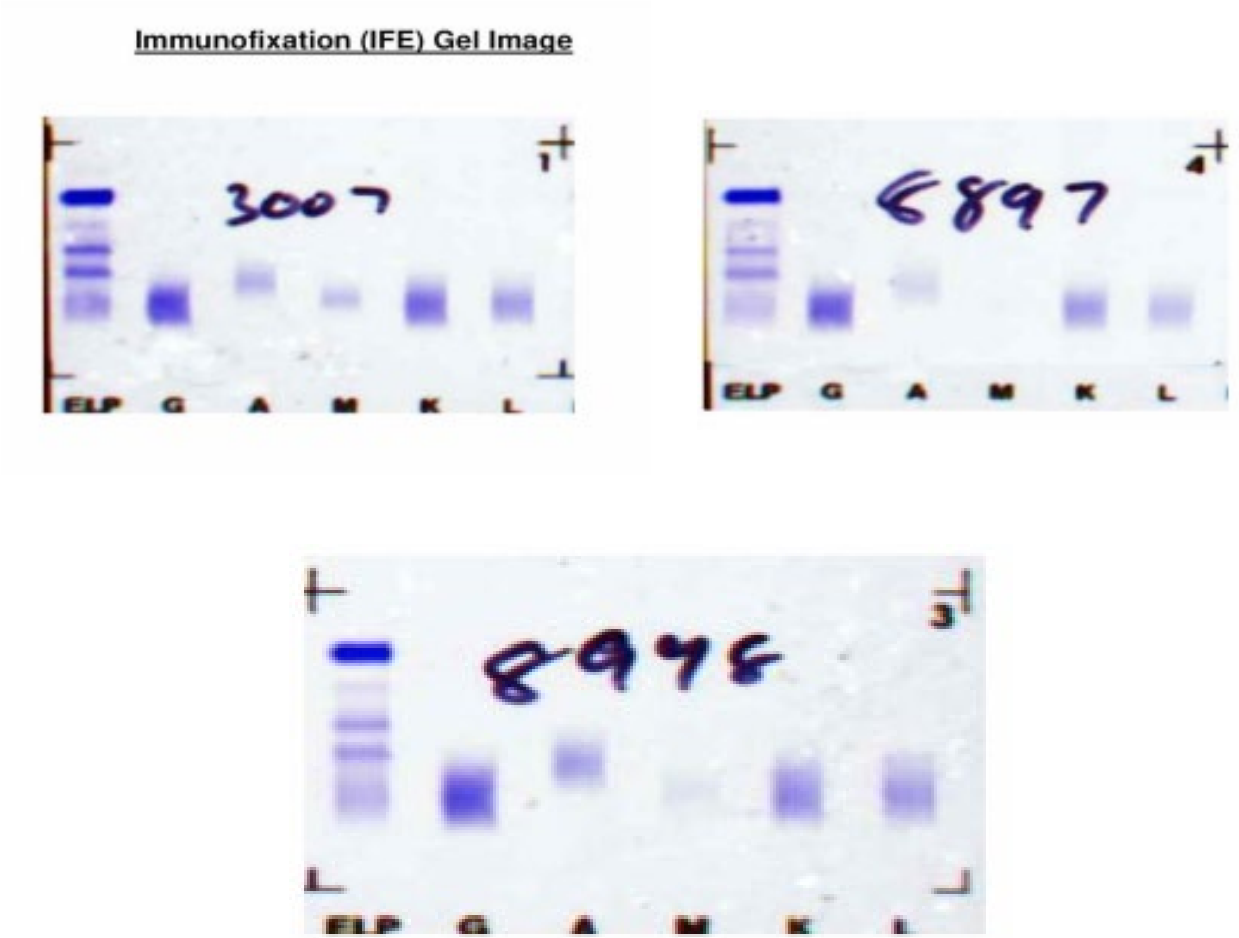
Immunofixation Electrophoresis

- Used to characterize the proteins within a sample
- Similar to protein electrophoresis, the proteins within a sample are separated within a gel matrix by applying an electric current and migrating based on electrical charge.
- After separation, an antisera is applied to the migration lanes and binds to the proteins.
- The lanes are then stained, and the gel is dried and can be evaluated for presence of discreet bands.



Monoclonal protein IFE

- IFE is used to characterize both the heavy and light chains when M-spikes are detected on SPEP.
- Most often a monoclonal heavy chain will have an associated monoclonal light chain, but heavy-chain only and light chain-only diseases are also seen.
- Urine IFE may be performed to evaluate for excess free light chain in additional to characterizing whether kappa or lambda light chains are produced.

Immunofixation Electrophoresis

- ARUP used Sebia Hydrasys Platform
- Patient sample is dilute and pipetted into combs that correspond to the lanes on the gel.
- After migration, antisera specific to IgG, IgA, IgM, Kappa LC, and Lambda LC, as well as non-specific protein antiserum is applied to the gel and incubated.
- The gel is then stained and dried.

https://www.abacusdx.com/electrophoresis/sebia-gel-electrophoresis/

Clinical Case #1, cont.

- SPEP/IFE, June 2023
- Quantitative Immunoglobulins
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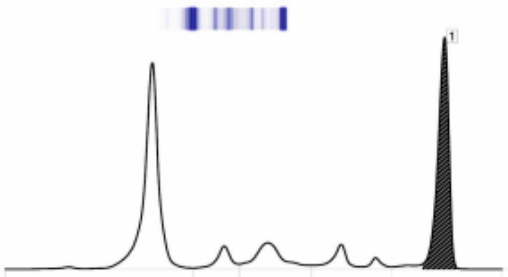
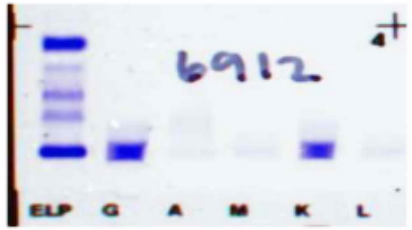
Gamma	**2.72 g/dL**	**H**	(Ref Interval: 0.62-1.51)
Monoclonal Protein	2.62 g/dL		
Immunofixation	IFE Done		

Electrophoresis Image



Immunofixation (IFE) Gel Image



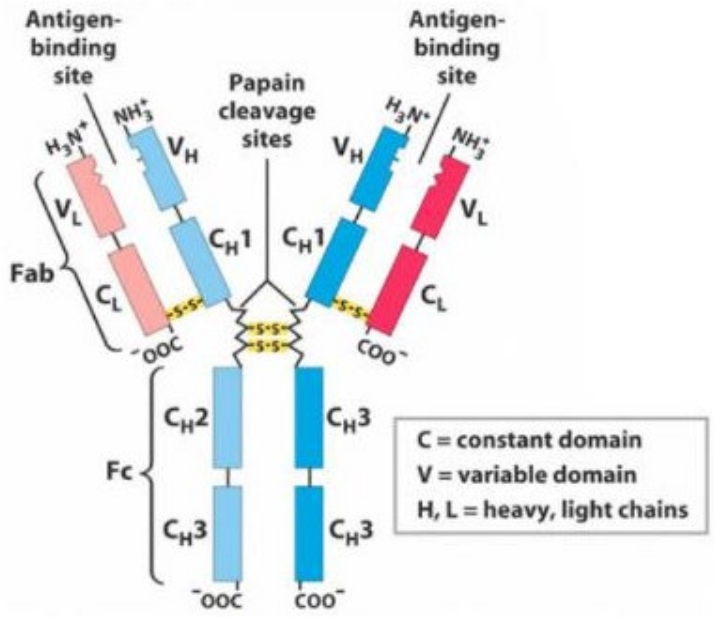
SPEP/IFE Interpretation

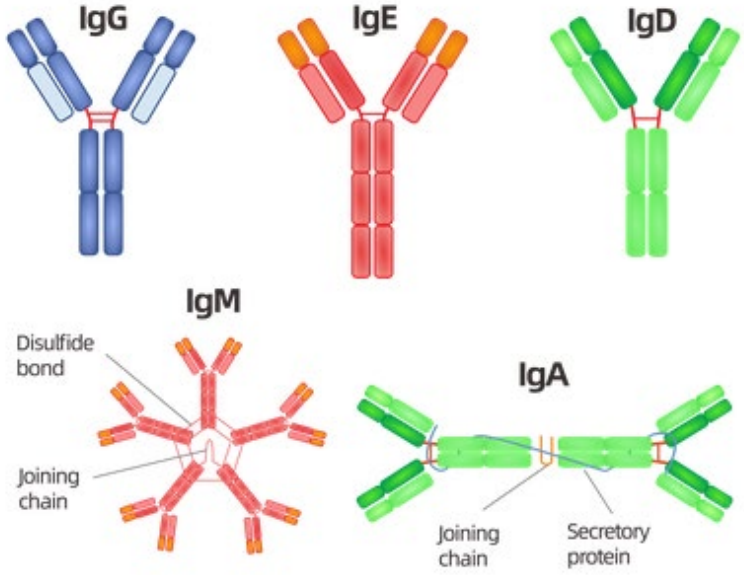
See Note

Monoclonal spike in the gamma region. IFE gel pattern shows an IgG type kappa monoclonal protein.
This test has been reviewed and approved by Abdulrahman Saadalla, M.B., B.Ch.

Antibody Structure

- Antibodies are produced by first by B-cells and expressed on the cell surface or freely circulating. Later produce by plasma cells.
- The heavy chain determines what isotype and includes the Fc and part of the Fab portion of the antibody.
- The light chain is only part of the Fab portion.
- Both Heavy and Light chain regions of the Fab portion contain a constant and variable region.
- IgM produced first and then cell signaling causes plasma cells to switch isotypes.

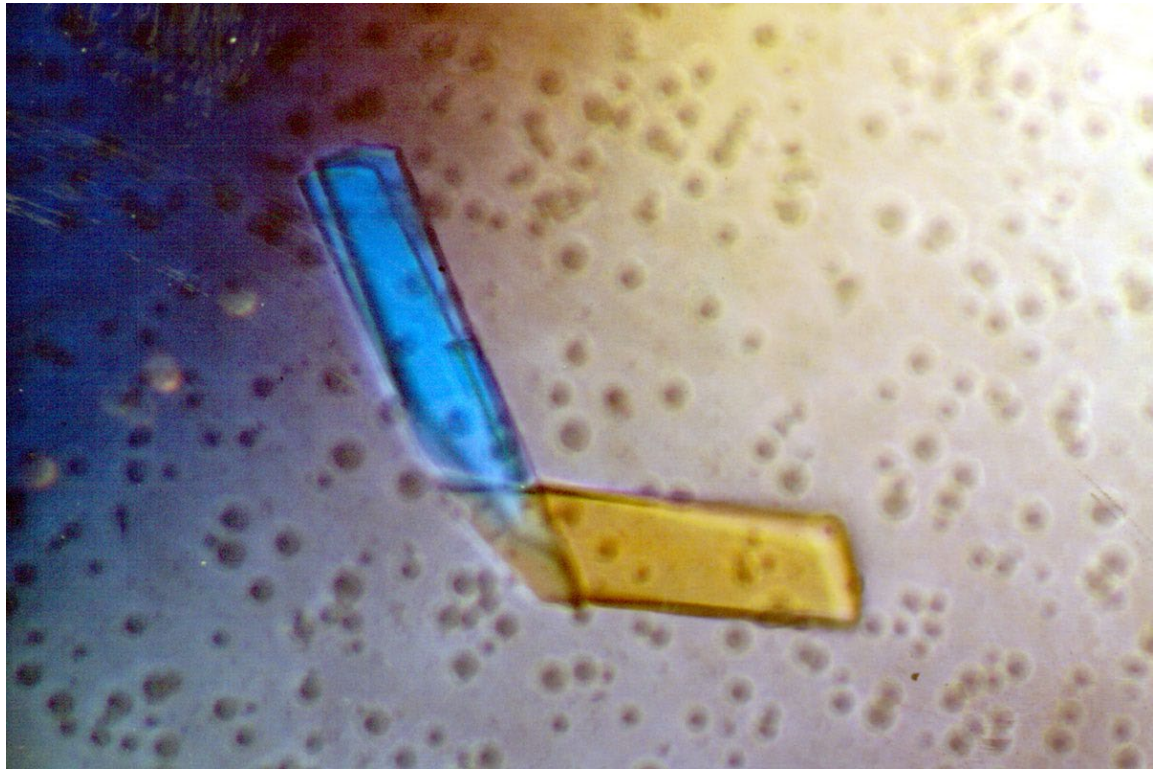




http://www.antibodysystem.com/archive/48.html

Bence -Jones Protein

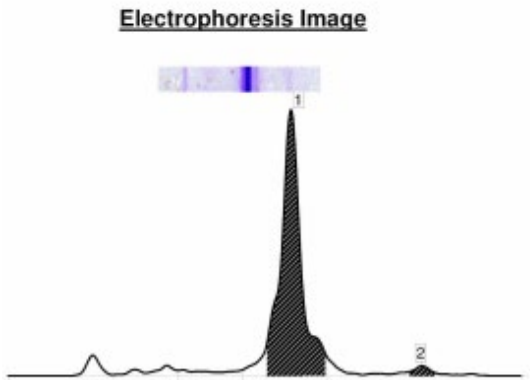
- Dysregulation of heavy and light chain production frequently occurs in plasma cell dyscrasias.
- Overproduction of monoclonal light chains by neoplastic plasma cells leads to increased unbound circulating free light chains: Bence-Jones Protein.
- Bence Jones protein are present in roughly two-thirds of myeloma cases.[2]
- Circulating free light chains may deposit in tissues or get trapped in glomeruli leading to kidney failure.
- Low levels of free light chains are reabsorbed in the kidney but will begin to spill into the urine as kidney function decreases.
- The ratio of kappa/lambda FLC important to evaluate in these disease. Normal ratio range is 0.26-1.65
- Different ranges for kidney disease patients (0.37-3.10)[2]

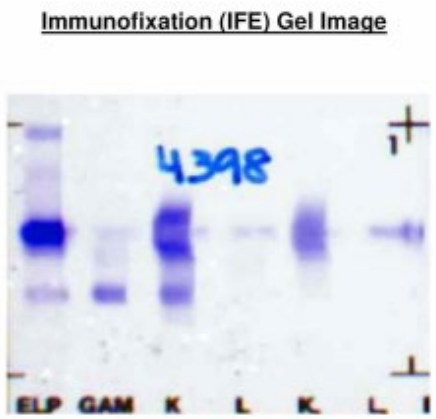


By Alex McPherson, University of California, Irvine - http://images.nigms.nih.gov/index.cfm?event=viewDetail&imageID=2399, Public Domain, https://commons.wikimedia.org/w/index.php?curid=5218720

Clinical Case #1, cont.

- UPEP/IFE
- 24hr Urine collection
- Total Urine Protein/24 hrs
  - » 4070 mg/day (40-150 mg/day)
- Paraprotein % - 79.7%
- Paraprotein excretion/24hrs
  - » 3243.8 mg/24 hrs



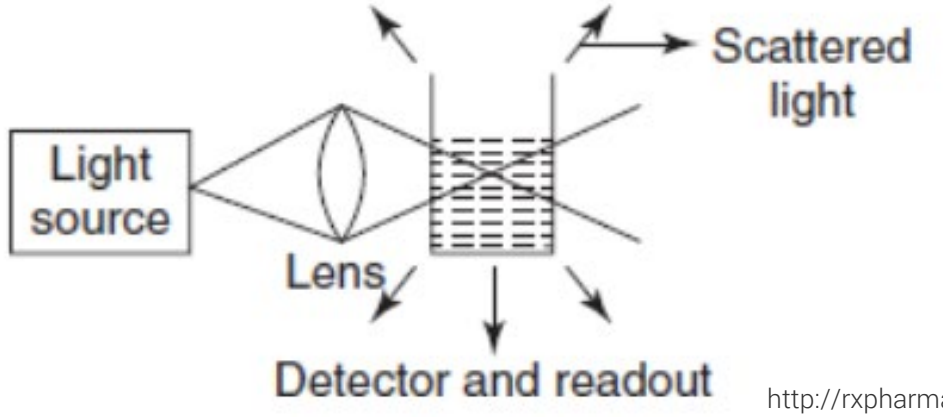


IFE Interpretation	See Note
	Monoclonal spike in the beta region. Monoclonal spike in the gamma region. Urine IFE is positive for monoclonal free kappa light chains (Bence Jones Protein). Urine IFE shows a monoclonal IgG heavy chain with associated kappa light chain and excess monoclonal free kappa light chains. This test has been reviewed and approved by Patricia Slev, PhD, D(ABCC).

Nephelometry

- Can be used to detect many different proteins in serum, urine, and other body fluids.
- Patient sample is diluted in a clear cuvette and light passes through the sample. The amount and type of protein with scatter the light and a detector picks up the scatter at varying angles from the incoming light.
- The amount and type of protein present can be calculated based of the amount of scatter detected.
- This method is used at ARUP to quantitate immunoglobulins as well as other serum proteins.
- This method is important to distinguish from turbidimetry

Nephlometry:

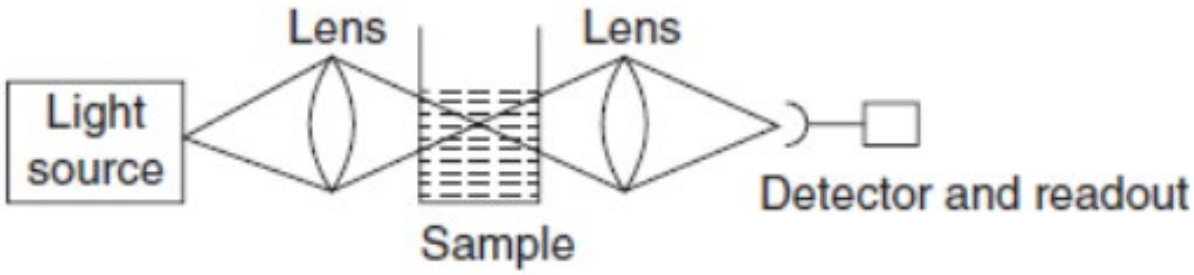


http://rxpharmaworld.blogspot.com/2016/12/nephelometry-and-turbidimetry.html

Turbidimetry

- In contrast to nephelometry, turbidometry is measures transmitted light through a solution, not light scatter.
- The patient sample is added to a solution and light is passed through the sample with the detector measuring the amount of light transmitted. Solutions with higher concentration of protein will transmit less light. (High lipemic or hemolyzed samples may interfere)
- We use this method at ARUP to measure Free Light Chains (formerly this test was performed by nephelometry)

Turbidimetry:



http://rxpharmaworld.blogspot.com/2016/12/nephelometry-and-turbidimetry.html

Methods Recap

- Four different test methods with associated tests discussed.
  - » Capillary Electrophoresis – Serum/Urine Protein Electrophoresis
  - » Immunofixation Electrophoresis – Heavy and Light Chain IFE
  - » Nephelometry & Turbidimetry – Free light chain quantitation, Quantitative Immunoglobulins
- Results from these tests are incorporated to generate our Result Interpretation. These tests are often ordered all together or separately. (Pros and cons to both)

Clinical Case #2

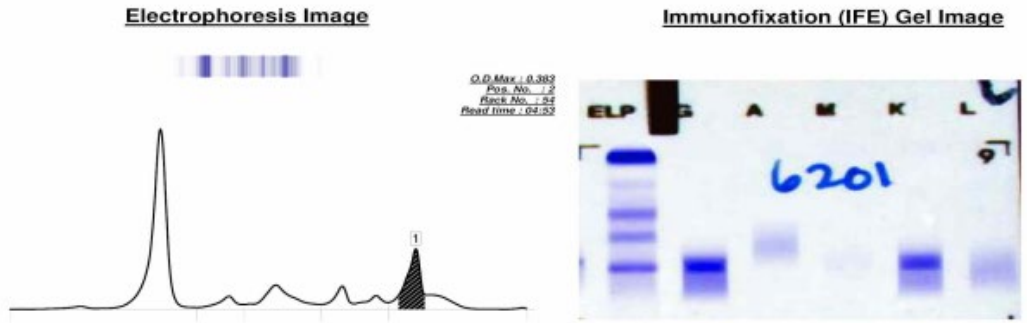
- 46yo M
- History of T2DM for 10 years, now with Stage 4 Chronic Kidney Disease
- July 2022 workup for kidney transplant. No complaints at exam, normal physical exam findings. SPEP/IFE and Serum Free Light Chains ordered as part of workup
- CBC, July 2022
  - » WBC 6.61 (4.30-11.30 k/uL)
  - » Hgb 12.9 (14.8-17.8 g/dL)
  - » Plt 312k (159k-439k)
- CMP, July 2022
  - » Normal except for elevated BUN, Creatinine

Clinical Case #2, cont.

- SPEP/IFE, July 2022
- Kappa/Lambda Free Light Chains
  - » Kappa LC – 89.16 (3.30-19.40 mg/L)
  - » Lambda LC – 57.61 (5.71-26.30 mg/L)
  - » K/L Ratio – 1.55 (0.26-1.65)
- Quantitative Immunoglobulins
  - » IgG – 1775 mg/dL (768-1632 mg/dL)
  - » IgA – 167 mg/dL (68-408 mg/dL)
  - » IgM – 49 mg/dL (35-263 mg/dL)

Total Protein, Serum	7.8 g/dL	(Ref Interval: 6.3-8.2)
Albumin	3.84 g/dL	(Ref Interval: 3.75-5.01)
Alpha 1 Globulin	0.35 g/dL	(Ref Interval: 0.19-0.46)
Alpha 2 Globulin	1.05 g/dL	(Ref Interval: 0.48-1.05)
Beta Globulin	0.83 g/dL	(Ref Interval: 0.48-1.10)
Gamma	**1.74 g/dL H**	(Ref Interval: 0.62-1.51)
Immunofixation Reflex	IFE Done	
SPEP/IFE Interpretation	See Note	

M-spike in the gamma region. The monoclonal protein peak accounts for 1.14 g/dL of the total 1.74 g/dL of protein in the gamma region. IFE gel pattern shows an IgG type kappa monoclonal protein. This test has been reviewed and approved by Vijayalakshmi Nandakumar, Ph.D., DABCC.



Diagnosis of Myeloma

- SLiM-CRAB findings are myeloma-defining events
  - » >60% PCs in bone marrow
  - » Free light chain ratio >100 or <0.01
    - Kappa or Lambda
  - » >1 focal lesion on MRI

  - » Calcium increased
  - » Renal insufficiency
  - » Anemia
  - » Bone lesions
- A small percentage of patients have non-secretory disease and will lack an M-spike.[3]

S. VINCENT RAJKUMAR

TABLE 1. International Myeloma Working Group Diagnostic Criteria for Multiple Myeloma and Related Plasma Cell Disorders

Disorder	Disease Definition
Non-IgM MGUS	All three criteria must be met:
	Serum monoclonal protein (non-IgM type) < 3 g/dL
	Clonal bone marrow plasma cells < 10%*
	Absence of end-organ damage such as CRAB features that can be attributed to the plasma cell proliferative disorder
Smoldering MM	Both criteria must be met:
	Serum monoclonal protein (IgG or IgA) ≥ 3 gm/dL, or urinary monoclonal protein ≥ 500 mg per 24 h and/or clonal bone marrow plasma cells 10%–60%
	Absence of myeloma-defining events or amyloidosis
MM	Both criteria must be met:
	Clonal bone marrow plasma cells ≥ 10% or biopsy-proven bony or extramedullary plasmacytoma
	Any one or more of the following myeloma defining events:
	Evidence of end organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically:
	Hypercalcemia: serum calcium > 0.25 mmol/L (> 1 mg/dL) higher than the upper limit of normal or > 2.75 mmol/L (> 11 mg/dL)
	Renal insufficiency: creatinine clearance < 40 mL/min or serum creatinine > 177 μmol/L (> 2 mg/dL)
	Anemia: hemoglobin value of > 2 g/dL below the lower limit of normal, or a hemoglobin value < 10 g/dL
	Bone lesions: one or more osteolytic lesions on skeletal radiography, CT, or PET-CT
	Clonal bone marrow plasma cell percentage ≥ 60%
	Involved: uninvolved serum FLC ratio ≥ 100 (involved FLC level must be ≥ 100 mg/L)
	> 1 focal lesion on MRI studies (at least 5 mm in size)

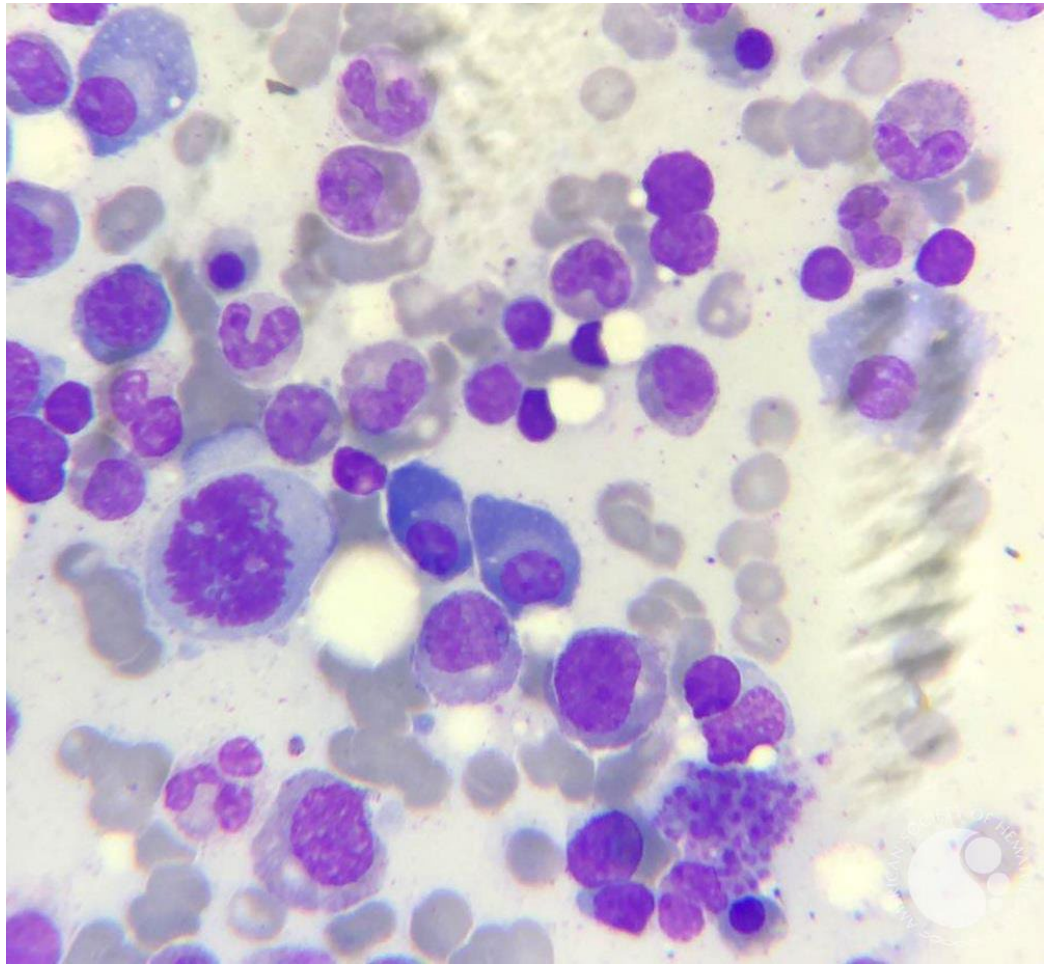
Criteria for Plasma Cell Dyscrasias

S. VINCENT RAJKUMAR

TABLE 1. International Myeloma Working Group Diagnostic Criteria for Multiple Myeloma and Related Plasma Cell Disorders

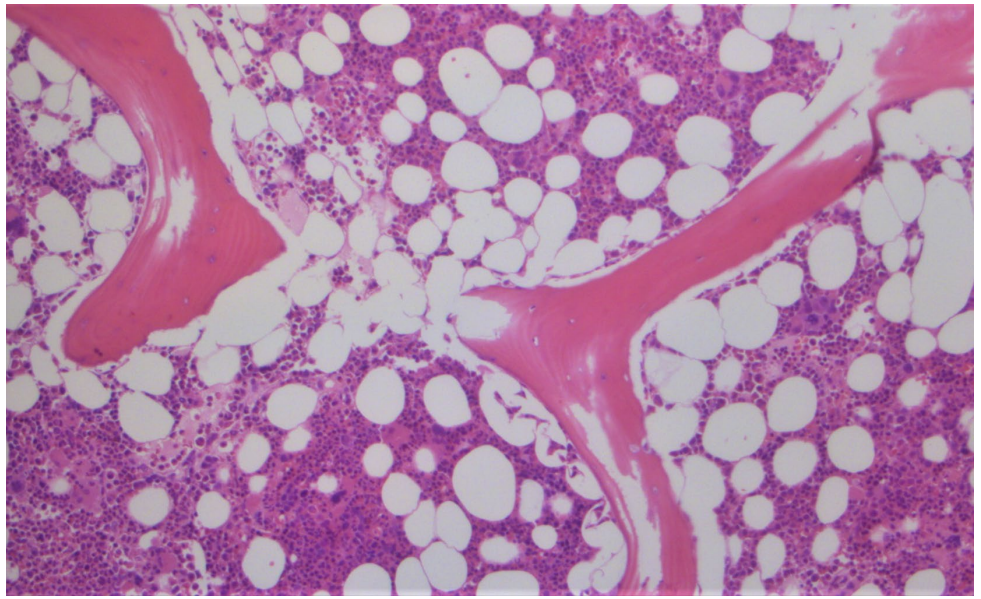
Disorder	Disease Definition
Non-IgM MGUS	All three criteria must be met:
	Serum monoclonal protein (non-IgM type) < 3 g/dL
	Clonal bone marrow plasma cells $< 10\%$*
	Absence of end-organ damage such as CRAB features that can be attributed to the plasma cell proliferative disorder
Smoldering MM	Both criteria must be met:
	Serum monoclonal protein (IgG or IgA) ≥ 3 gm/dL, or urinary monoclonal protein ≥ 500 mg per 24 h and/or clonal bone marrow plasma cells 10%–60%
	Absence of myeloma-defining events or amyloidosis
MM	Both criteria must be met:
	Clonal bone marrow plasma cells $\geq 10\%$ or biopsy-proven bony or extramedullary plasmacytoma
	Any one or more of the following myeloma defining events:
	Evidence of end organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically:
	Hypercalcemia: serum calcium > 0.25 mmol/L (> 1 mg/dL) higher than the upper limit of normal or > 2.75 mmol/L (> 11 mg/dL)
	Renal insufficiency: creatinine clearance < 40 mL/min or serum creatinine > 177 µmol/L (> 2 mg/dL)
	Anemia: hemoglobin value of > 2 g/dL below the lower limit of normal, or a hemoglobin value < 10 g/dL
	Bone lesions: one or more osteolytic lesions on skeletal radiography, CT, or PET-CT
	Clonal bone marrow plasma cell percentage $\geq 60\%$
	Involved: uninvolved serum FLC ratio ≥ 100 (involved FLC level must be ≥ 100 mg/L)
	> 1 focal lesion on MRI studies (at least 5 mm in size)

Bone Marrow Aspirate

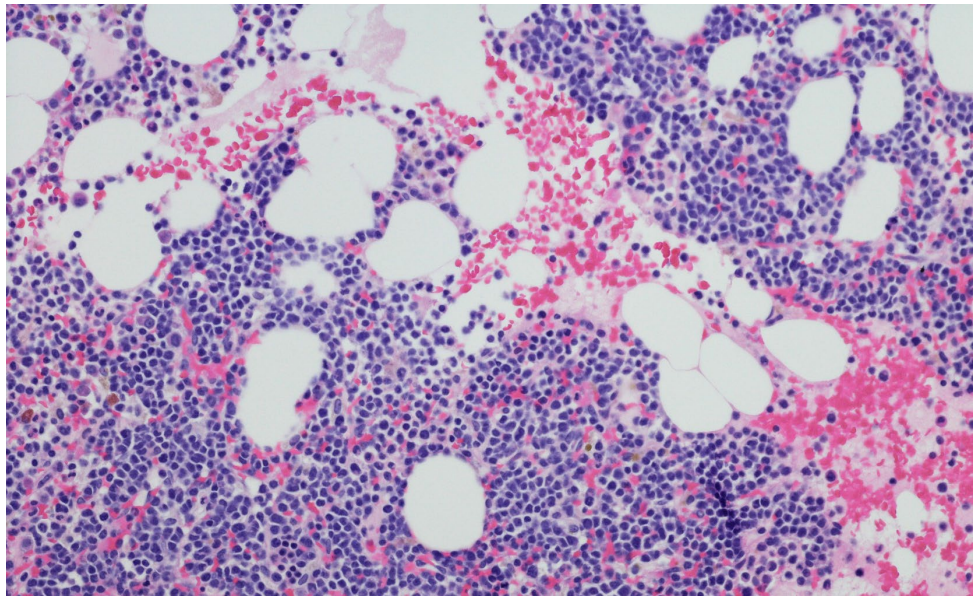


Diagnosis of Myeloma

- Presence of M-spike, monoclonal immunoglobulins, or increased FLC
- Bone Marrow Biopsy
  - » Normal biopsy should show trilineage hematopoiesis with normal amount of fat intermixed.
  - » Abnormal biopsy will typically show increased replacement by neoplastic plasma cells with a decrease in the other hematopoietic lineages.
- Myeloma cells cause removal of calcium from the bones and lead to hypercalcemia and may cause pathologic fractures.

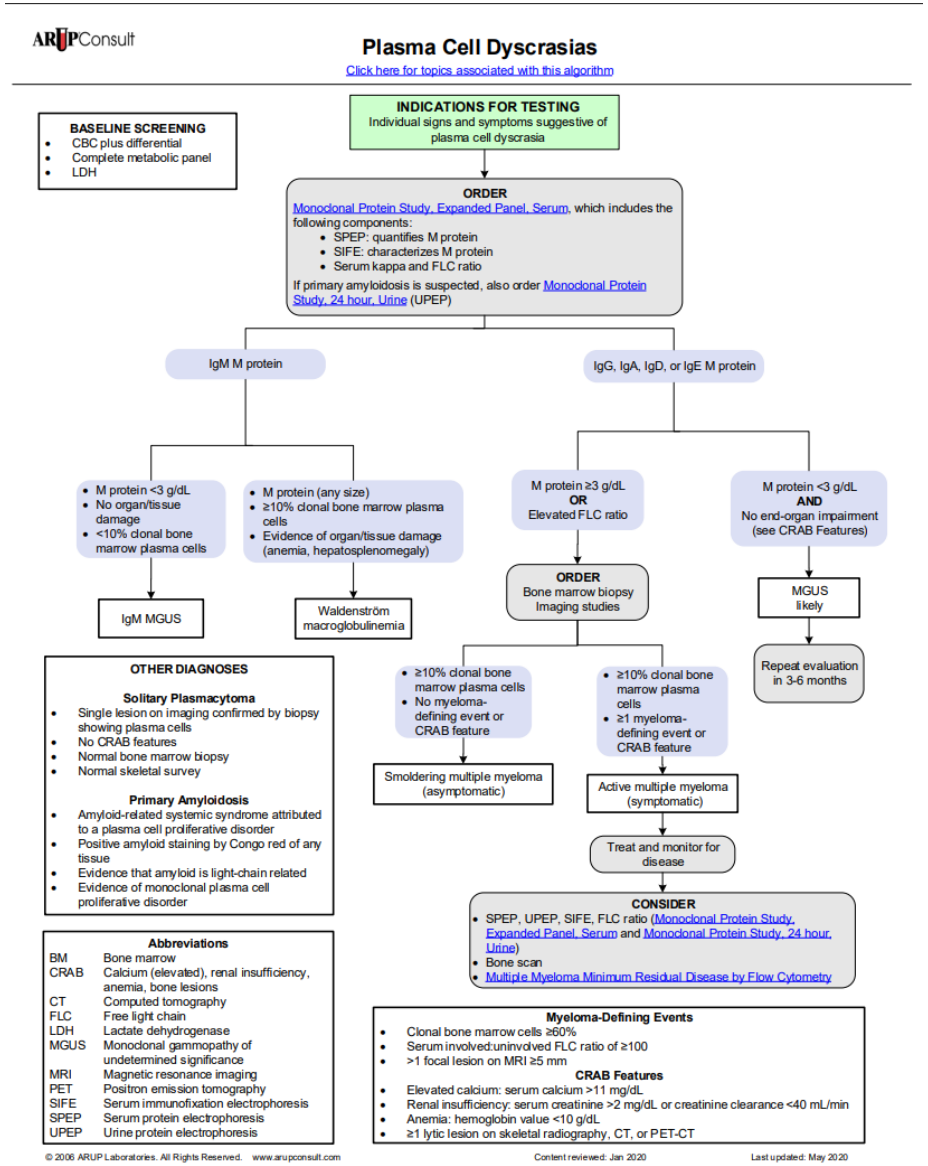


https://www.mypathologyreport.ca/how-to-read-your-bone-marrow-report/



https://www.genengnews.com/news/antisense-oligo-targets-irf4-gene-to-treat-multiple-myeloma/

Test algorithm for Plasma Cell Dyscrasias



https://arupconsult.com/algorithm/plasma-cell-dyscrasias-testing-algorithm

Other Plasma Cell Dyscrasias

IgM MGUS	All three criteria must be met:
	Serum IgM monoclonal protein < 3 gm/dL
	Bone marrow lymphoplasmacytic infiltration < 10%
	No evidence of anemia, constitutional symptoms, hyperviscosity, lymphadenopathy, or hepatosplenomegaly that can be attributed to the underlying lymphoproliferative disorder.
Light-Chain MGUS	All criteria must be met:
	Abnormal FLC ratio (< 0.26 or > 1.65)
	Increased level of the appropriate involved light chain (increased kappa FLC in patients with ratio > 1.65 and increased lambda FLC in patients with ratio < 0.26)
	No immunoglobulin heavy-chain expression on immunofixation
	Absence of end-organ damage that can be attributed to the plasma cell proliferative disorder
	Clonal bone marrow plasma cells < 10%
	Urinary monoclonal protein < 500 mg/24 h
Solitary Plasmacytoma	All four criteria must be met:
	Biopsy-proven solitary lesion of bone or soft tissue with evidence of clonal plasma cells
	Normal bone marrow with no evidence of clonal plasma cells
	Normal skeletal survey and MRI (or CT) of spine and pelvis (except for the primary solitary lesion)
	Absence of end-organ damage such as CRAB features that can be attributed to a lympho-plasma cell proliferative disorder
Solitary Plasmacytoma With Minimal Marrow Involvement**	All four criteria must be met:
	Biopsy-proven solitary lesion of bone or soft tissue with evidence of clonal plasma cells
	Clonal bone marrow plasma cells < 10%
	Normal skeletal survey and MRI (or CT) of spine and pelvis (except for the primary solitary lesion)
	Absence of end-organ damage such as CRAB features that can be attributed to a lympho-plasma cell proliferative disorder

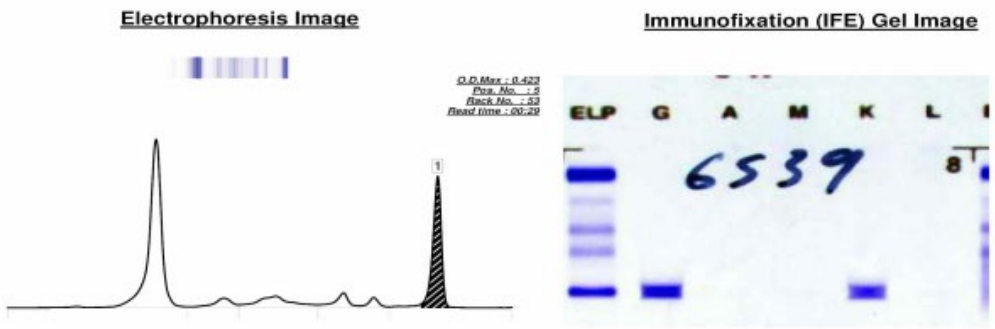
Clinical Case #3

- 63yo F
- History of Type 2 Diabetes Mellitus with neuropathy. Seen by Rheumatology for polyarthritis. SPEP/IFE ordered as part of workup
- Referred to Hematology in March 2021 for evaluation after discovery of an abnormal SPEP/IFE in Oct 2020
- CBC findings March 2021
  - » WBC 6.62 (4.30-11.30 k/uL)
  - » Hgb 10.9 (12-6-15.9 g/dL)
  - » Plts 243k (159k-439k)
- CMP normal

Clinical Case #3, cont.

- SPEP/IFE, Oct 2020
- Quantitative Immunoglobulins
  - » IgG – 2580 mg/dL (768-1632 mg/dL)
  - » IgA – 8 mg/dL (68-408 mg/dL)
  - » IgM – <5 mg/dL (35-263 mg/dL)
- Serum Free Light Chains, Dec 2020
  - » Kappa LC – 717 mg/L (3.30-19.40 mg/L)
  - » Lambda LC – 4.57 mg/L (5.71-26.30 mg/L)
  - » K/L Ratio – 156.89 (0.26-1.65)

Total Protein, Serum	7.5 g/dL		(Ref Interval: 6.3-8.2)
Albumin	3.79 g/dL		(Ref Interval: 3.75-5.01)
Alpha 1 Globulin	0.34 g/dL		(Ref Interval: 0.19-0.46)
Alpha 2 Globulin	0.74 g/dL		(Ref Interval: 0.48-1.05)
Beta Globulin	0.58 g/dL		(Ref Interval: 0.48-1.10)
Gamma	**2.06 g/dL**	**H**	(Ref Interval: 0.62-1.51)
Immunofixation Reflex	IFE Done		
SPEP/IFE Interpretation	See Note M-spike in the gamma region. The monoclonal protein peak accounts for 1.97 g/dL of the total 2.06 g/dL of protein in the gamma region. IFE pattern shows an IgG type kappa monoclonal protein. Other immunoglobulin levels (IgA and IgM) are decreased. This test has been reviewed and approved by Anne Tebo, Ph.D.		



Daratumumab

- Monoclonal antibody directed against CD38
- CD38 expressed highly on myeloma cells
- Binding by daratumumab induces apoptosis
- Can interfere with SPEP/IFE (monoclonal protein)
- Also creates challenges in the Blood bank: antibody screens appear pan-reactive up to 5-6 months[5]
- This can be mitigated by treating patient blood with dithiothreitol (DTT) which denatures CD38 by cleaving disulfide bonds.
  - » This will also destroy the Kell antigen. May require transfusion of Kell-negative units.

Clinical Case #1 Review

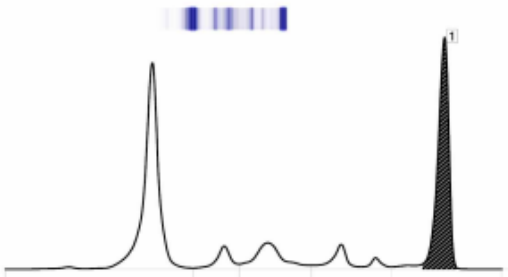
- On presentation to the ED
  - » CMP: Hyponatremic, Hypercalcemia, Increased BUN and Creatinine, High total protein
  - » CBC: Normal WBC, Anemic, Low Platelets, 21% plasma cells on differential
  - » CT Head and Chest: Multiple lytic bone lesions
- Following hospital admission
  - » 24 hr Urine Monoclonal Protein Study
  - » Serum Protein Electrophoresis with Immunofixation Electrophoresis and Quantitative Immunoglobulins
  - » Serum Free light chain study

Clinical Case #1, Review

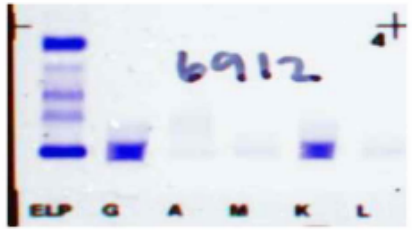
- SPEP/IFE, June 2023
- Quantitative Immunoglobulins
  - IgG – 3005 mg/dL (768-1632 mg/dL)
  - IgA – 24 mg/dL (68-408 mg/dL)
  - IgM – 17 mg/dL (35-263 mg/dL)
- Serum Free Light Chains
  - Kappa LC – 260.57 mg/L (3.30-19.40 mg/dL)
  - Lambda LC – 7.81 mg/dL (5.71-26.30 mg/dL)
  - K/L Ratio – 33.36 (0.26-1.65)

Total Protein, Serum	7.5 g/dL		(Ref Interval: 6.3-8.2)
Albumin	**3.14 g/dL**	**L**	(Ref Interval: 3.75-5.01)
Alpha 1 Globulin	0.35 g/dL		(Ref Interval: 0.19-0.46)
Alpha 2 Globulin	0.76 g/dL		(Ref Interval: 0.48-1.05)
Beta Globulin	0.53 g/dL		(Ref Interval: 0.48-1.10)
Gamma	**2.72 g/dL**	**H**	(Ref Interval: 0.62-1.51)
Monoclonal Protein	2.62 g/dL		
Immunofixation	IFE Done		

Electrophoresis Image



Immunofixation (IFE) Gel Image



SPEP/IFE Interpretation

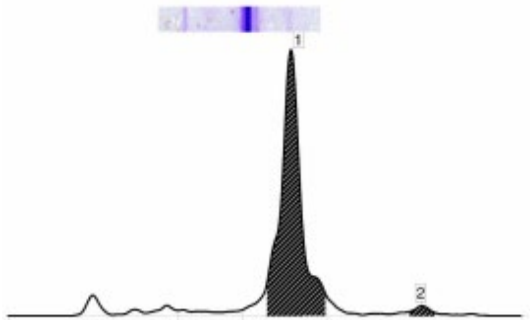
See Note

Monoclonal spike in the gamma region. IFE gel pattern shows an IgG type kappa monoclonal protein.
This test has been reviewed and approved by Abdulrahman Saadalla, M.B., B.Ch.

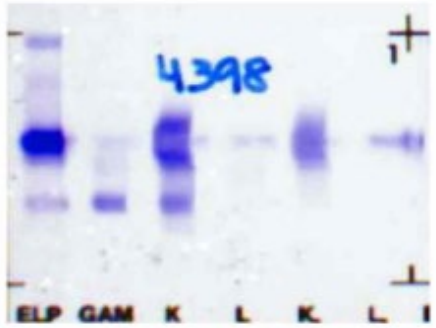
Clinical Case #1
Re vie w

- UPEP/IFE
- 24hr Urine collection
- Total Urine Protein/24 hrs
  - » 4070 mg/day (40-150 mg/day)
- Paraprotein % - 79.7%
- Paraprotein excretion/24hrs
  - » 3243.8 mg/24 hrs

Electrophoresis Image



Immunofixation (IFE) Gel Image



IFE Interpretation	See Note
	Monoclonal spike in the beta region. Monoclonal spike in the gamma region. Urine IFE is positive for monoclonal free kappa light chains (Bence Jones Protein). Urine IFE shows a monoclonal IgG heavy chain with associated kappa light chain and excess monoclonal free kappa light chains. This test has been reviewed and approved by Patricia Slev, PhD, D(ABCC).

Clinical Case Updates

- Case #1: Chemotherapy followed by hematopoietic stem cell transplant ~6 months after diagnosis.
  - » Most recent BMBx showed normocellular marrow with no monoclonal plasma cells and MRD negative by flow cytometry
- Case #2: Waiting for kidney transplant
  - » His SMM markers remain stable, stable mild anemia
- Case #3: Changed to daratumumab monotherapy in Oct 2023.
  - » Recent hospitalization for pneumonia
  - » Most recent SPEP showed M-spike of 0.65 g/dL (from a high of 2.61 g/dL in 2021)

Follow-up Testing

- Patients can be followed with repeat testing
- Current recommendation for MGUS and SMM patients is to be retested after 6 months and then yearly after that except for low-risk patients (IgG type, <1.5 g/dL, and normal FLC ratio).[1]
- Risk of progression of MGUS is highest within the first year (2.1%)[2]
- Follow-up testing can be discontinued if >80 years old or life expectancy is <5 years.

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ARUP is a nonprofit enterprise of the University of Utah and its Department of Pathology.