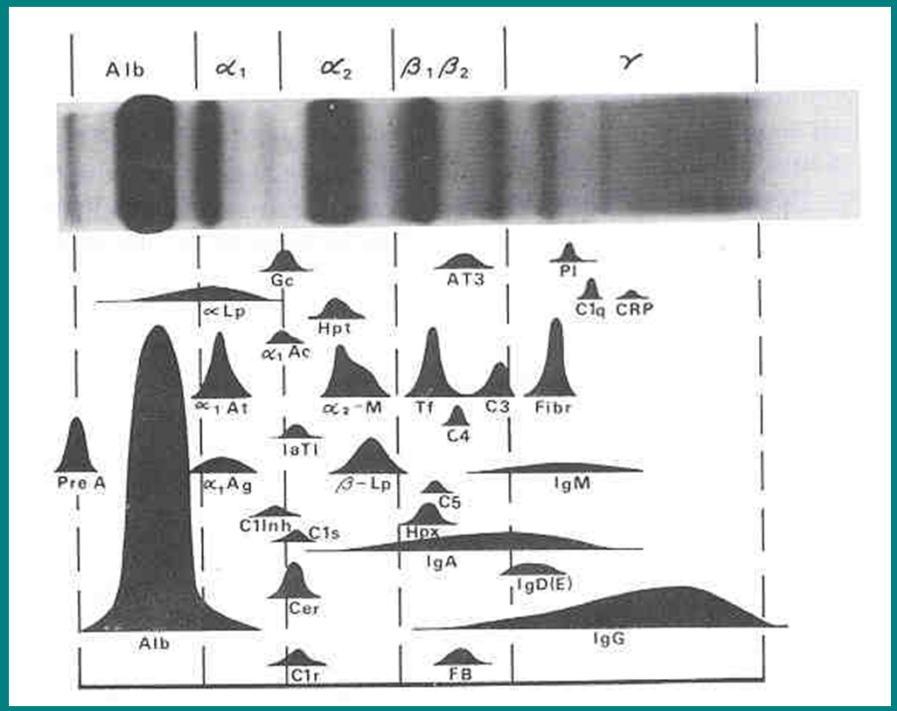
What Clinical Chemists Should Know About Monoclonal Proteins



Gary L. Horowitz, MD
Beth Israel Deaconess Medical Center
Boston, MA

Objectives

- Explain the importance of evaluating both urine and serum in screening for monoclonal proteins
- Differentiate MGUS from multiple myeloma
- Explain why the free light chain ratio is (usually) more important than the absolute concentration of either free light chain

Topics for Today

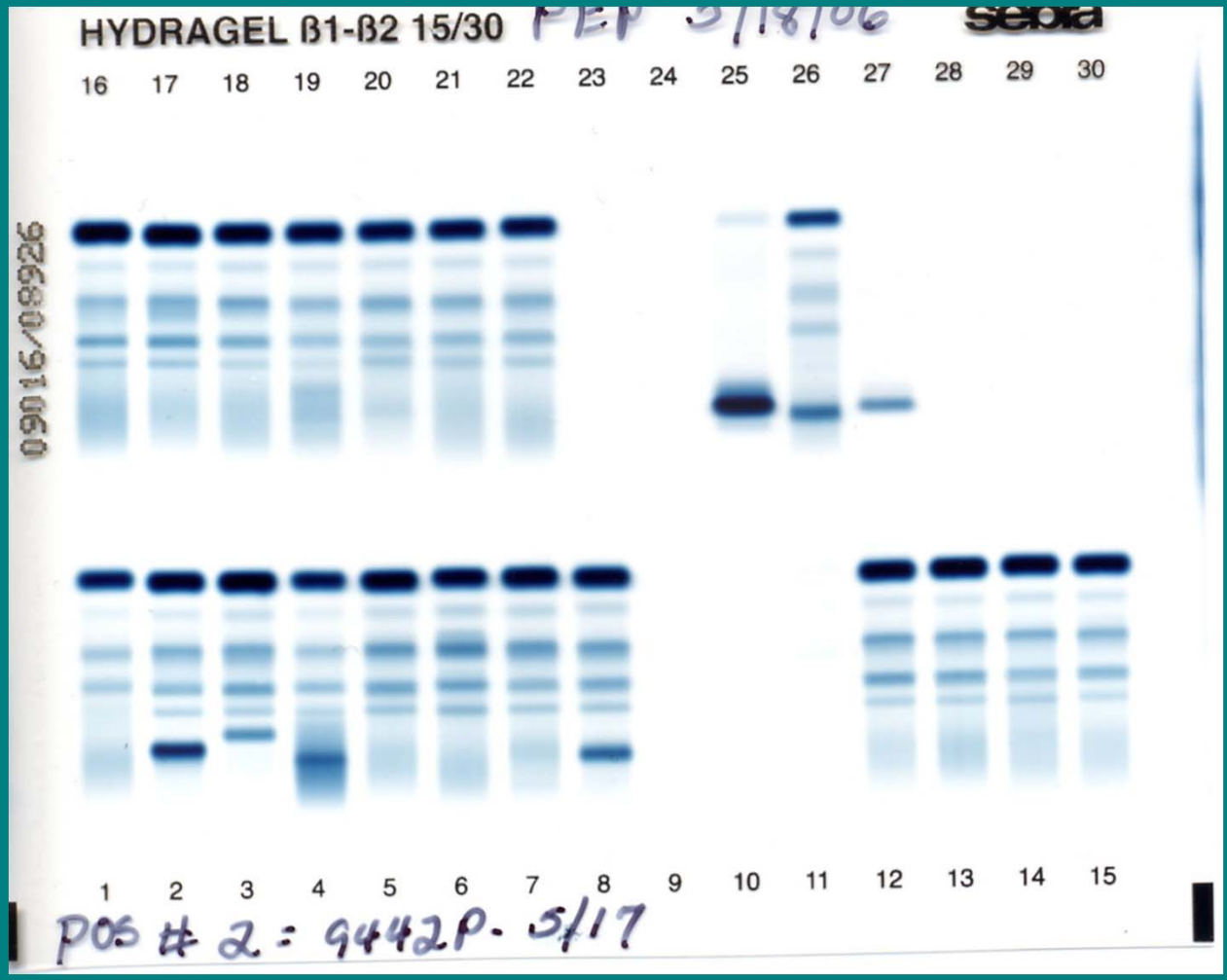
- Techniques
  - Protein Electrophoresis
  - Immunofixation Electrophoresis
  - Quantitative Immunoglobulins
- Concepts
  - MGUS
  - Bence-Jones Proteins
  - Serum Free Light Chains

Protein Electrophoresis
Size Doesn't Matter (much)

- separation is charge-dependent
- net charge is (virtually) all that matters
- secondary factors include
  - size & shape of molecule
  - *electric field strength*
  - *supporting medium*
  - *temperature*

Resolution

- traditional SPEP (low resolution)
  - 5 bands:
    - albumin, alpha1, alpha2, beta, gamma
    - multiple proteins in each “zone”
- now, high resolution
  - 10-16 bands!
    - do we really need it?
- CAP recommendation: beta1/beta2 separation


HYDRAGEL ß1-ß2 15/30 PEP 5/18/06
sebia
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
09016/08926
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
POS # 2 : 9442P - 5/17

Guidelines for Clinical and Laboratory Evaluation of Patients With Monoclonal Gammopathies

David F. Keren, MD; Raymond Alexanian, MD; James A. Goeken, MD; Peter D. Gorevic, MD; Robert A. Kyle, MD; Russell H. Tomar, MD

• This guideline provides the recommendations of an expert panel for the clinical and laboratory evaluation of patients suspected of having a clinical condition that produces a monoclonal protein in serum or urine. The recommendations describe the clinical conditions in which a monoclonal protein should be sought, the optimal sequence of testing to diagnose and monitor these patients, and the most effective laboratory procedures.

(*Arch Pathol Lab Med.* 1999;123:106–107)

Clinical conditions associated with a monoclonal protein (M-protein) in serum or urine may be grouped separately as plasmacytic, lymphocytic, protein infiltrative, or miscellaneous disorders. Incidence data for the most common disorders are listed in Table 1. The clinical features of multiple myeloma are listed in Table 2. A dialogue between the laboratory and the clinician is strongly recommended.

GUIDELINE 1

Serum and urine electrophoresis of high resolution is indicated for all patients suspected of having a plasma cell dyscrasia. The gel should be examined directly by the interpreter. This applies most commonly to clinical disorders that suggest multiple myeloma, Waldenström's macroglobulinemia, or amyloidosis (AL), but also includes less frequent conditions, such as solitary plasmacytoma, POEMS syndrome, heavy-chain diseases, and immunoglobulin deposition disease. The quantitative level of M-protein should be defined precisely by densitometry measurement of the M-protein peak. We discourage the use of electrophoresis of low resolution.

GUIDELINE 2

Immunofixation is indicated to define the abnormal protein type. In addition, even when high-resolution electrophoresis is negative, immunofixation with κ and λ light chain antisera may be useful to detect small M-proteins in cases where there is a suspicion of a plasma cell dyscrasia. Immunofixation is not indicated in cases of obvious polyclonal gammopathies on high-resolution electrophoresis. When there is asymmetry of a polyclonal elevation of gamma globulin, an immunofixation may be useful after contact between the individual interpreting the electrophoresis and the clinician. We discourage the use of immunoelectrophoresis.

GUIDELINE 3

The M-protein should be followed by using densitometric quantitation, unless a low-level M-protein is obscured by other proteins. In such cases, quantitation of immunoglobulins by nephelometry may be more accurate. Immunofixation should not be repeated unless there is a change in the electrophoretic migration, there is an additional spike, or for confirmation of complete remission after treatment.

GUIDELINE 4

For all patients with a plasma cell dyscrasia, direct measurement of immunoglobulins by nephelometry is indicated at diagnosis to define the level of uninvolved immunoglobulins. Nephelometric measurement of immunoglobulins should never be used as the sole means to screen patients for an M-protein. We discourage the use of radial immunodiffusion procedures.

GUIDELINE 5

All patients with multiple myeloma, Waldenström's macroglobulinemia, amyloidosis (AL), and related disorders should be assessed for the presence, type, and daily excretion of monoclonal free light chains. This is best done by the quantitation of 24-hour urine protein excretion, densitometry measurements of the light chain peak in a ≥100× concentrated aliquot, and immunofixation. Screening for monoclonal free light chain by dipstick, sulfosalicylic acid, or acidified heat precipitation tests is not useful.

GUIDELINE 6

Changes in level of a previously identified monoclonal protein in serum or urine should be assayed by high-resolution electrophoresis at regular intervals that vary from every 1 to 2 months for patients being treated for multiple myeloma, Waldenström's macroglobulinemia, or amyloidosis (AL), to every year for patients with low level monoclonal gammopathy of undetermined significance.

Accepted for publication August 27, 1998.

From the Warde Medical Laboratory, Ann Arbor, Mich (Dr Keren); the Texas Medical Center, Houston (Dr Alexanian); The University of Iowa, Iowa City (Dr Goeken); Mt Sinai Medical Center, New York, NY (Dr Gorevic); the Mayo Medical School, Mayo Clinic and Foundation, Rochester, Minn (Dr Kyle); and The University of Wisconsin, Madison (Dr Tomar).

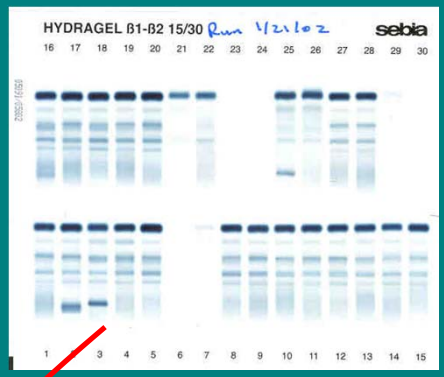
Presented at the College of American Pathologists Conference XXXII, Guidelines for Laboratory Evaluation and Use of Antinuclear Antibodies and Laboratory Diagnosis and Monitoring of Monoclonal Gammopathies, Chicago, Ill, May 29–31, 1998.

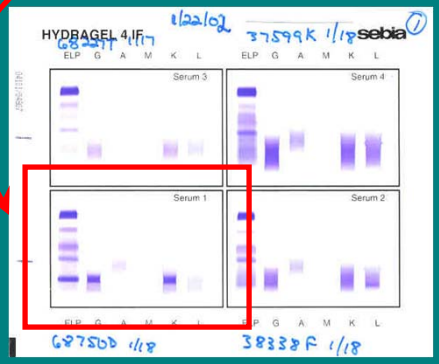
Reprints: David F. Keren, MD, Warde Medical Laboratory, 5025 Venture Dr, Ann Arbor, MI 48108.

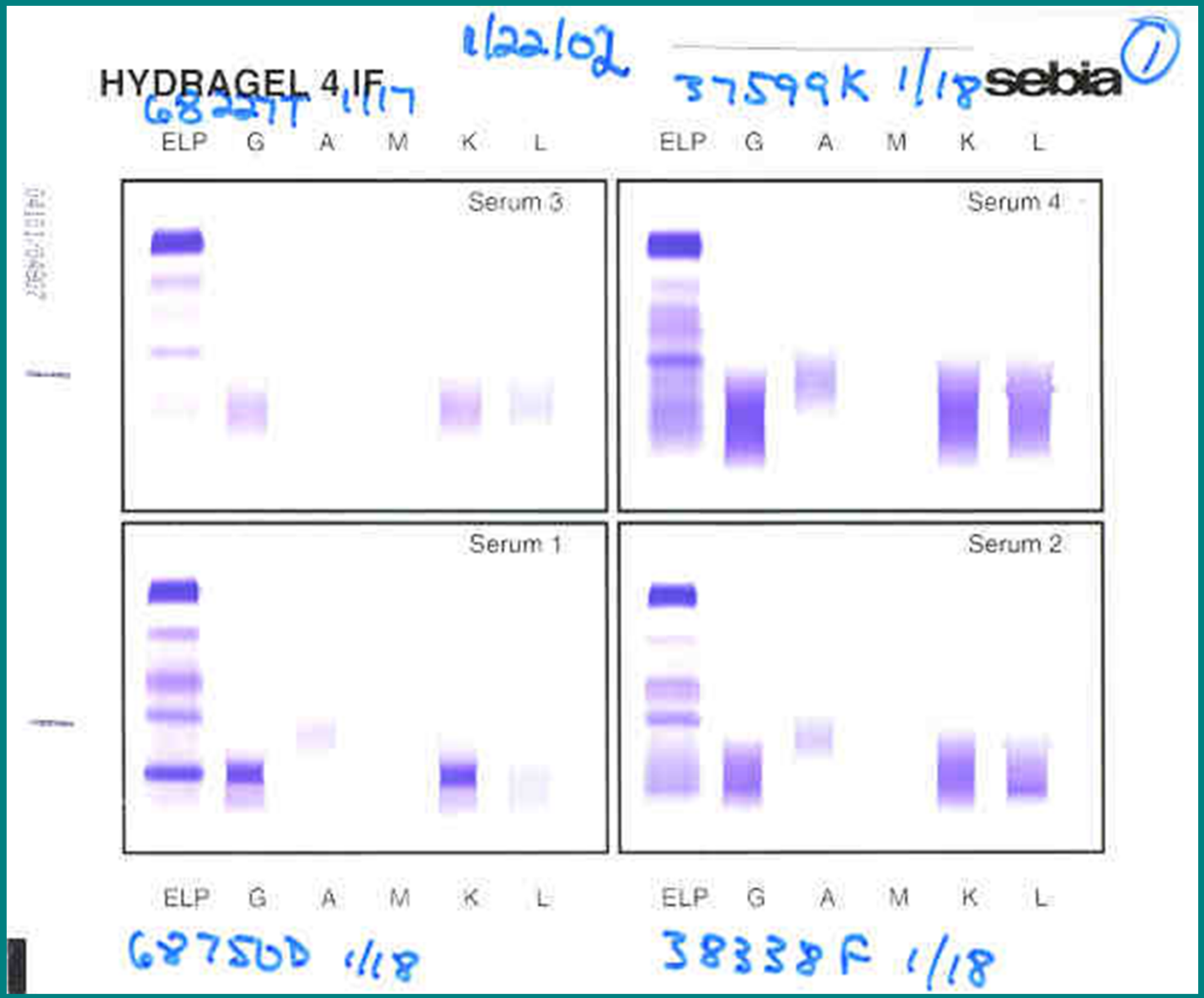
Report of the Consensus Conference on Monoclonal Gammopathies. Arch Pathol Lab Med. 1999; 123:104-132.

Immunofixation Electrophoresis (IFE)

- run multiple PEPs of same sample
- step 2
  - precipitate all proteins in Lane 1
  - in Lanes 2-6, overlay antisera to G, A, M, k, l
- wash entire plate
  - only precipitated proteins remain
- stain entire plate
- look for precipitates that line up



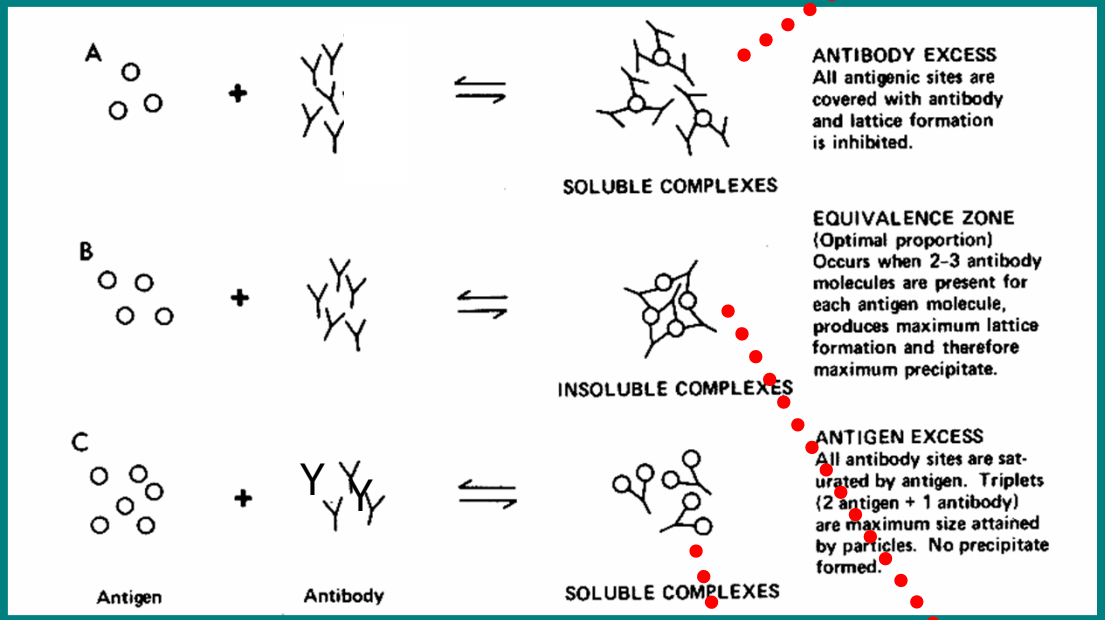


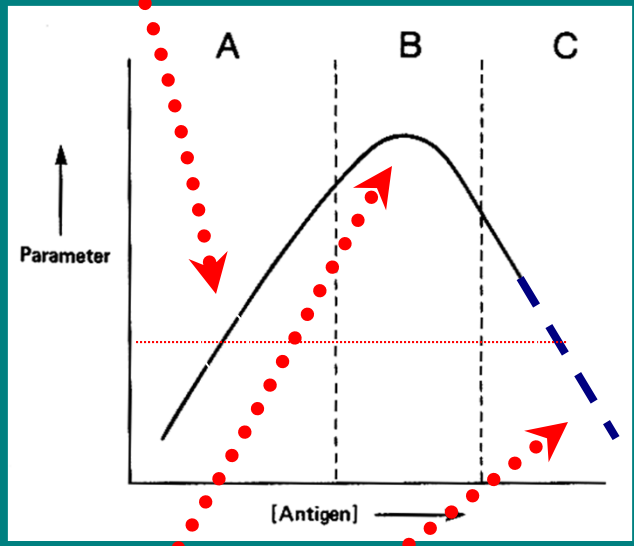

HYDRAGEL 4 IF
sebia
ELP G A M K L
Serum 3
Serum 4
Serum 1
Serum 2
ELP G A M K L
ELP G A M K L

Antigen Excess (Hook Effect)

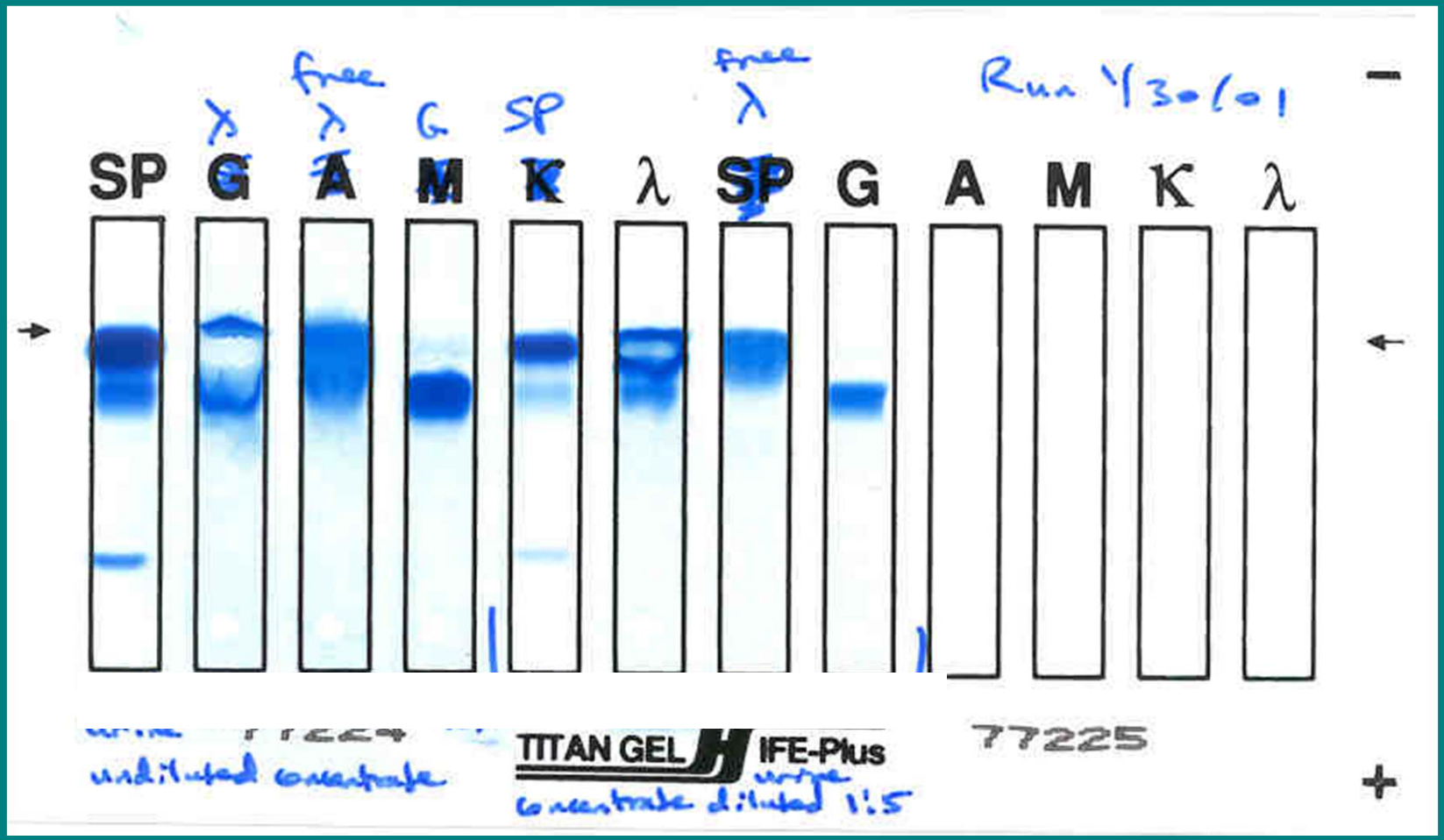
- nomenclature
  - antigen here is antibody
- in homogeneous immunoassay ([IgG]):
  - you may get falsely low results (with no error flag!)
- in IFE:
  - you get “donuts”
  - with Sebia, get “hourglass” effect

Hook Effect: What Is It?



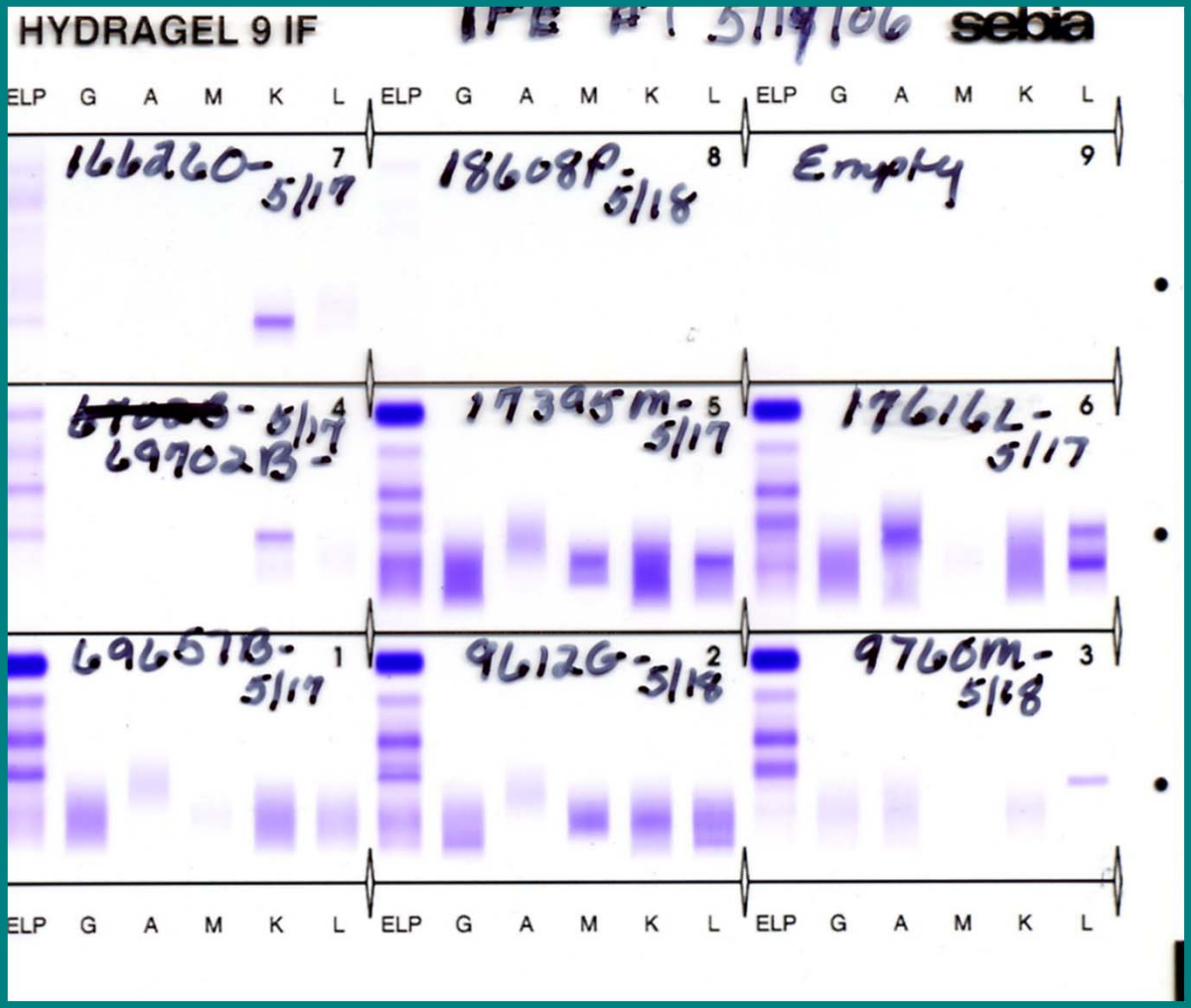


Adapted from Burtis, CA & Ashwood, ER.
Tietz Fundamentals of Clinical Chemistry (4th Edition). Philadelphia: W.B Saunders, 1996, p.136.


free λ
free λ
Run Y30101
SP G A M K λ SP G A M K λ
TITAN GEL IFE-Plus
77225
undiluted concentrate
concentrate diluted 1:5

M Protein Concentration

- two alternatives
  - densitometry (recommended)
  - quantitative immunoglobulin levels
- each has its place
  - if both polyclonal IgX and monoclonal IgX present, [IgX] will OVERestimate
  - to assess suppression of other immunoglobulins, need [IgG], [IgA], [IgM]


HYDRAGEL 9 IF
sebia
ELP G A M K L
16626O- 5/17
7
18608P- 5/18
8
Empty
9
17395m- 5/17
5
17616L- 5/17
6
6970ZB- 5/17
4
9612G- 5/18
2
9760m- 5/18
3

Recommended Reporting Language

- On protein electrophoresis, there is an abnormal band in the gamma region, representing 10% of the total protein, or 700 mg/dL (7 g/L)
- When subjected to immunofixation electrophoresis, this band is identified as monoclonal IgG lambda
- There is no suppression of IgA or IgM levels, indicating that this is probably an MGUS
- The total IgG concentration is 1200 mg/dL (12 g/L)

Clonality is Qualitative

- CAP recommendations
  - do **not** use immunoglobulin levels for screening
    - high immunoglobulin levels may be polyclonal
    - normal immunoglobulin levels can include clonal populations
  - screen with PEP (not immunoglobulin levels)

Clonality May Not Be Myeloma

- monoclonal gammopathy (M protein):
  - occurs in diseases other than multiple myeloma
    - Waldenstrom's, amyloidosis, …
  - occurs in entities that may not even be "malignant"
    - monoclonal gammopathy of undetermined significance (MGUS)
- when one reports M protein:
  - what do you think clinician's next step should be?

Case History

A 70 year old man visits his primary care physician (PCP) with complaints of fatigue over the past three months. As part of his initial work-up, he's found to be anemic, so his PCP orders a battery of follow-up tests, including a serum protein electrophoresis (SPEP) to rule out multiple myeloma.

The SPEP is reported as howing a trace monoclonal band, representing roughly 1% of serum protein (70 mg/dL). In addition, his immunoglobulin levels are reported as follows:

IgG	400 mg/dL	(reference interval 700-1600)
IgA	57 mg/dL	(70-400)
IgM	32 mg/dL	(40-230)

The PCP infers that, with such a low concentration M-protein, it's almost certainly an "MGUS", that should be followed annually.

Diseases Associated with “M Proteins”

- Myeloma
  - Solitary
  - Asymptomatic
  - Multiple Myeloma
  - POEMS
- Waldenstrom’s
- Amyloidosis
- MGUS

Incidence/Survival in United States

disease	cases/year	median survival (years)
Myeloma	13,000	3
Waldenstrom's	3,000	5
Amyloidosis	2,000	1
MGUS	750,000	12

MGUS

- prevalence
  - 1% over age 50
  - 3% over age 70
- roughly 1.5% per year progress
- majority die of unrelated disease

Features of MGUS

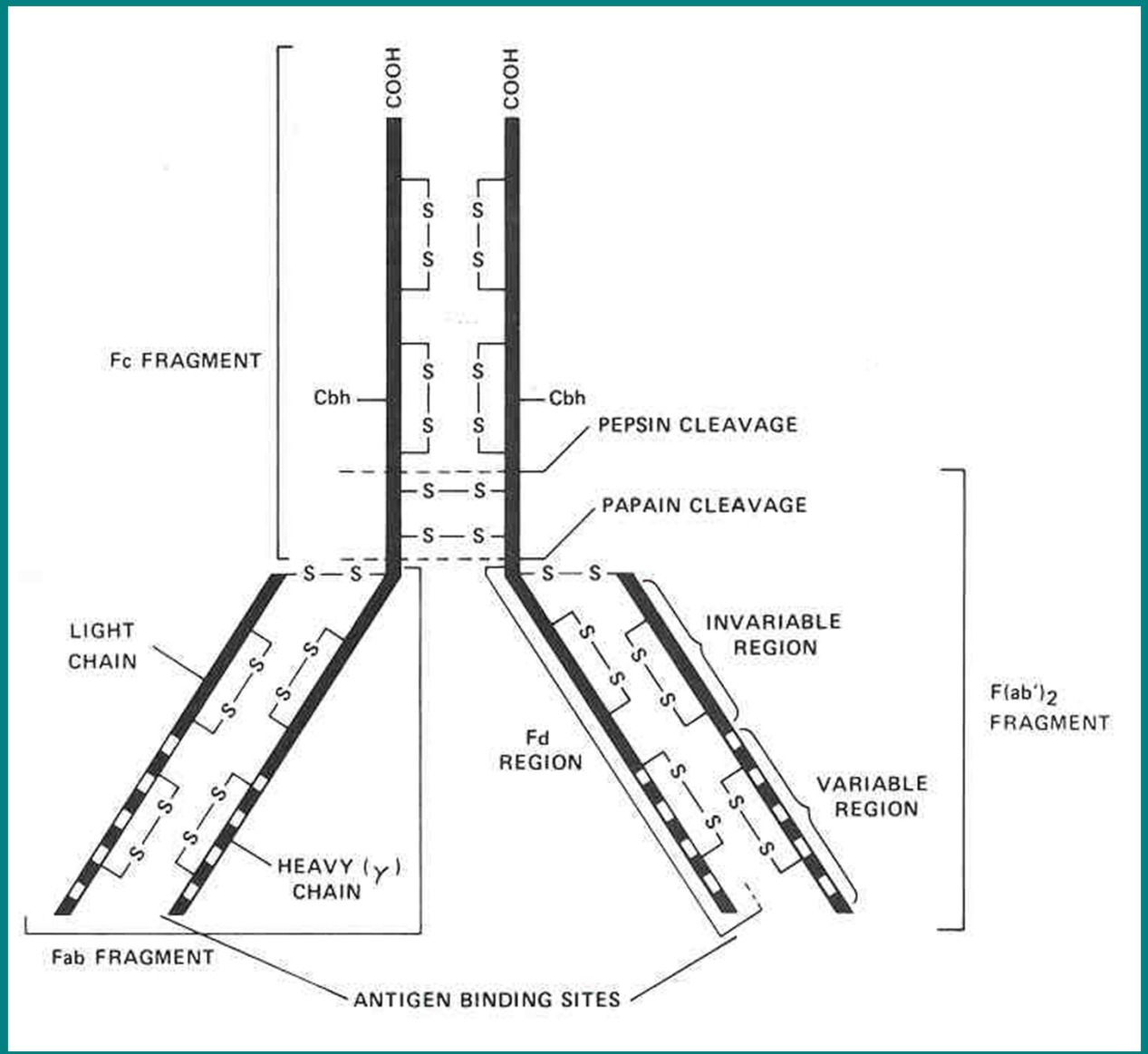
- asymptomatic, older individuals
- [M protein] < 2500 mg/dL (<25 g/L)
- marrow plasma cells < 10%
- no bone lesions
- BJP < 50 mg/day (<0.050 g/day)
- other [immunoglobulin] preserved

Urine Proteins: Situations Where PEP Is Less Important

- “micro”albuminuria
- glomerular pattern
  - non-selective proteinuria
  - everything filtered, small proteins reabsorbed
- tubular pattern
  - failure to reabsorb low molecular proteins
  - e.g., beta2microglobulin

Urine Proteins: Situation Where PEP Is Critical

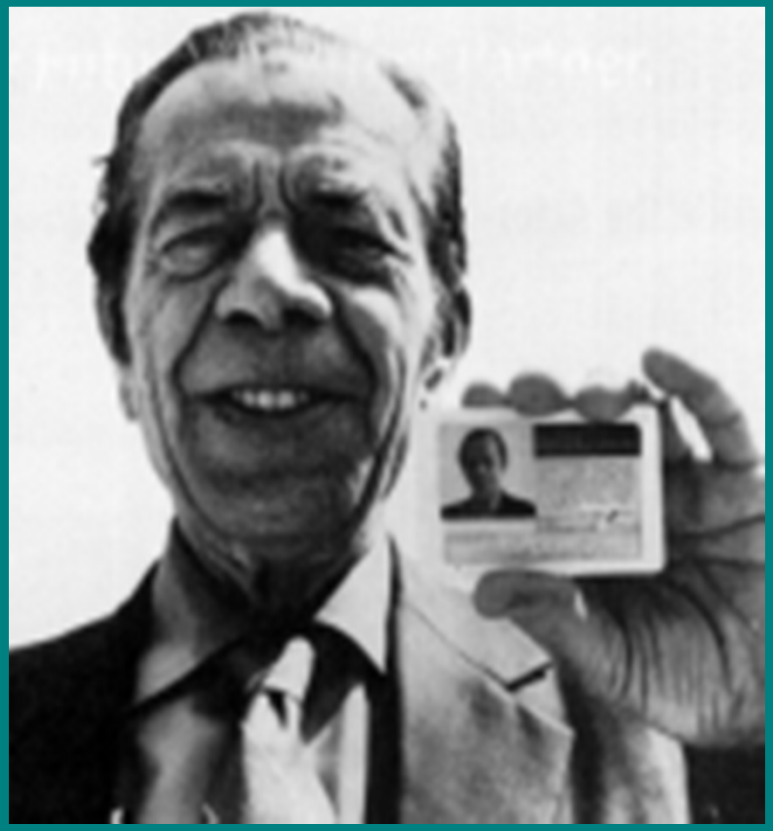
- Bence-Jones Protein (BJP)
  - 3-part definition:
    - free
    - monoclonal
    - light chains
  - precipitation characteristics are **not** diagnostic
    - precipitate @ 40-60^{o}C
    - re-dissolve @ 100^{o}C


COOH
COOH
Fc FRAGMENT
Cbh
Cbh
PEPSIN CLEAVAGE
PAPAIN CLEAVAGE
S—S
LIGHT CHAIN
INVARIABLE REGION
Fd REGION
VARIABLE REGION
F(ab')2 FRAGMENT
HEAVY (γ) CHAIN
Fab FRAGMENT
ANTIGEN BINDING SITES

Clinical & Diagnostic Significance of BJP

- false negative dipstick
- SPEP may show no “M protein” (probably shows hypogammaglobulinemia)
- think of Willie Sutton, famous bank robber

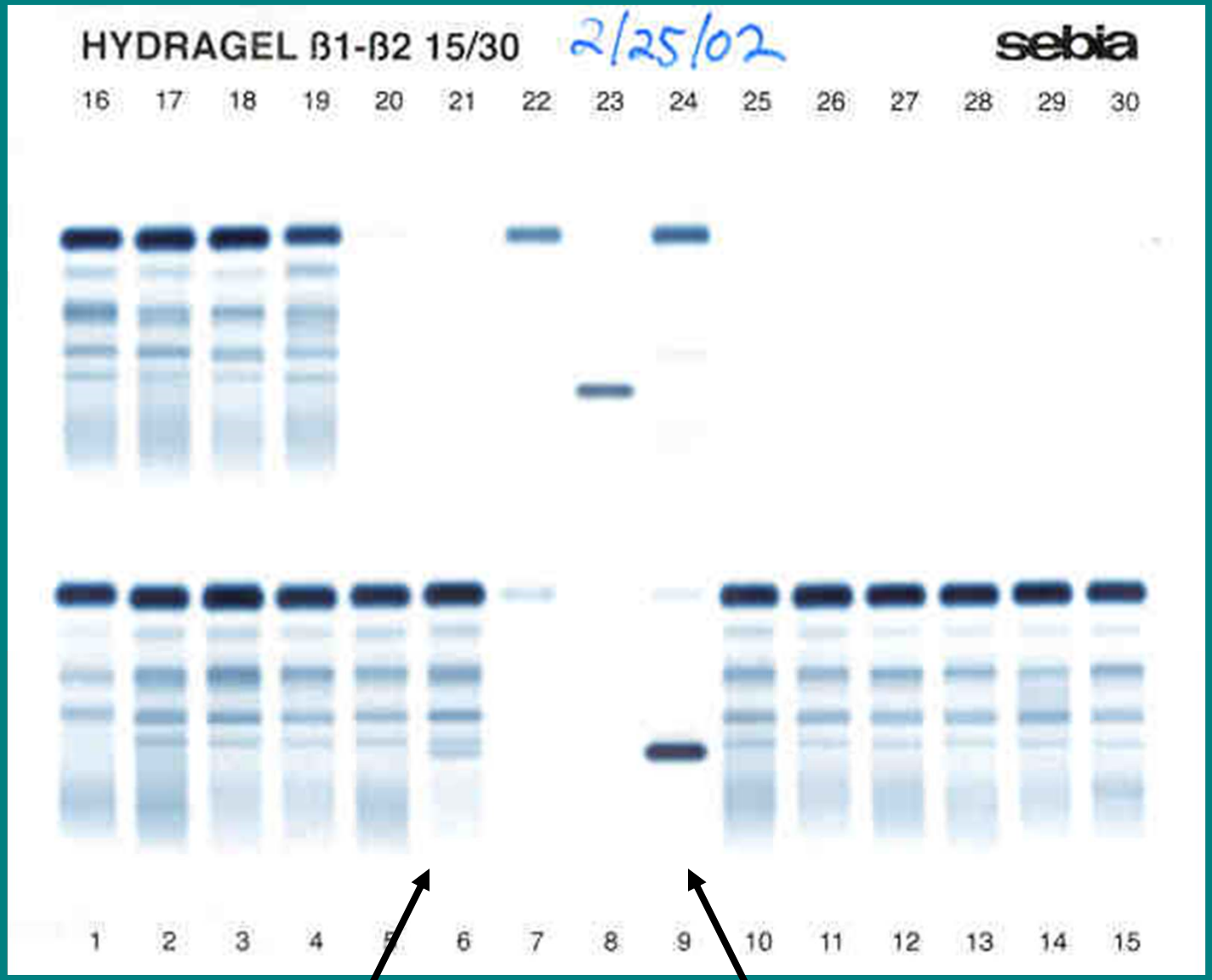
Willie Sutton

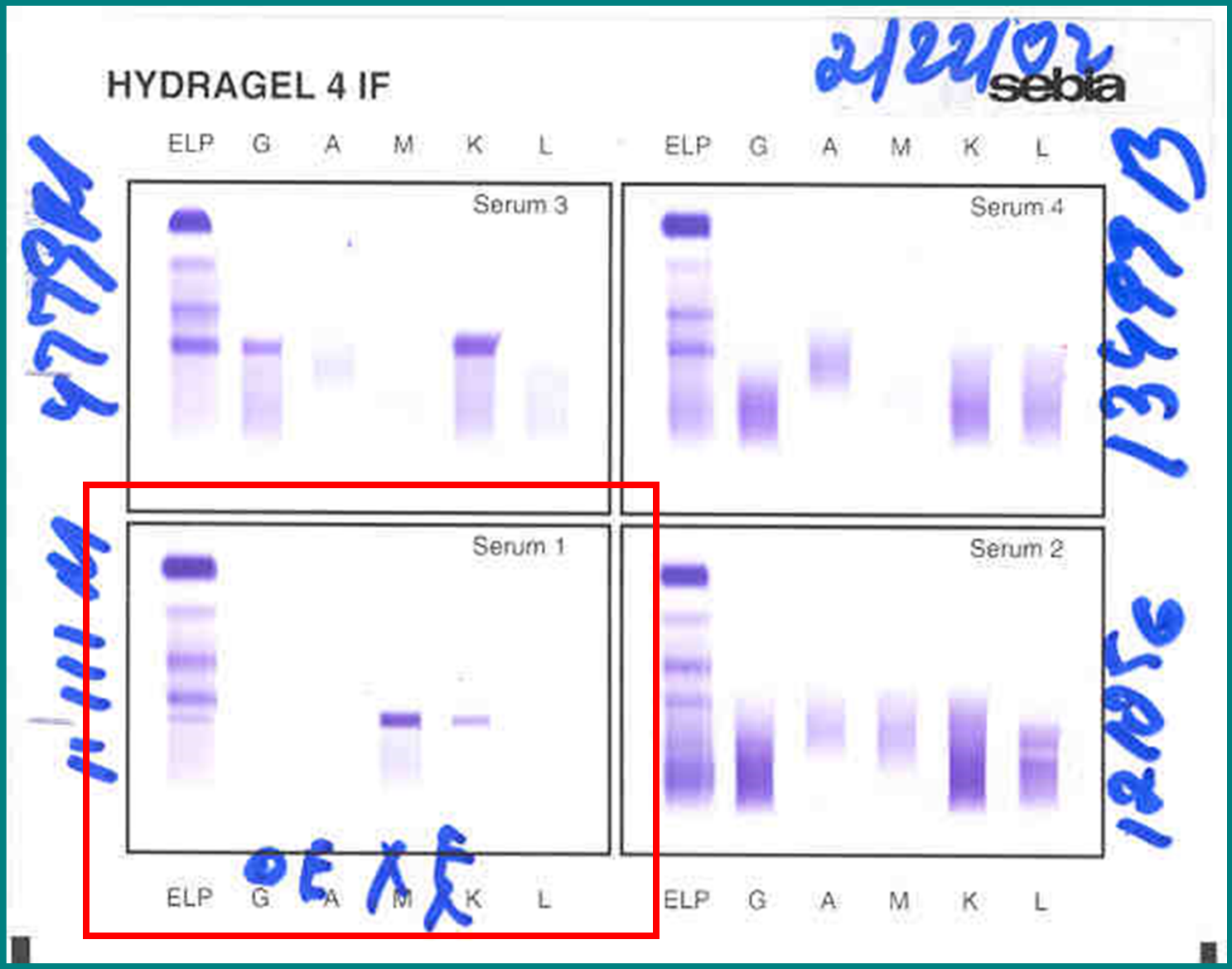


- one of the most famous bank robbers of the twentieth century
- a favorite of newspaper reporters, who could count on him for the kind of quote that makes a headline bounce
- spent most of his adult life in prison
- though he escaped more than once, his short bursts of freedom always ended with an arrest for bank robbery
- in an attempt to learn why he continued along such a futile course, one reporter asked, "Willie, why do you keep robbing banks?"
- "Because," Sutton said smoothly, "that's where the money is."

Clinical & Diagnostic Significance of BJP

- false negative dipstick
- SPEP may show no “M protein” (probably shows hypogammaglobulinemia)
- think of Willie Sutton, famous bank robber
  - look in the urine
  - not in the blood
  - BJP may be the ONLY evidence of disease


HYDRAGEL ß1-ß2 15/30
2/25/02
sebia
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
serum
urine


HYDRAGEL 4 IF
sebia
ELP G A M K L
Serum 3
Serum 4
Serum 1
Serum 2
ELP G A M K L
ELP G A M K L

Back to Our Patient

The SPEP is reported as howing a trace monoclonal band, representing roughly 1% of serum protein (70 mg/dL). In addition, his immunoglobulin levels are all low.

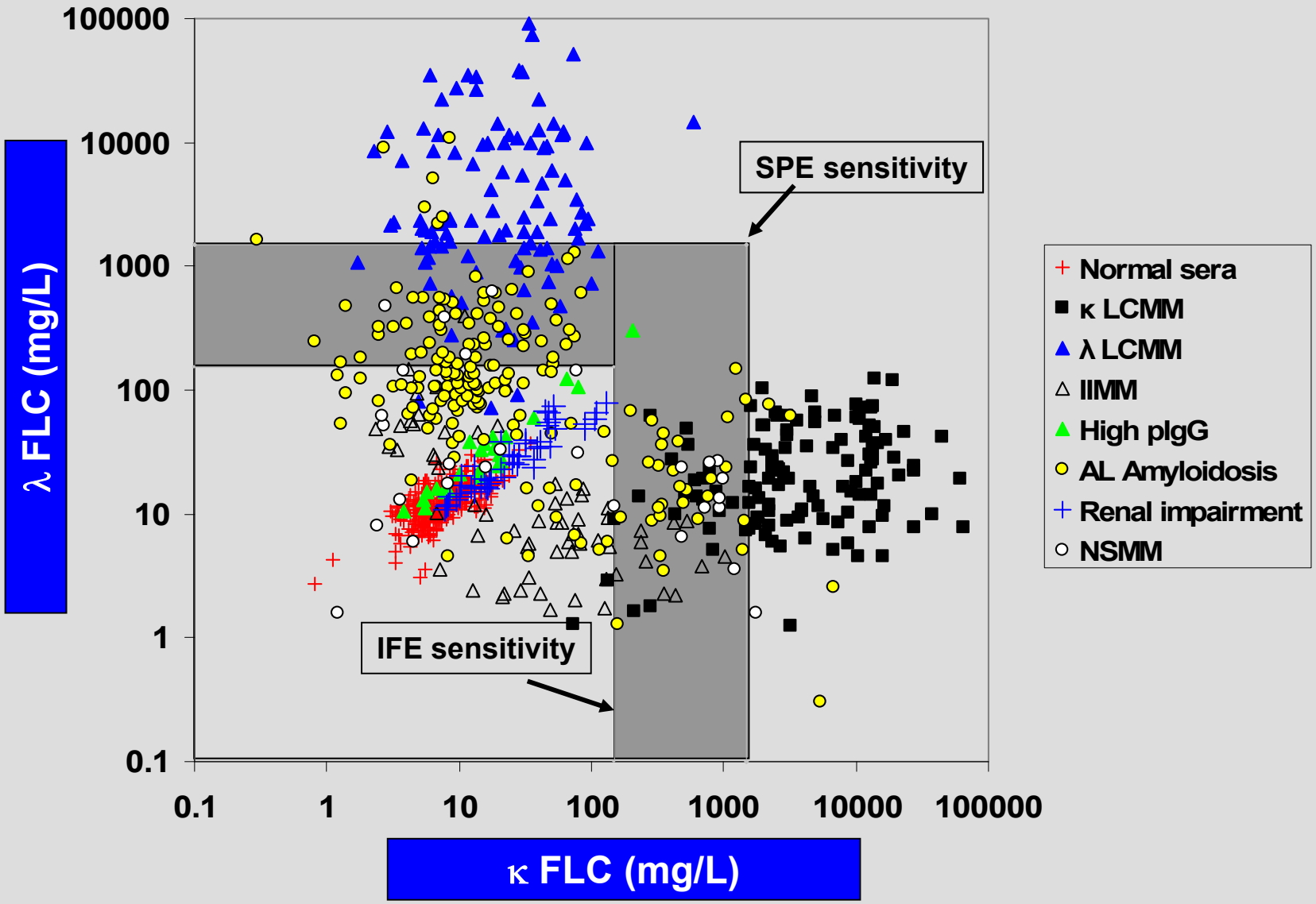
The PCP infers that, with such a low concentration M-protein, it's almost certainly an "MGUS", that should be followed annually.

- The M-protein in the serum was free kappa (Bence-Jones kappa).
- Its concentration was low because it was excreted in the urine.
- A 24-hour collection showed that the daily excretion was 4200 mg BJk, strongly suggestive of light chain myeloma.
- The diagnosis was confirmed on bone marrow biopsy, which showed abnormal plasma cells replacing the marrow, which explained the hypogammaglobulinemia

Take Home Messages

- **Always submit *urine* as well as *blood* for PEPs**
- **Role of PEPs**
  - Screening
  - Quantitating
    - Densitometry
    - [M-protein]=(PEP %) x [TP]
  - Monitoring
    - Change in migration
    - Change in amount

- **Role of IFE**
  - PEP abnormality: initial ID
  - PEP change: re-ID
  - High clinical suspicion
    - even with negative PEP
    - order IFE in addition to PEP
- **Role of [IgG], [IgA], [IgM]**
  - Can be misleading
  - More for suppression than elevation
  - ***Except*** when M-protein overlies normal proteins (e.g., beta region)



Bradwell, AR. Serum Free Light Chain Analysis [4th Edition]. 2006.

Self-Assessment Question 1

The best combination of tests to screen for monoclonal proteins is:

A) Serum immunoglobulin concentrations (IgG, IgA, IgM)
B) Serum protein electrophoresis and immunofixation electrophoresis
C) Serum and urine protein electrophoresis
D) Urine protein electrophoresis and immunofixation electrophoresis

Self-Assessment Question 2

Typical findings in Light Chain Myeloma include all of the following EXCEPT:

A) A discrete band in the serum protein electrophoresis
B) (Serum) hypogammaglobulinemia
C) A discrete band in the urine protein electrophoresis
D) A negative urine dipstick for protein

Self-Assessment Question 3

What is the most common diagnosis associated with monoclonal proteins?

A) Amyloidosis
B) Multiple Myeloma
C) Monoclonal Gammopathy of Undetermined Significance
D) Waldenstrom's Macroglobulinemia