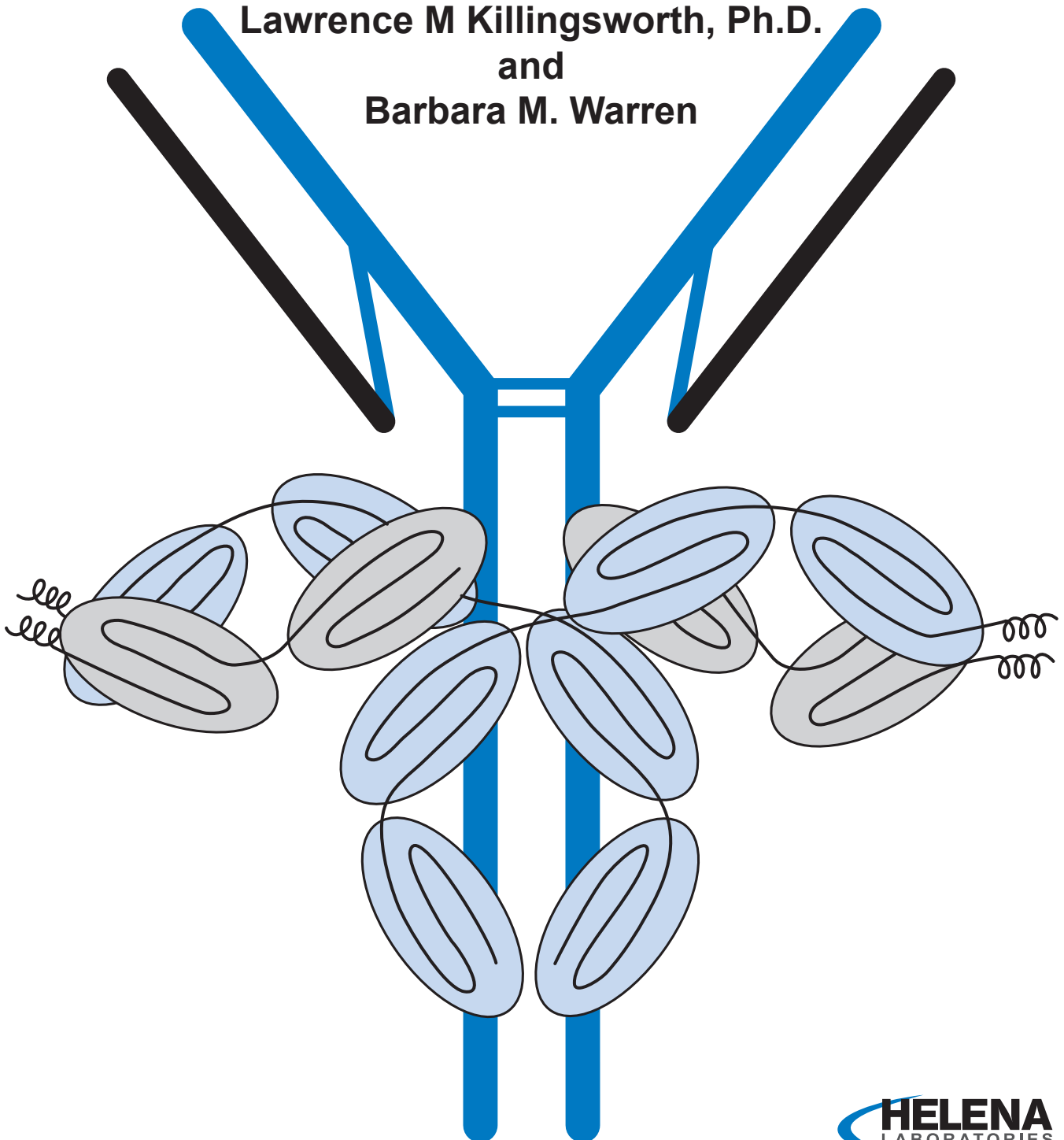


IMMUNOFIXATION

For the Identification of
Monoclonal Gammopathies

Lawrence M Killingsworth, Ph.D.
and
Barbara M. Warren



An Educational Service From



A stylized graphic of an antibody molecule, rendered in light blue and grey. It features two heavy chains (inner, blue) and two light chains (outer, grey) joined at their C-termini. The structure is symmetrical and Y-shaped, with the heavy chains extending downwards and the light chains extending outwards and upwards.

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Immunofixation for the Identification of Monoclonal Gammopathies

Introduction

Immunofixation electrophoresis (IFE) is a two stage procedure using agarose gel protein electrophoresis in the first stage and immunoprecipitation in the second. The specimen may be serum, urine, cerebrospinal fluid or other body fluids. There are numerous applications for IFE in research, forensic medicine, genetic studies and clinical laboratory procedures. The primary use of IFE is in clinical laboratories for the characterization of monoclonal immunoglobulins.

Methodology

Sample Application and Electrophoresis:

In the first stage of immunofixation, a single sample is applied to six different positions on an agarose plate and the proteins are separated by electrophoresis, according to their net charge.

Antisera Application and Incubation:

In the second phase of the procedure monospecific antisera are applied to five of the electrophoresis patterns (IgG, IgA, IgM and Kappa and Lambda antisera are used). A protein fixative solution is applied to the sixth pattern to provide a complete protein reference pattern. The plate is incubated for 10 minutes. If the complementary antigen is present in proper proportion in the test sample, an antigen/antibody complex forms and precipitates. The formation of a stable antigen/antibody precipitate fixes the protein in the gel. Three variables of the protein can be determined using immunofixation: (1) antigenic specificity; (2) electrophoretic mobility (3) quantity or ratio of the protein to other proteins under study.

Deproteination:

After fixation, the electrophoresis gel is washed in a deproteination solution such as dilute sodium chloride and the nonprecipitated proteins are washed out of the agarose leaving only the antigen/antibody complex.

Staining:

The protein reference pattern and the antigen/antibody precipitation bands are stained with a protein sensitive stain such as Amido Black.

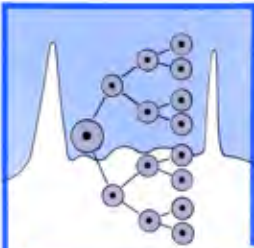
Alfonso first described immunofixation in the literature in 1964. Alper and Johnson published a more practical procedure in 1969 as a result of their work devoted to the detection of genetic polymorphisms of ceruloplasmin and Gc globulin and the conversion of C3 during activation. They later extended their studies to genetic polymorphisms of complement components and the identification of alpha₁-antitrypsin phenotypes. Immunofixation was introduced as a procedure for the study of immunoglobulins in 1976.

The Advantages of Immunofixation

Immunofixation electrophoresis (IFE) combines the protein separating powers of high resolution protein electrophoresis with the inherent specificity of the antigen-antibody reaction, providing a powerful analytical tool for investigating proteins. Immunofixation is replacing traditional immunoelectrophoresis (IEP) in many laboratories as the method of choice for characterizing and typing monoclonal immunoglobulins.

The widespread use of IFE can be attributed to several features incorporated into the procedure:

1. **Fast turnaround time**
Time is the first area where IFE really excels. A complete IFE work-up can be performed within 1½ to 2 hours where IEP requires overnight incubation.
2. **Sensitivity**
Immunofixation is a very sensitive system. It can detect and characterize monoclonal proteins at levels as low as 50 to 150 mg/dL compared to one gram for IEP. As routine electrophoresis has improved, laboratories are detecting more small monoclonal bands. Immunofix provides a method with the sensitivity to characterize any monoclonal band seen on electrophoresis.
3. **Resolution**
IFE utilizes the full capability of agarose to separate monoclonal bands with close electrophoretic mobility. This feature is especially useful with proteins that tend to polymerize, like IgA and light chains and those that break down into several monoclonal bands, as is sometimes the case with IgM.
4. **Ease of interpretation**
Results on IFE are easier to interpret than IEP because interpretation is based on examination of a precipitin pattern directly analogous to the routine electrophoresis pattern and does not depend on detecting slight deviations in the shape of a precipitin arc.



Monoclonal Gammopathies

Monoclonal gammopathies are characterized by an uncontrolled proliferation of a single clone of plasma cells at the expense of other clones. This dysfunction often leads to the synthesis of large amounts of one homogeneous immunoglobulin or immunoglobulin subunit with decreased levels of normal immunoglobulins. The stimulus for proliferation is unknown but it is probably not antigenic.

Since normal immunoglobulins are often suppressed in these disorders, there can be a functional immunodeficiency in association with increased gamma globulin levels. This immunosuppression can have life-threatening consequences in a patient whose condition is already compromised by the primary disease.

Electrophoretic patterns and immunoglobulin test results can be strikingly abnormal in patients with multiple myeloma and other B-cell-related neoplasms. As a result, protein analysis has been a valuable tool in the diagnosis and monitoring of these lymphoproliferative diseases.

Immunochemical methods can quantitate the abnormal protein production that marks these disorders, but only electrophoresis can demonstrate its monoclonal nature. The gamma region of the electrophoretic strip can show a dense, highly restricted band from uncontrolled proliferation of one cell clone, with decreased background staining

due to the shutdown of normal immunoglobulin synthesis.

Even though monoclonal gammopathies can have a dramatic pattern of protein change in some instances, their clinical interpretation can be difficult. It is important to consider that one-third of the patients with immunochemical evidence of monoclonal gammopathy are asymptomatic. These could be patients with benign or transient monoclonal proteins, especially in an older population. They could also represent an early or relatively dormant stage of the disease that might later accelerate.

Some patients show symptoms of a plasma cell dyscrasia or other lymphoproliferative disorder, but do not exhibit the characteristic monoclonal band or spike in their serum protein patterns. This is often the case with light chain disease where only kappa or lambda monoclonal light chains are synthesized by the clone. These low molecular weight immunoglobulin fragments are filtered through the glomerulus and into the urine, giving a serum electrophoresis pattern that shows hypogammaglobulinemia with either a very faint monoclonal band or no band at all. There is also the possibility of a non-secretory clone which produces no monoclonal immunoglobulin. The serum protein patterns in patients with non-secretory clones frequently show hypogammaglobulinemia due to the inhibition of normal clones.

Suggested Protocol for Monoclonal Gammopathy Evaluation

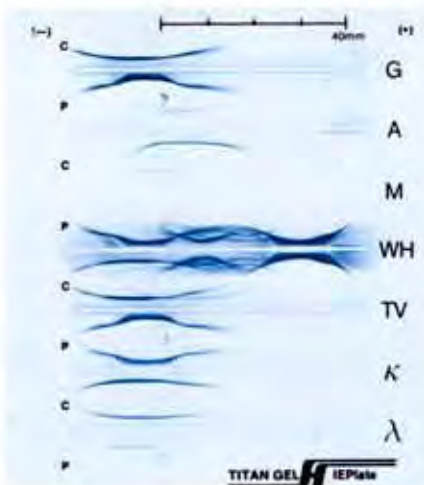
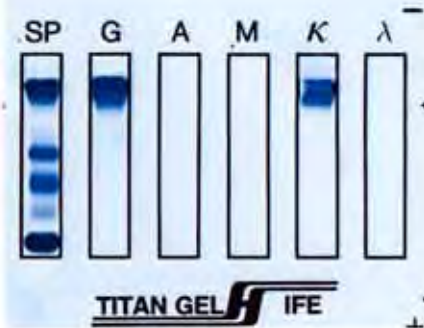
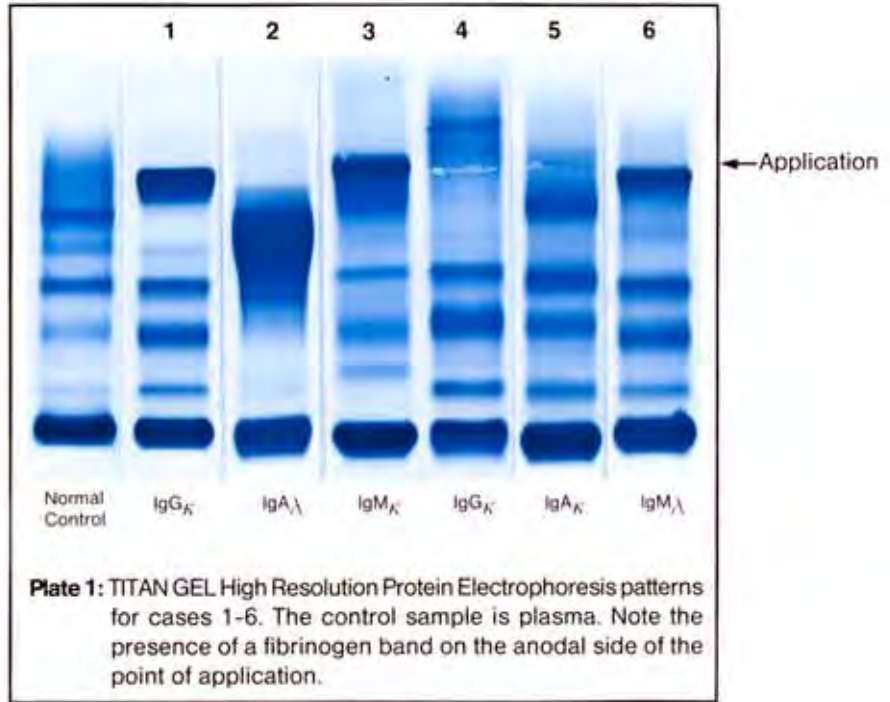
Initial Work-up

- | | |
|---|---|
| 1. Serum and urine high resolution protein electrophoresis.
A 24-hour urine specimen is preferable but a random specimen is adequate to characterize the monoclonal protein. | Only electrophoresis can demonstrate the monoclonal nature of the protein. |
| 2. Quantitative serum immunoglobulins | To assess general immune competence and provide base values of immunoglobulin concentrations for follow-up. |
| 3. Serum and urine immunoelectrophoresis or immunofixation. | Definitive identification of the monoclonal protein. |

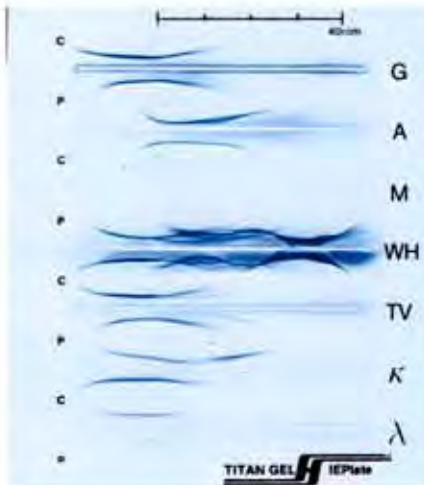
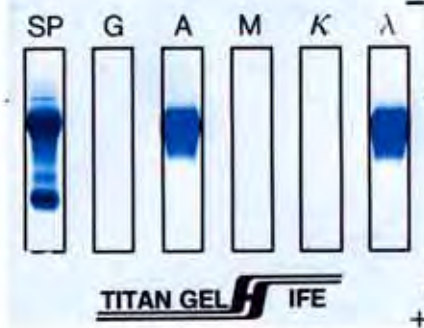
Follow-Up Studies

1. Serum and urine high resolution electrophoresis.
2. Quantitative serum immunoglobulins.
3. Immunoelectrophoresis or immunofixation may be helpful in follow-up studies for very rare cases in which the immunoglobulin class changes during the course of the disease.

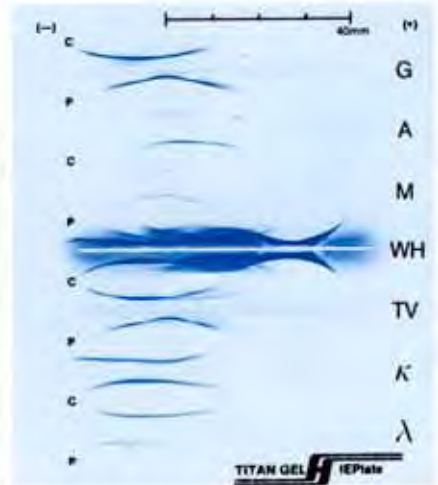
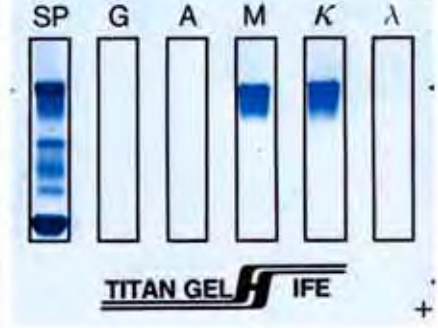
The Monoclonal Gammopathies



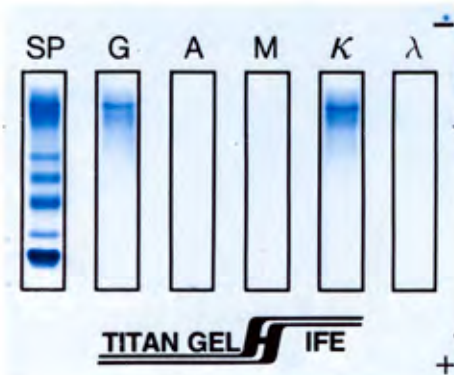
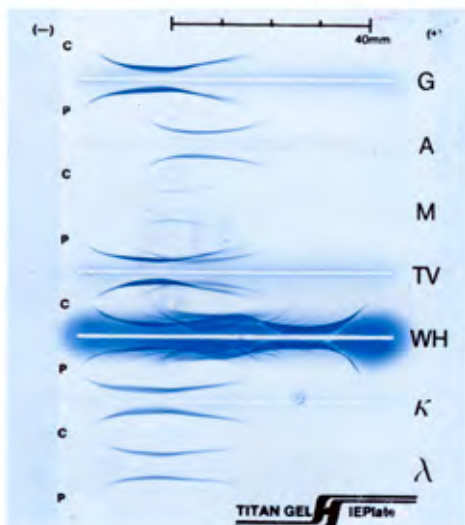
Case 1
IgG Kappa monoclonal gammopathy
 Ig quantitations by RID:
 IgG 3524 mg/dL
 IgA < 40 mg/dL
 IgM 20 mg/dL



Case 2
IgA Lambda monoclonal gammopathy
 Ig quantitations by RID:
 IgG 200 mg/dL
 IgA 4520 mg/dL
 IgM 35 mg/dL



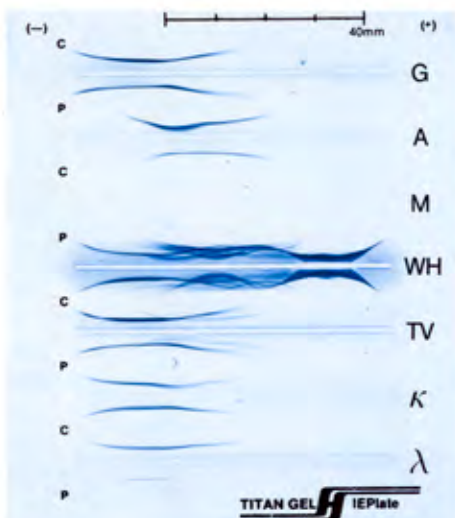
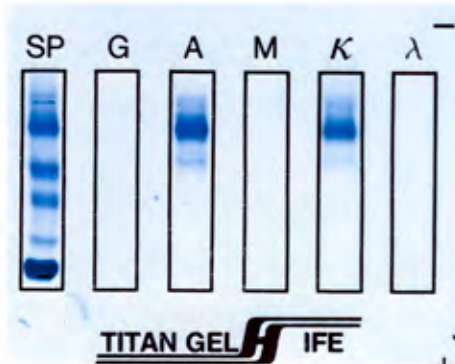
Case 3
IgM Kappa monoclonal gammopathy
 Ig quantitations by RID:
 IgG 645 mg/dL
 IgA 74 mg/dL
 IgM 6515 mg/dL



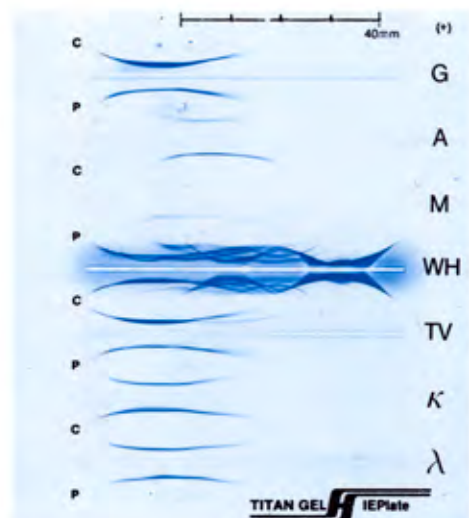
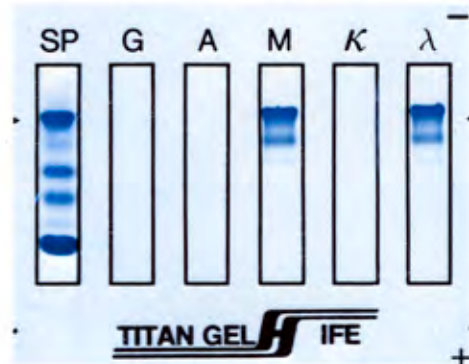
Case 4
IgG Kappa monoclonal gammopathy

This case demonstrates the advantage of using IFE to identify minimonoclonal bands. Due to the low level of monoclonal IgG, the IEP results are not definitive. However, the IFE results provide definitive identification of the small monoclonal band in the mid-zone of the gamma region.

When low concentrations of the monoclonal protein are present as in this sample, the IFE band may appear in the stained background of the polyclonal immunoglobulins. A stained background may also appear when the M-protein is present along with a large polyclonal increase.



Case 5
IgA Kappa monoclonal gammopathy
Multiple bands are probably due to the polymerization of IgA molecules. The formation of dimers and trimers through polymerization results in proteins with different migration patterns.



Case 6
IgM Lambda monoclonal gammopathy
The double bands are most likely due to the presence of intact 19s IgM pentamers and 7s (monomer) subunits.

Bence Jones Proteins and Free Light Chain Disease



Bence Jones Proteins (BJP) are monoclonal kappa or lambda immunoglobulin light chains which are not attached to the heavy chain portion of the immunoglobulin molecule. Bence Jones proteins are seen in two types of syndromes (1) in conjunction with a typical monoclonal gammopathy or (2) in free light chain disease.

Light chain disease is a monoclonal gammopathy in which only kappa or lambda monoclonal light chains, or Bence Jones proteins, are produced. Light chain disease comprises 10% to 15% of monoclonal gammopathies. It ranks behind IgG myeloma (about 60%) and IgA myeloma (about 15%) in incidence and occurs about as often as Waldenstrom's macroglobulinemia. Its diagnosis presents various difficulties not associated with other common monoclonal gammopathies and requires considerable clinical skill and sophisticated analytic techniques.

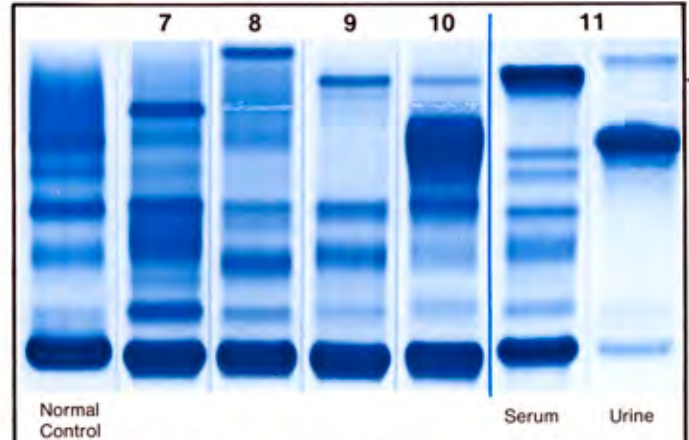
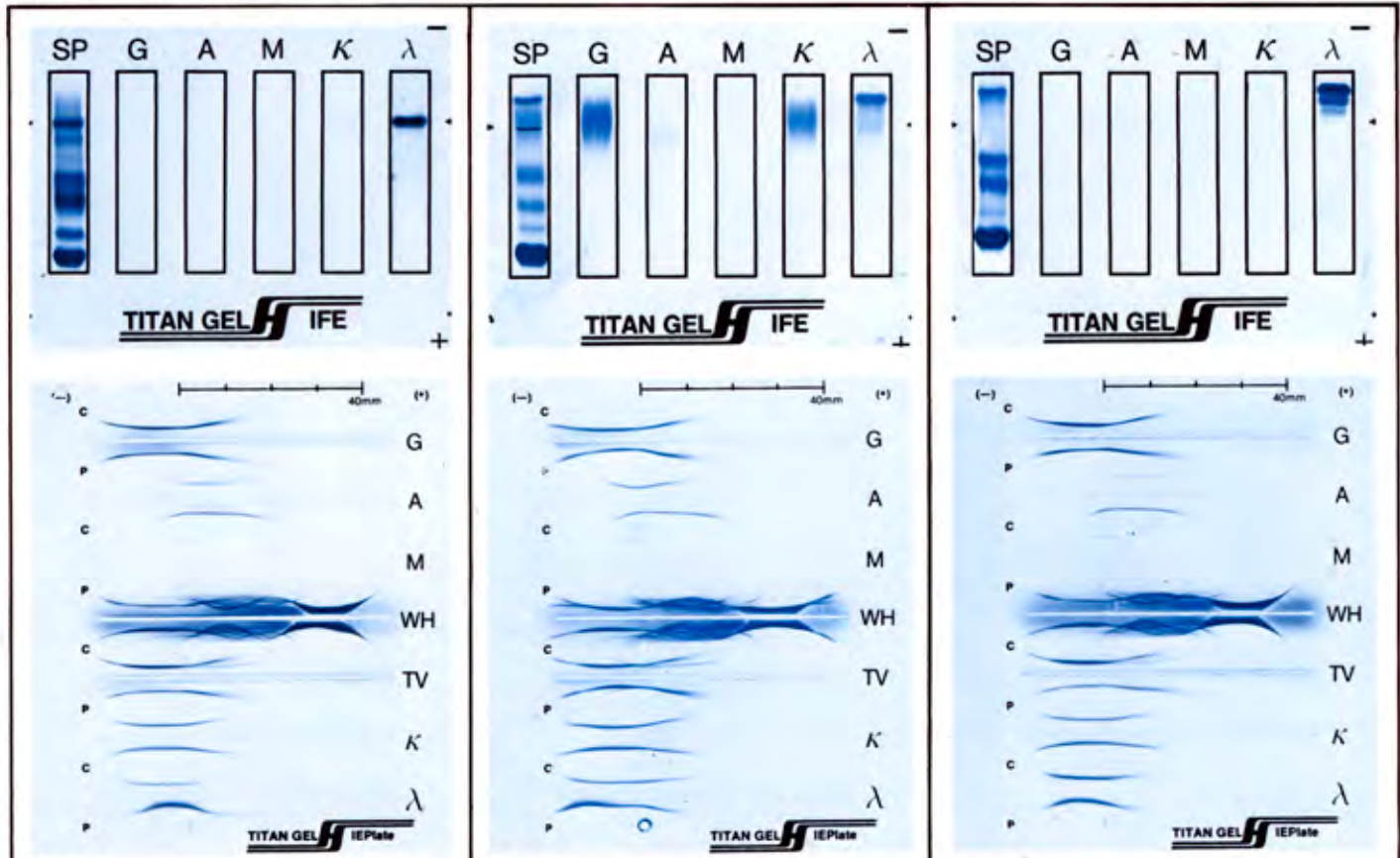


Plate 2: TITAN GEL High Resolution protein electrophoresis patterns for cases 7-11. The normal control is plasma.



Case 7
Free Lambda Light Chain Disease
 The monoclonal immunoglobulin is identified as a monoclonal free light chain. Note that the sample contains a second prominent band slightly anodic to the free lambda band, which did not react with IgG, A, M, kappa or lambda antisera. The band was identified as fibrinogen.

Case 8
Free Lambda Light Chain Disease
 The monoclonal Ig is identified as free lambda light chain. Note that due to increased polyclonal IgG Kappa, a reaction also occurred in the IgG and kappa positions on the IFE plate. However, the broad diffuse polyclonal increase is easily differentiated from a restricted monoclonal band.

Case 9
Free Lambda Light Chain Disease
 The monoclonal Ig is identified as free lambda light chain. Multiple bands are probably due to the polymerization of the lambda chains. The monomers, dimer and trimers, have different migration patterns.

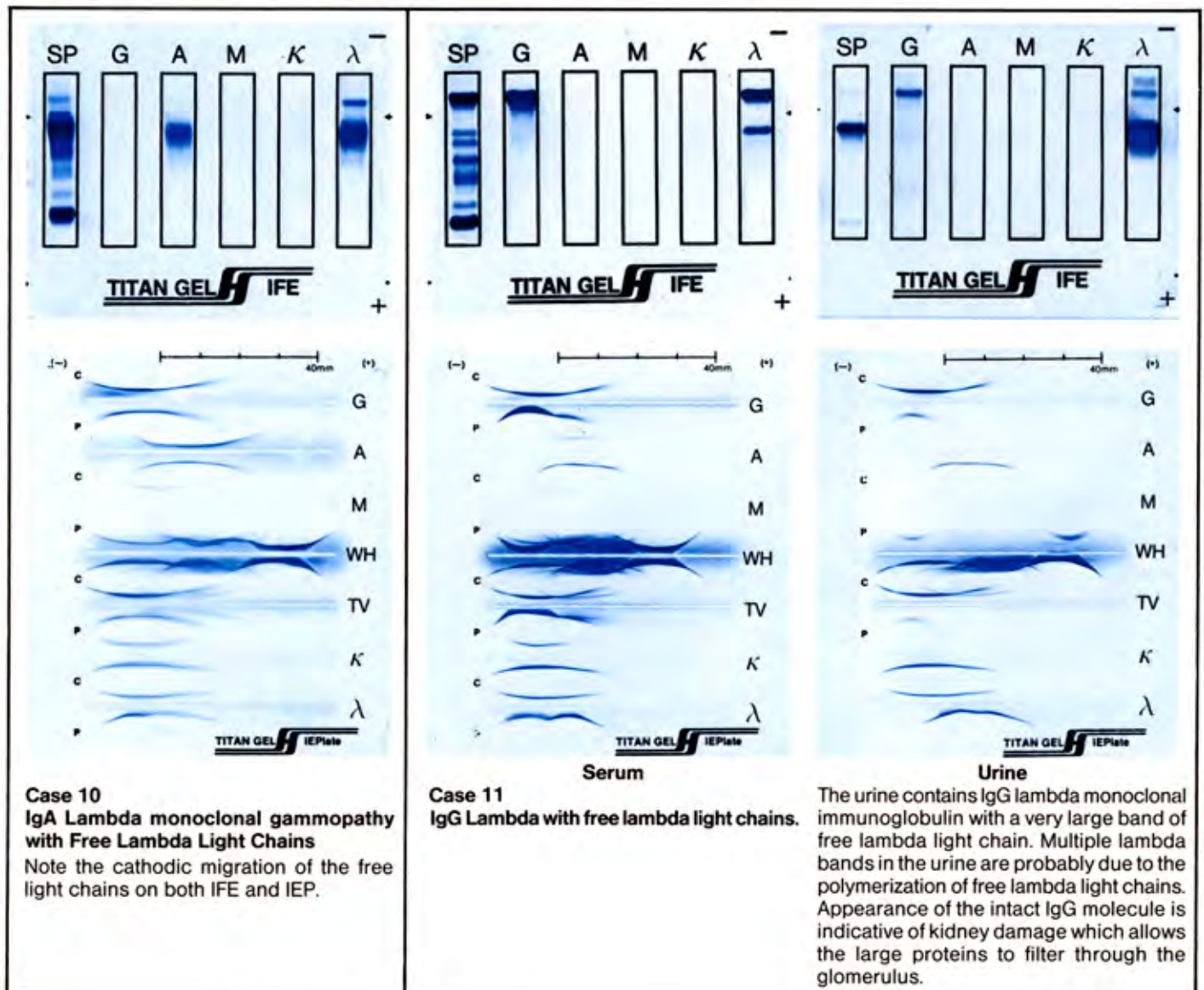
Patients with B-cell malignancies other than light chain disease may be asymptomatic until serum levels of monoclonal protein are very high (about 3 gm/dL is an accepted cutoff, above which patients are usually symptomatic). On the other hand, very small amounts of Bence Jones protein in serum can be associated with significant clinical problems, especially pathologic renal changes. Free light chains filter through the glomerulus almost without obstruction due to their small molecular size, and accumulate in the tubules. Renal impairment can result from the toxicity of the light chains. Pathological changes can range from relatively benign tubular proteinuria to acute renal failure or amyloidosis.

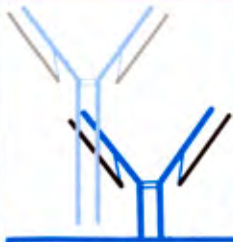
BJP may be detected in serum, urine or both. The level of monoclonal light chain in serum or urine is related to filtration, reabsorption or catabolism of the protein by the kidney. During the early stages of the disease when the kidney is only mildly affected, excretion and reabsorption continue normally but only partial catabolism occurs. At this point, BJP may be detected in the serum but not urine. Progressive renal involvement impairs reabsorption so that diminished reabsorption with decreased catabolism results in free light chains in both serum and urine. Later, as reabsorption is totally blocked light chains are present in urine only. In terminal stages of the disease uremia

occurs and renal clearance is affected and BJP again appears in serum.

The laboratory workup of a patient with suspected light chain disease is similar to the workup for any lymphoproliferative disorder, but there are certain changes in approach due to low levels of paraprotein that can be involved. Agarose high resolution protein electrophoresis of serum and urine should be done to determine overall protein status (as little as 50 mg/dL of the monoclonal protein can be detected visually). Even a urine specimen with a normal 24-hour protein excretion rate should be examined electrophoretically since almost all protein may be Bence Jones protein. Densitometric scanning of electrophoretic patterns provides at least a semi-quantitative estimate of a monoclonal band if it is large enough, but small light chain bands often show no significant peak on a scan. Visual examination of the original pattern is thus an essential part of interpretation.

Serum protein electrophoresis patterns from patients with monoclonal free light chains may show: (1) The typical well defined monoclonal band, (2) A somewhat broad, diffuse band (due to polymerization of monoclonal proteins), (3) A normal gamma region, (4) Hypogammaglobulinemia.





Gammopathies With More Than One Band

Circumstances that can lead to difficulties in interpretation of protein patterns include those conditions in which more than one monoclonal band is produced. Advanced gammopathies are often associated with the asynchronous production of the components of the immunoglobulin molecule. This can lead to synthesis of an intact monoclonal immunoglobulin plus excess monoclonal light chains, for instance. Monoclonal IgA molecules have a tendency to dimerize, and the resulting dimer often has a different mobility than the monomer parent molecule. The pentameric IgM, on the other hand, can break down into 7s subunits which show up on electrophoresis as one or more "extra" monoclonal bands. There is also the possibility that more than one clone could be producing monoclonal immunoglobulins, a true biclonal gammopathy. Although it is tempting to call gammopathies with two bands "biclonal," routine laboratory techniques cannot distinguish between the various mechanisms which could produce two (or more) monoclonal bands. For example, a serum sample with an IgG Kappa and an IgA lambda band should be appropriately reported "Gammopathy with IgG kappa and IgA lambda monoclonal bands."

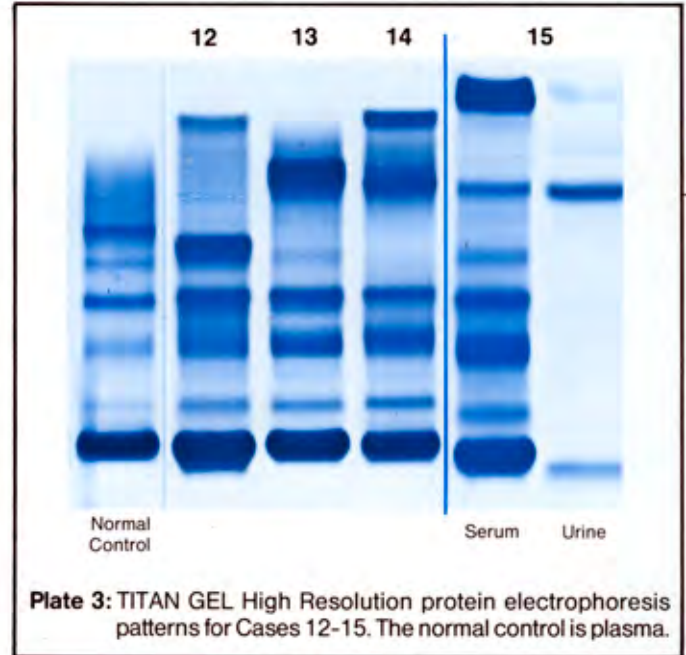
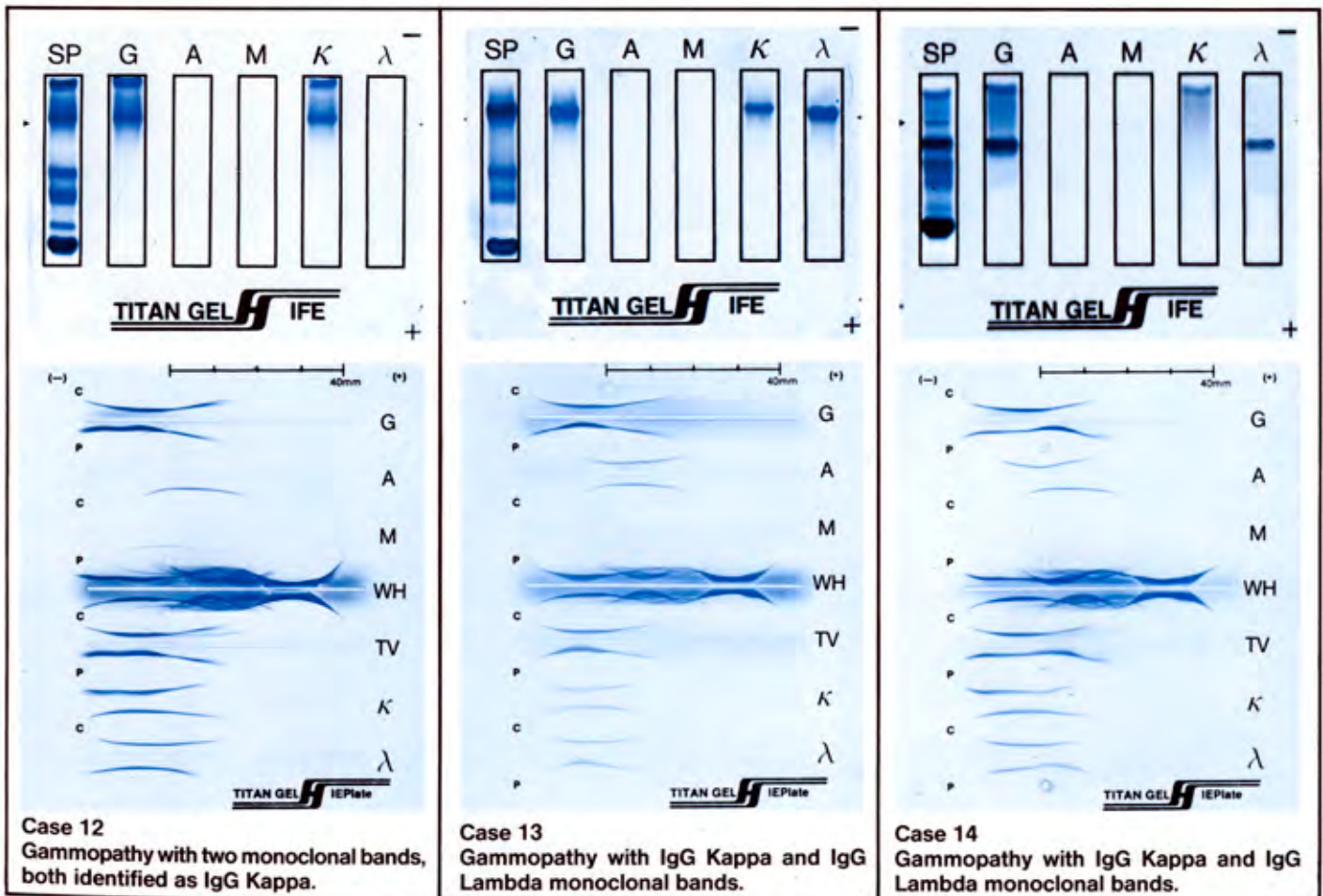


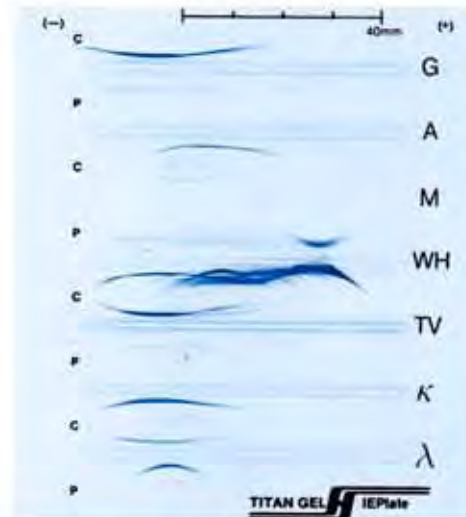
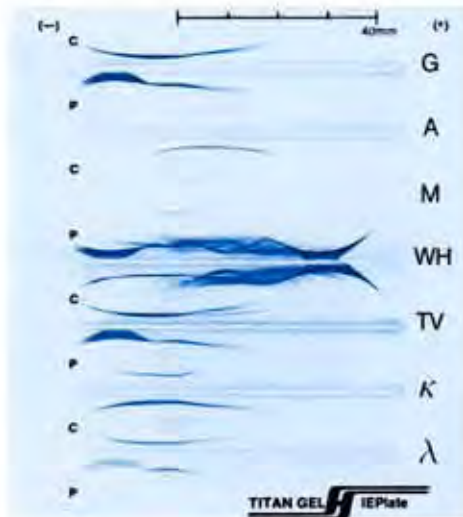
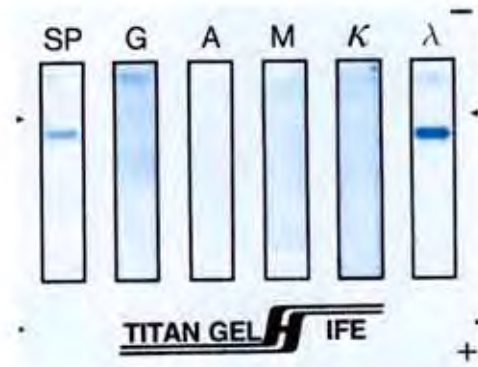
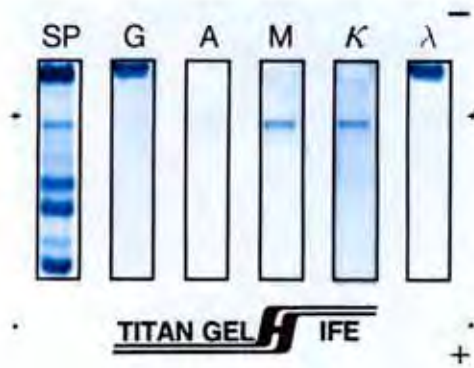
Plate 3: TITAN GEL High Resolution protein electrophoresis patterns for Cases 12-15. The normal control is plasma.



Case 12
Gammopathy with two monoclonal bands, both identified as IgG Kappa.

Case 13
Gammopathy with IgG Kappa and IgG Lambda monoclonal bands.

Case 14
Gammopathy with IgG Kappa and IgG Lambda monoclonal bands.

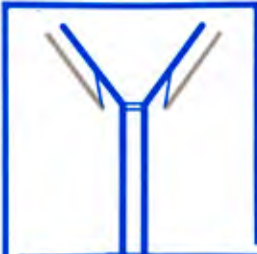


Serum

Case 15
 Gammopathy with IgG Lambda and IgM
 Kappa monoclonal bands.

Urine

The urine contains IgG Lambda and free
 lambda light chain monoclonal immuno-
 globulins.



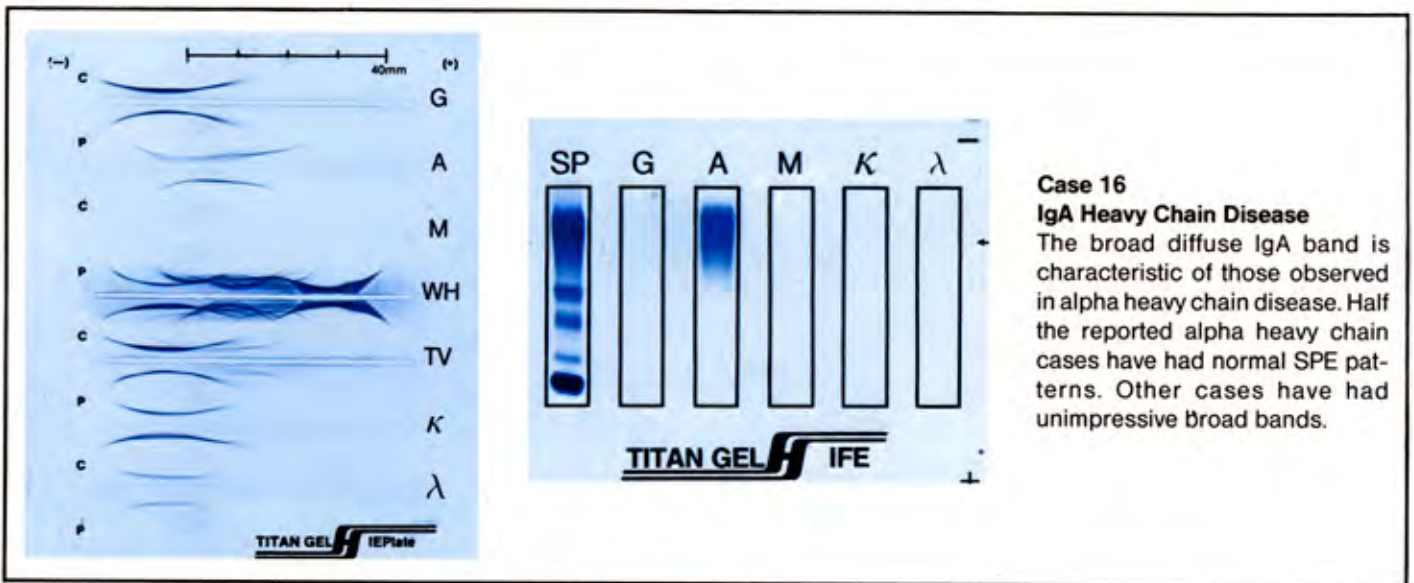
Heavy Chain Disease

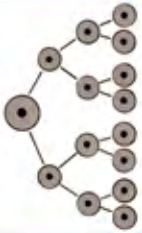
The heavy chain diseases are characterized by the presence of monoclonal proteins comprised of the heavy chain portion of the immunoglobulin molecule. They may be detected in serum or urine or both (depending on the class of heavy chain involved).

Franklin reported the first case of heavy chain disease, which involved a gamma chain. Franklin's disease is synonymous with gamma heavy chain disease. Alpha heavy chain disease is the most frequent of the heavy chain

gammopathies with approximately 60 cases reported in the literature, most of which have been in men of Mediterranean decent. Mu heavy chain disease is rare, with less than 10 cases reported in the literature.

When heavy chain disease is suspected, monospecific anti-FAB antisera should be used for definitive testing. The serum sample should also be diluted and re-tested with kappa and lambda light chain antisera to rule out prozoning due to antigen excess.





Monoclonal Proteins in CSF

Appropriate Use of Immunofixation

This case illustrates the usefulness of immunofixation to identify a small monoclonal IgM Kappa band in CSF and serum. As with this case, most monoclonal bands in CSF result from leakage of the protein from the plasma across the blood-CSF barrier.

Immunofixation has been used in some research studies to show that the oligoclonal bands seen in CSF protein patterns are made up primarily of IgG. While this may be of academic interest, characterization of the immunoglobulin in the bands does not significantly improve the diagnostic usefulness of the test. Exceptions to this general rule could occur in cases where banding might be caused by some protein other than IgG, but this would be exceedingly rare. Immunofixation of CSF electrophoresis patterns is therefore not usually indicated in the routine laboratory. Immunoelectrophoresis of CSF provides no useful information on oligoclonal bands, since the technique has neither the resolution nor the sensitivity required to detect them.

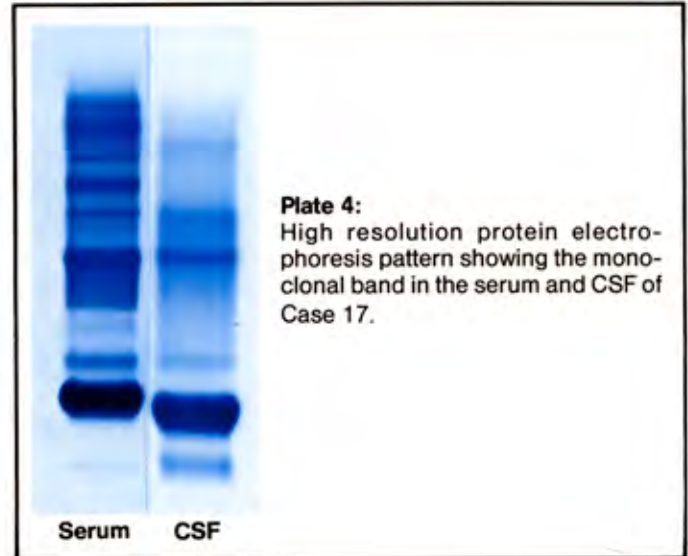
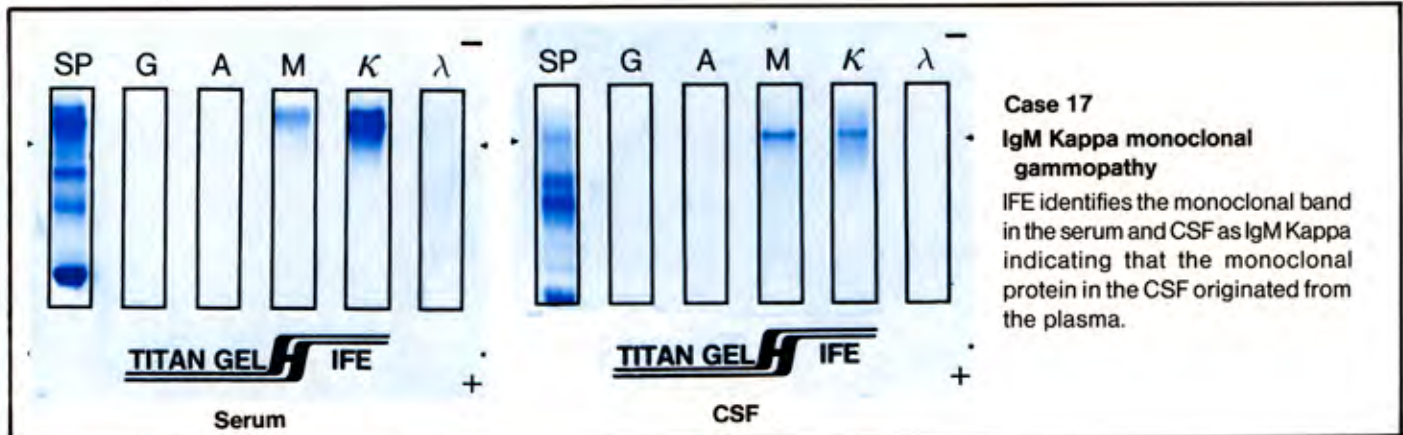
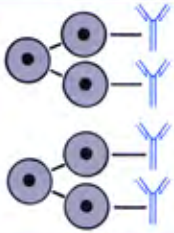


Plate 4:
High resolution protein electrophoresis pattern showing the monoclonal band in the serum and CSF of Case 17.



Case 17
IgM Kappa monoclonal gammopathy
IFE identifies the monoclonal band in the serum and CSF as IgM Kappa indicating that the monoclonal protein in the CSF originated from the plasma.



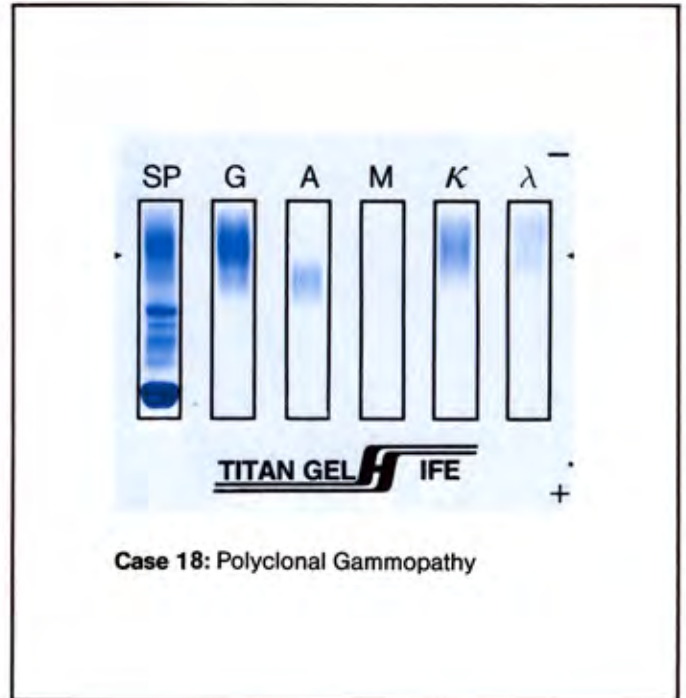
Polyclonal Gammopathy

A polyclonal gammopathy is seen on protein electrophoresis as a very broad diffuse and intense band in the gamma region. Simply stated a polyclonal gammopathy resembles a normal pattern, but stains more intensely.

A polyclonal gammopathy is a common protein abnormality, defined as an increase in more than one immunoglobulin and involves several clones of plasma cells. Polyclonal increases are secondary due to inflammation or infection. They are commonly seen in chronic infections, chronic liver disease and collagen disorders.

Selective polyclonal increases are of special interest. A selective polyclonal increase means that only one class of immunoglobulin is significantly elevated. But the increase is polyclonal because production is by several clones of plasma cells and both kappa and lambda types are produced. Quantitation of the immunoglobulins determines which immunoglobulin is increased.

IFE results on a sample containing a polyclonal gammopathy is presented to illustrate the difference in a polyclonal increase and a monoclonal band. However, the use of immunofixation is not recommended in cases of a polyclonal gammopathy. It presents no additional information.





TITAN GEL ImmunoFix Procedure

Procedure Summary

Plate.....	TITAN GEL IFE Plate
Buffer	Dissolved in 1500 mL purified water
Buffer Volume.....	40 mL each side (inner sections)
Serum Dilution.....	1:2 (0.85% saline) for SPE pattern 1:10 (0.85% saline) for immunoglobulin identification
Blot Application Area.....	Blotter A
Sample Volume	3 μ L
Sample Absorption Time.....	5 minutes
Application Point.....	Cathode (at arrows)
Electrophoresis Time.....	20 minutes
Voltage.....	120 volts
Fixative/Antisera Volume	1 drop from applicator vial (50 μ L)
Incubation Time	10 minutes
First Press Step.....	5 minutes 1 Blotter C & 1 Blotter X
Wash Time	4 minutes
Second Press Step.....	1 minute 1 Blotter C & 1 Blotter D
Drying Time/Temp.....	1-2 minutes/56-60°C
Staining Time	4 minutes
Destaining Time.....	2 x 1 minute

Specimen Collection and Handling

Specimen: The specimen may be serum, cerebrospinal fluid (CSF) or urine.

Specimen preparation:

A. Serum

Routinely two sample dilutions are prepared with 0.85% saline as follows:

1. Serum protein reference pattern: Dilute serum 1:2 (1 part serum + 1 part 0.85% saline)
2. Immunofixation patterns (IgG, A, M, kappa and lambda): Dilute serum 1:10 (1 part serum + 9 parts 0.85% saline).

Specimens with minimonoclonal bands:

When testing specimens with minimonoclonal bands, if the sample IgG level exceeds 1500 mg/dL dilute the sample 1:20 (1 part serum + 19 parts 0.85% saline) for the IgG pattern only.

B. Urine

Depending on the protein level, urine may be used neat or may need to be concentrated. In rare cases urine may need to be diluted in order to avoid prozoning. Remember that the monoclonal protein in urine may not correlate well with the total protein value. Studies should be performed even when total protein levels are within normal ranges.

1. Detection of Bence Jones proteins (free kappa and lambda light chains): Determine the total protein on the urine sample. If necessary, concentrate the urine sample to 100 mg/dL of total protein. Use the concentrated sample for all patterns.
2. Detection of intact immunoglobulins: Concentrate the urine specimen to 800-1000 mg/dL total protein for the detection of immunoglobulins. Use the concentrated sample for all patterns.

C. CSF

Concentrate CSF to an IgG level of 100-200 mg/dL for typing oligoclonal bands in CSF. Refer to page 11 for discussion of using IFE on CSF samples.

Storage and Stability of specimens: Fresh serum, CSF or urine is the specimen of choice. If storage is necessary, samples may be stored covered at 2 to 6°C for up to 72 hours. The complement proteins may change under these storage conditions but the immunoglobulins will not be affected. Samples stored for prolonged periods will exhibit breakdown products which may precipitate at the point of application.

Sensitivity and Specificity of TITAN GEL IFE Antisera

The TITAN GEL IFE Antisera are monospecific for the immunoglobulin specificities indicated on each vial. Kappa and lambda light chain antisera react with both bound and free light chains. The antisera titers are optimized to detect monoclonal immunoglobulins in minimum concentrations of 50-150 mg/dL. The antisera will react with immunoglobulins to at least 4000 mg/dL without prozoning.

Troubleshooting

Antigen Excess

Antigen excess will occur if there is not a slight antibody excess or antigen/antibody equivalency at the site of precipitation. Antigen excess in IFE is usually due to an excess of the immunoglobulin in the patient sample. The dissolution of immunoprecipitation is manifested by a loss of protein at the point of highest antigen concentration resulting in staining in the margins and leaving the central area with little demonstrable protein stain. In this case it may be necessary to adjust the protein content of the sample by making additional dilutions.

Electrophoresing excessive amounts of antigen decreases resolution and requires higher concentrations of antibody. For optimum separation and sufficient intensity for visual detection, the antibody content, sample concentration, time and voltage must be adjusted carefully. The TITAN GEL Immunofix method has been optimally developed to minimize the prozoning phenomenon.

Non-specific Precipitation in all Patterns Across the Plate

Occasionally a completed IFE plate exhibits a precipitate band in the same position in every pattern across the plate. The phenomenon may result from:

1. IgM Monoclonal Immunoglobulins
IgM monoclonal proteins occasionally adhere to the gel matrix. A band will appear in all five antisera reaction areas of the gel. However, where the band reacts with a specific antisera for the heavy chain and light chain there will be a marked increase in size and staining activity allowing the band to be identified.
2. High Titers of RF or Immune Complexes
Samples with high titers of Rheumatoid Factor or with immune complexes may have a precipitate band across the plate at the point of origin. Reducing the sample with β -2-mercaptoethanol will usually break the complexes and eliminate the non-specific precipitation. Reduction with β -2-mercaptoethanol:
Add 100 μ L sample to 10 μ L of 1:10 dilution of β -2-ME in water. Perform the IFE procedure as usual. Always work under a hood when using 2-ME.
3. Fibrinogen
If fibrinogen is present in the sample it may adhere to the gel matrix. Fibrinogen is always present in plasma and sometimes in the serum of patients on anti-coagulant therapy.

Reaction with Kappa or Lambda Antisera with no Reactivity Against IgG, IgA, or IgM Antisera

Serum samples which have a precipitin band with kappa or lambda light chain antisera but no corresponding band with IgG, IgA, or IgM antisera may have a free light chain monoclonal gammopathy or they may have an IgD or IgE monoclonal protein. Such sera should be tested with IgD and IgE antisera by immunoelectrophoresis. Failure to get

a reaction with IgD or IgE antisera is indicative of the patient having free light chain disease.

Band in Cathodic End of Gamma Region showing no Reactivity with IFE Antisera

Using agarose high resolution protein electrophoresis a CRP band may be detected in patients with acute inflammatory response. CRP appears as a narrow band on the most cathodic end of the protein electrophoresis pattern. Elevated α_1 antitrypsin and haptoglobin (acute phase proteins) are supportive evidence for the presence of a CRP band. Patients with a CRP band will probably have a positive CRP by latex agglutination or an elevated quantitative CRP. Immunofixation is an excellent method for differentiating a CRP band from a small monoclonal immunoglobulin in the cathodic end of the gamma region.

Non-Reactvity with Kappa and Lambda Antisera

Occasionally a sample will have a reaction with a heavy chain antiserum but no light chain reaction is noted. In this event we must rule out (1) heavy chain disease, (2) very high concentrations of light chains resulting in antigen excess, (3) light chains present in very low concentration, (4) atypical light chain that does not react with the antisera used, (5) light chains with "hidden" light chain determinants (as sometimes seen with IgA or IgD). To obtain definitive results testing may include using (1) a higher dilution of the specimen to determine if antigen excess has occurred, (2) a lesser dilution of the specimen to determine if the light chains were present in low concentration, (3) antisera from more than one manufacturer to aid in identification of atypical immunoglobulin, and (4) treat the sample with β -2-mercaptoethanol to "reveal" the light chains.

Time Parameters

The time parameters established for the procedure have been selected to provide optimal test results and maximum laboratory convenience. Electrophoresis times beyond 20 minutes may result in loss of the albumin band or a cathodically migrating monoclonal protein. Electrophoresis of less time will result in inadequate separation of the proteins. The incubation time of 10 minutes is the minimum time in which adequate precipitation of the proteins occurs. However, a slightly longer incubation time should not present problems.

Antisera Application

The first step in proper antisera application is the alignment of the antisera template. Align the template on the agarose plate so that the slits in the template are aligned over the antisera application areas on the plate. Make sure the template makes good contact with the agarose of the sample plate by running the tip of your finger along the edges of the individual slits. In applying antisera, avoid cutting the agarose with the vial tip and spillage into other areas of the plate.



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