Introduction to High Resolution Protein Electrophoresis and Associated Techniques

LEARNING ACTIVITY PACKAGE

Helena Laboratories

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SECTION I

High Resolution Protein Electrophoresis

Introduction

Study and interpretation of protein electrophoresis patterns takes considerable effort. Interpretation is a skill which must be consistently practiced so that patterns are interpreted accurately and no abnormalities missed.

There are several excellent references (see Reference section) to which laboratory personnel may refer for study of patterns representing significant protein abnormalities. The patterns and exercises contained in this study guide are not intended to replace these references.

The focus of this study guide is to provide a summary of high resolution electrophoresis findings in various sample types, to demonstrate how interpretive reporting might be done in the laboratory, and to give the laboratory worker some practice patterns to interpret.

No single protein study guide can provide examples of every abnormality seen in the lab. Nearly each week, new pattern abnormalities are seen. We have seen that the laboratory worker new at high resolution electrophoresis interpretation is quickly overwhelmed by the number of bands seen and doesn't know where to start to turn the seemingly complex patterns into simple and useful comments that are meaningful to the clinician. This learning package provides an approach to the successful interpretation of high resolution electrophoresis patterns.

High Resolution Electrophoresis

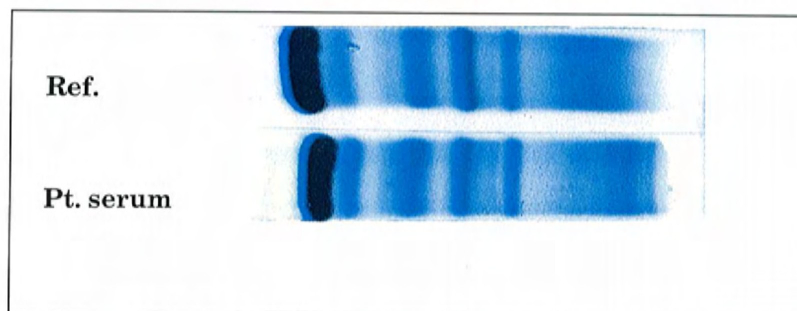
High Resolution Electrophoresis (HRE) is a very sensitive electrophoretic method which resolves a protein pattern into multiple zones. As many as 12 proteins are readily identified by their location and appearance, but several others are recognized when found in abnormal concentration. The high resolution is accomplished by use of agarose gel as the medium, higher voltage coupled with a cooling system in the electrophoretic apparatus, and modification of the electrophoretic buffer.

To obtain the HRE patterns, samples are applied on the agarose gel, which is then electrophoresed in a chamber cooled by a special gel block. The agarose gels are stained with Amido black or Coomasie blue stains, dried, and then inspected.

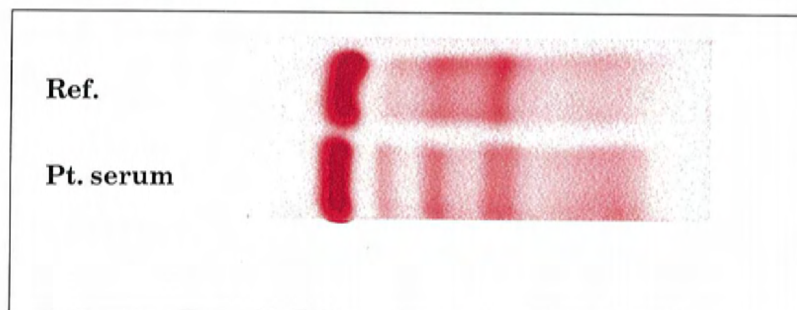
Visual inspection of the agarose patterns is an essential step in their interpretation. Each zone is compared to the same zone on a reference pattern for color density, appearance, migration rates, and appearance of abnormal bands or regions of density. Because the proteins are so clearly separated, a great deal of information about the proteins is learned by this visual inspection step.

In addition, the patterns may be scanned with a densitometer to obtain semiquantitative estimates of the proteins found in each zone. Because there is so much information gained by visual inspection, we have found that the semiquantitative values are best used as an indicator of the quantity of monoclonal proteins present.

In the example shown, a patient serum with multiple bands in the gamma region was electrophoresed using HRE and 5-zone electrophoretic methods.



High Resolution Electrophoresis



5-Zone Electrophoresis

Serum Patterns

A normal serum HRE pattern contains both discrete bands of protein as well as less distinct and homogeneous areas of protein. So that interpretation can be standardized, these areas can be divided into 12 distinct zones and interzones, retaining a similar classification scheme used with 5-zone electrophoresis. Each zone is denoted by pointers 1-12 on the pattern shown. In the table which follows, the serum proteins which commonly migrate in each zone are noted. Prealbumin migrates the fastest (most anodic) of the proteins listed. IgG and C-reactive protein are the slowest (most cathodic).

The serum shown as "normal" represents a pool of fresh sera from normal human volunteers. Since a range of normal values may exist for each protein, an individual pattern may have slightly different densities of protein. Other normal variations include non-pathologic genetic variants, seen particularly with haptoglobin.

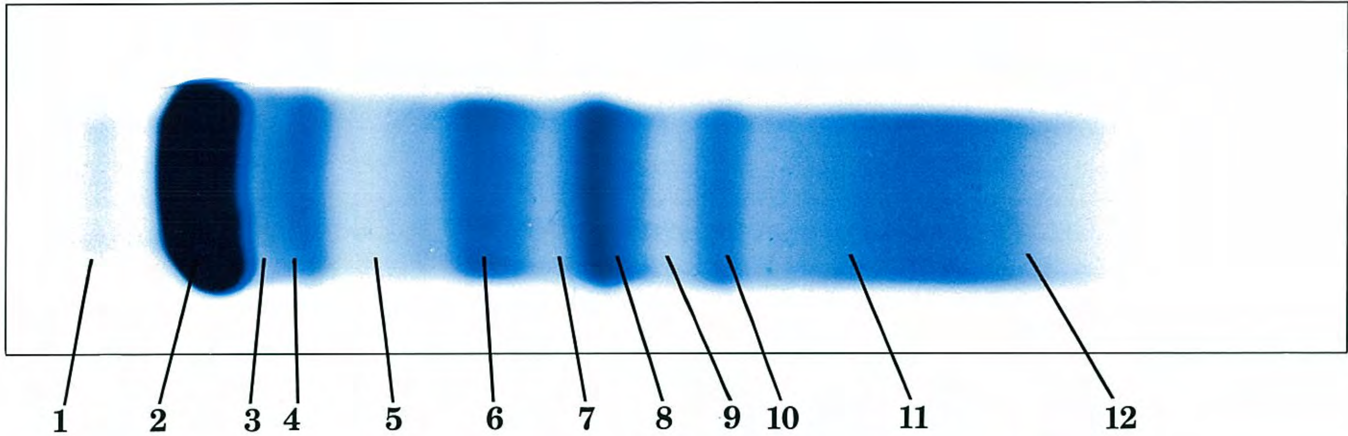
Proteins seen in an HRE pattern can become increased or decreased in concentration in disease states and will thus appear more or less dense than a reference pattern. Proteins not normally visible in a pattern can become increased in concentration and contribute to the pattern density. Proteins present as homogeneous areas or discrete bands can be altered in appearance or migration by changes in protein binding due to drugs or other interactions or the presence of abnormal clones of proteins.

It is not within the scope of this study guide to discuss in detail all functions or clinical situations in which these, or other, proteins are increased and decreased. A brief description of each of the proteins mentioned is provided in the glossary at the end of this manual.

Serum protein electrophoresis may be used as a screen; in particular, it can be used to screen for abnormalities in the immunoglobulin fractions.

Serum Patterns

Normal HRE Pattern



Zones	Serum Proteins Found in Zones
1. PREALBUMIN ZONE	- Prealbumin
2. ALBUMIN ZONE	- Albumin
3. ALBUMIN-ALPHA-1 INTERZONE	- Alpha-lipoprotein, (Alpha-fetoprotein)
4. ALPHA-1 ZONE	- Alpha-1-antitrypsin, Alpha-1-acid glycoprotein
5. ALPHA-1-ALPHA-2 INTERZONE	- Gc-globulin, Inter-alpha-trypsin inhibitor, Alpha-1-antichymotrypsin
6. ALPHA-2 ZONE	- Alpha-2-macroglobulin, Haptoglobin
7. ALPHA-2-BETA-1 INTERZONE	- Cold insoluble globulin, (Hemoglobin)
8. BETA-1 ZONE	- Transferrin
9. BETA-1-BETA-2 INTERZONE	- Beta-lipoprotein
10. BETA-2 ZONE	- C3
11. GAMMA-1 ZONE	- IgA, (Fibrinogen), IgM (Monoclonal Ig's, light chains)
12. GAMMA-2 ZONE	- IgG, (C-reactive protein) (Monoclonal Ig's, light chains)

Proteins listed in ( ) are normally found in too low a concentration to be visible in a normal pattern.

Urine Patterns

Normal urine contains very small amounts of protein and requires concentration 50x or more. Electrophoresis of the concentrated sample will yield the same high resolution of the proteins present as seen in serum. The pattern shown was obtained with a sample of urine from a normal subject. Note that only albumin is visible.

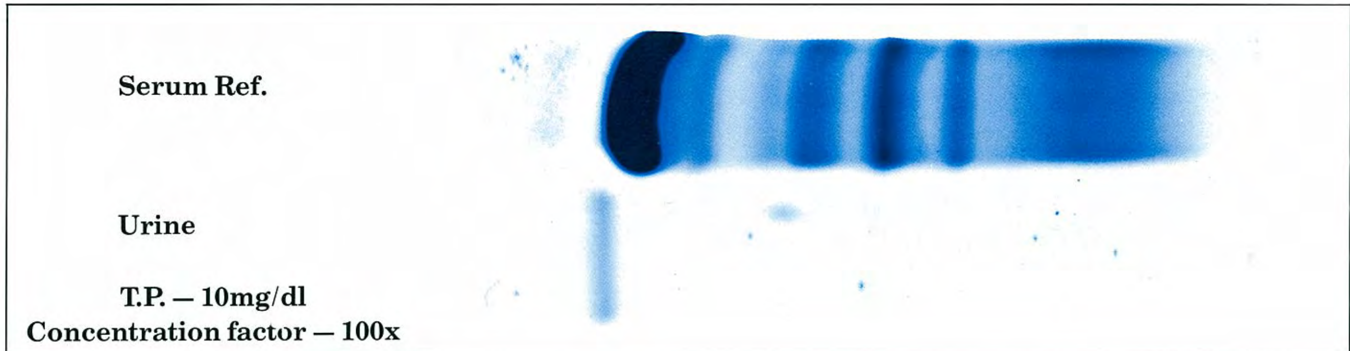
Proteins in the urine can be increased with either glomerular or tubular dysfunction, as well as in conditions of 'overflow,' seen with light chain disease, inflammation, etc. Urine HRE is frequently performed to screen for monoclonal bands or light chains. In light chain disease, the abnormal amounts of the monoclonal protein are filtered through the glomerulus. The monoclonal proteins are usually found in the gamma region of the HRE, but may be found in alpha or beta zones.

In selective glomerular proteinuria, the urine pattern reflects the loss of selectivity found in the glomerulus, with albumin, alpha-1- antitrypsin, alpha-1-acid glycoprotein and transferrin (MW 39,500- 76,500) excreted. In tubular proteinuria, the reabsorptive capacity of the tubules for low molecular weight proteins (MW < 20,000) is compromised. The urine pattern generally shows the presence of a small amount of albumin, 2 bands in the alpha-2 region (alpha-2 microglobulin) and a band in the mid-beta region (beta-2 microglobulin). In non-selective proteinuria, the urine pattern can resemble that of serum.

In the table which follows, the proteins seen in urines are noted. For consistency of interpretation, these zones are denoted similarly to serum patterns except that the gamma region is not split into 2 zones. The proteins commonly found in the zones differ slightly from the serum proteins.

Urine Patterns

Normal Urine Pattern



Zones	Urine Proteins Found in Zones
1. PREALBUMIN ZONE	-
2. ALBUMIN ZONE	- Albumin
3. ALBUMIN-ALPHA-1 INTERZONE	-
4. ALPHA-1 ZONE	- (Alpha-1-antitrypsin) (Alpha-1-acid glycoprotein)
5. ALPHA-1-ALPHA-2 INTERZONE	- (Zn-alpha-2-glycoprotein)
6. ALPHA-2 ZONE	- (Alpha-2-microglobulin, seen as two bands)
7. ALPHA-2-BETA-1 INTERZONE	-
8. BETA-1 ZONE	- (Transferrin)
9. BETA-1-BETA-2 INTERZONE	- (Beta-2-microglobulin)
10. BETA-2 ZONE	-
11. GAMMA ZONE	- (IgG, IgA, light chains, lysozyme, gamma trace)

Proteins listed in ( ) are normally found in too low a concentration to be visible in a normal pattern.

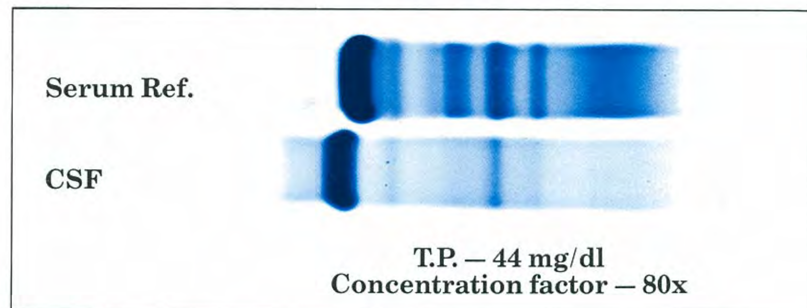
C S F Patterns

Cerebrospinal fluid (CSF) is a clear, colorless fluid that bathes and protects the brain and spinal cord. The fluid is essentially an ultrafiltrate of plasma. The amount of protein in normal CSF is much less than in plasma; CSF thus requires concentration (usually 80x) before the the HRE is done. Approximately 80% of spinal fluid protein is derived from plasma and the remaining 20% is synthesized locally. Normal CSF concentrated 80x for HRE predominantly demonstrates prealbumin, albumin, and transferrin along with small quantities of other low molecular weight proteins. In the pattern shown, the CSF sample, concentrated 80x, is shown next to the normal serum reference.

Electrophoresis of spinal fluid is most often performed to detect oligoclonal banding (many bands) in the gamma region. These CSF bands are immunoglobulins that are produced in the central nervous system and may be diagnostic of multiple sclerosis (MS). CSF Oligoclonal bands can sometimes be seen in viral infections, but these are transient bands where bands from MS are often persistent. Monoclonal CSF bands can be seen, and are most often of serum origin (e.g. from multiple myeloma).

The following table shows the proteins commonly found in a normal CSF. For consistency of interpretation, these zones are denoted similarly to serum patterns except that the gamma region is not split into 2 zones. The proteins found in the zones differ slightly from serum proteins.

Normal CSF Pattern



T.P. — 44 mg/dl
Concentration factor — 80x

Zones	CSF Proteins Found in Zones
1. PREALBUMIN ZONE	-Prealbumin
2. ALBUMIN ZONE	- Albumin
3. ALBUMIN-ALPHA-1 INTERZONE	-
4. ALPHA-1 ZONE	- Alpha-1-antitrypsin
5. ALPHA-1-ALPHA-2 INTERZONE	-
6. ALPHA-2 ZONE	-
7. ALPHA-2-BETA-1 INTERZONE	-
8. BETA-1 ZONE	- Transferrin
9. BETA-1-BETA-2 INTERZONE	-
10. BETA-2 ZONE	- Beta-2-transferrin
11. GAMMA ZONE	- IgG (oligoclonal bands) (Gamma trace seen as a distinct band at the extreme cathodal end)

Proteins listed in ( ) are normally found in too low a concentration to be visible in a normal pattern.

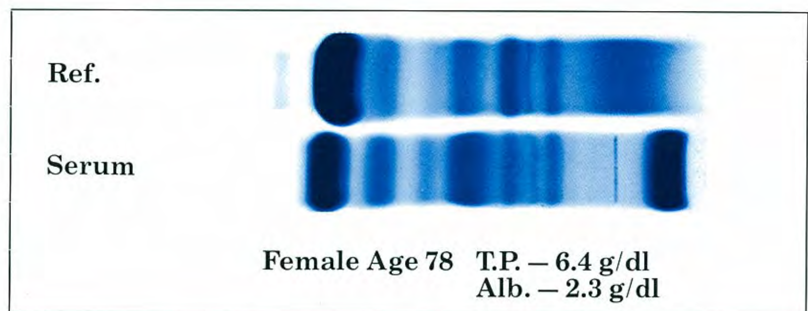
Supporting Methods - HRE,IFE

Because high resolution electrophoresis is so frequently used to screen for abnormalities in the gamma region, it has become one of a battery of protein tests used for monoclonal gammopathy evaluation. There are 3 complementary techniques used by the laboratory which aid in the interpretation of electrophoresis results. These are immunofixation electrophoresis, immunoelectrophoresis, and immunoglobulin quantitation.

Immunofixation electrophoresis (IFE) is a two-staged process consisting of agarose gel protein electrophoresis in the first stage and precipitation with specific antibodies in the second. Immunoelectrophoresis (IEP) is a two-staged process consisting of agarose gel protein electrophoresis in the first stage and immunodiffusion with resultant precipitation arcs in the second.

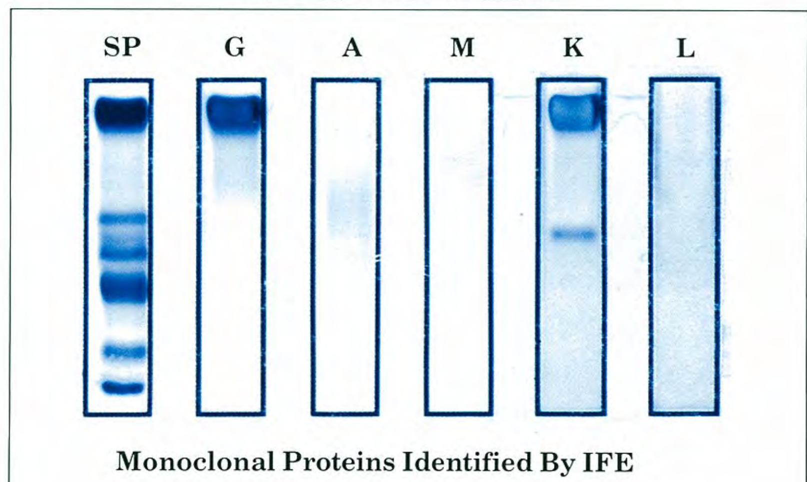
The third technique which provides useful information in interpretation is specific protein quantitation. This is frequently done by rate nephelometry.

In the HRE pattern shown, there is an abnormal band of protein in the gamma-2 zone. The location of the band on the pattern suggests that it is a monoclonal immunoglobulin. IFE and IEP were performed on the specimen.



Abnormal HRE Pattern

To obtain the IFE pattern, the sample was first applied to the six positions on the agarose plate and the proteins separated by electrophoresis. In the second step, specific antisera was applied to 5 of the electrophoretic lanes, and a protein fixative was applied to the sixth lane. The different antisera included anti-IgG, anti-IgA, anti-IgM, anti-kappa, and anti-lambda. Antigen-antibody formation and precipitation occured in the gel when the specific immunoglobulin was present.

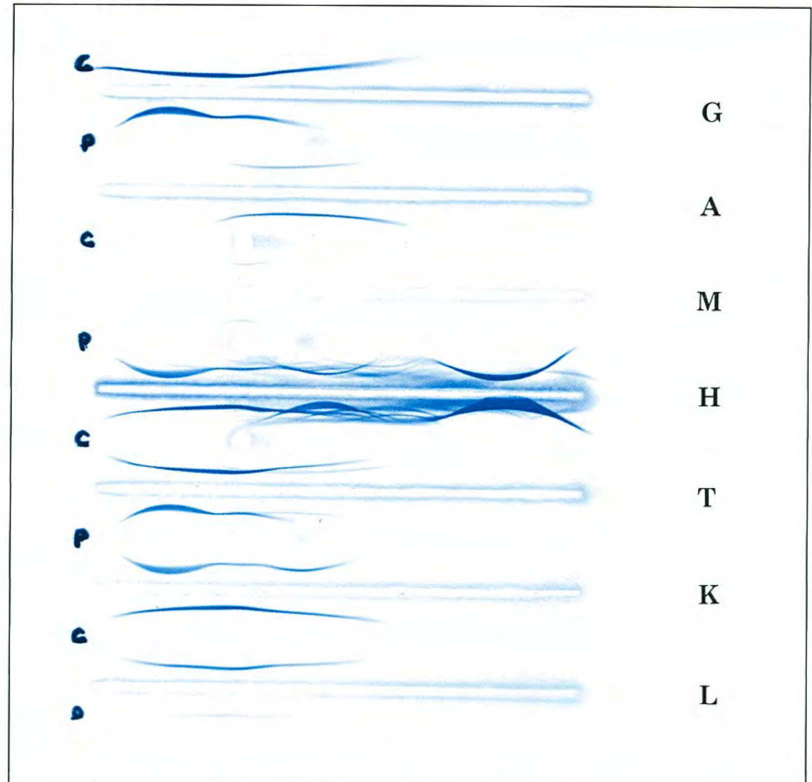


Monoclonal Proteins Identified By IFE

In the IFE pattern shown, the monoclonal band seen in the serum (SP) was identified as IgG/K by the presence of stained, precipitated antigen-antibody complex in the IgG and kappa lanes. There were no IgA, IgM, or lambda antigen-antibody complexes seen, suggesting reduced quantities of these immunoglobulins. Polyclonal immunoglobulin complexes appear as stained background. Two additional pieces of information can be learned from the pattern. First, an additional band of precipitation appeared in the kappa lane in the mid-beta region. There was no corresponding band in the IgG, IgA, or IgM lanes, suggesting the presence of free kappa light chains. On the high resolution electrophoresis pattern, this band was masked by other proteins in the beta region. Secondly, the precipitated complexes in the IgG and kappa lanes appear somewhat hollowed. This is due to a phenomenon known as prozoning, a result of antigen excess and dissolution of the immunoprecipitate.

Supporting Methods - IEP

To obtain the IEP pattern, the sample was first applied in four alternating application points on an agarose plate. A control (reference sera) was applied to the other four application points. The proteins in each were separated by electrophoresis. In the second step, specific antisera was placed in the troughs between the samples and immunodiffusion occured. The antisera used were anti-IgG, anti-IgA, anti-IgM, anti-kappa, and anti-lambda, as well as a trivalent antisera to IgG, IgA, and IgM and an antisera to whole human serum. After the plate was washed and stained, the precipitin lines were analyzed for density and the presence of abnormal arcs when compared to the control samples.



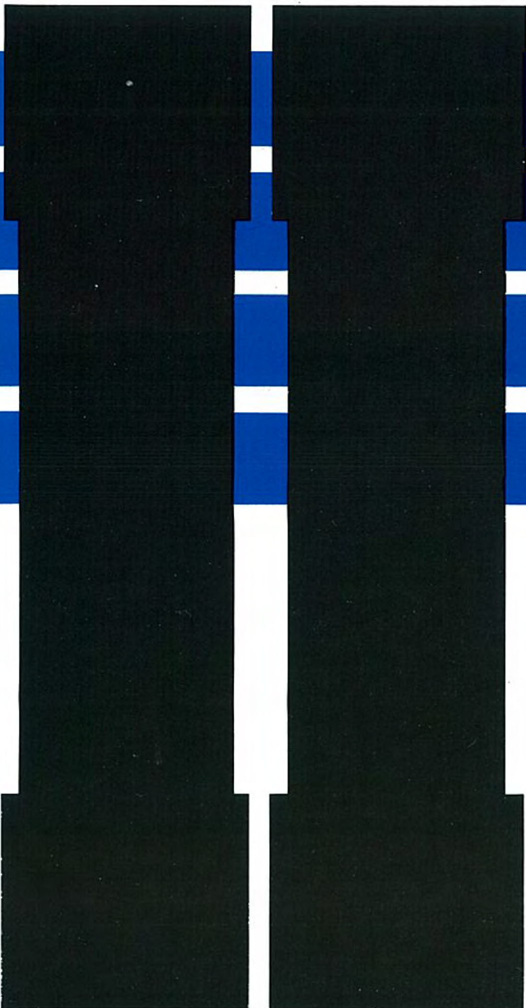
Immunoelectrophoresis

In the IEP pattern shown, the control (C) and patient (P) are seen in alternating lanes on the IEP pattern. The troughs contain anti-IgG (G), anti-IgA (A), anti-IgM (M), anti-whole human serum (H), trivalent antisera (T), anti-kappa (K), and anti-lambda (L). Analysis of the precipitin lines shows the presence of the IgG/K immunoglobulin and the monoclonal free kappa light chain arcs as well as reduced quantities of IgA, IgM and lambda immunoglobulins.

Notes

SECTION II

Interpretive Reporting



Introduction

Electrophoresis patterns can be reported in two ways, a) quantitation by densitometric scanning, and b) visual inspection of the pattern. Densitometric scanning of high resolution patterns is preferably used only to monitor patients with monoclonal gammopathies who are treated with chemical agents. In these patients, the semiquantitative M protein value determined by scanning is used by the physician to determine changes needed in treatment. The values obtained are semiquantitative particularly because of a) differences in binding of proteins to dye, and b) non-linearity of the densitometer (eg. at high protein concentrations).

Visual interpretation of patterns is done by a direct comparison of each zone and interzone to a pooled human reference serum. First, the appearance of the proteins found in each zone and interzone are noted. Second, a subjective interpretation of the amount of protein in each zone is made. Third, pattern recognition and comparison to clinical conditions is done. The first two steps are done by the medical technologist; the third, by a physician consultant (immunologist). To aid in interpretation, a file is kept on each patient with chronological interpretive reports saved for all HRE, IFE, and IEP patterns run.

Interpretive Reporting

Interpretation of protein electrophoresis patterns is a multi-stage process. Each step is described in detail below.

1. Reference sample:

   For HRE, a reference sample is prepared from a pool of normal human sera, aliquoted and stored at -70 C. An aliquot of this reference is assayed next to each sample. After the electrophoresis, staining, and drying steps are complete, the gel is cut apart. Each gel section contains both a reference and patient pattern. In this way, the patterns can easily be stored by patient rather than by date or gel.

2. Standardized report form:

   One report form is used with each sample assayed. Steps 1-6 and step 8 are done by the medical technologist performing the assay and the report is reviewed by the supervisor. Step 7 is done by a physician consultant. The form includes:

   (1) Patient demographics - patient name, sex, age or date of birth and hospital number are filled in on each form.

   (2) Specimen information - the lab accession number and the specimen type (serum, urine, or CSF) are added.

   (3) Gel section - a space is provided to attach the gel section containing the patient and reference patterns. (Note - an instant photograph is attached to the charted copy while the original gel remains in the lab.)

   (4) Quantitative results - the total protein and albumin results measured by chemical methods are recorded. For urine, the volume, collection period, and excretion per day are recorded.

   (5) Protein list - a list of the 12 zones and interzones which divide a protein pattern along with the proteins normally (and abnormally) found in each is printed on each form.

   (6) Pattern results - for each zone and interzone listed the pattern is compared to the same zone on the reference sample by the medical technologist.

   For each serum zone, a qualitative estimate of the amount of protein is made with one of the following comments:

   marked decrease
   moderate decrease
   slight decrease
   normal (or blank)
   slight increase
   moderate increase
   marked increase

   For each serum interzone, a qualitative estimate of the amount of protein is made with one of the following comments:

   decreased *normal (or blank)* *increased*

   For urine and CSF samples in all zones and interzones, the presence and absence of proteins are noted with one of the following comments:

   present *absent (or blank)*

Interpretive Reporting

For all sample types in all zones and interzones, any abnormal appearance of the proteins is noted as well as the presence of proteins not normally seen. Typical comments include:

fast albumin present
marked monoclonal band present
restricted band in the gamma-2 zone
faint banding
split alpha-1 band present

Additional comments which describe the pattern may be indicated as needed.

(7) Clinical interpretation of the pattern - a review of the pattern abnormalitites and clinical interpretation by the physician plus suggestions for any followup lab work is written here.

(8) Monoclonal protein estimate - when a monoclonal immunoglobulin is present in the pattern, a densitometric scan is done, and the semiquantitative value of the monoclonal protein is listed on the report. If more than one monoclonal protein exists, values are provided for each. The densitometric tracing is attached to the report form.

3. Patient data file:

For each patient, a file folder is created to store the original report forms. Copies of the reports are sent to the patient chart. Because HRE is frequently performed sequentially on patients with gammopathies or other disease processes, a patient file is of inestimable help in following changes on an individual patient. After steps 1-6 and 8 of the standardized report form are filled in, the patient folder is reviewed before final clinical interpretation is done.

Examples

Four examples of interpretive reports are shown on the next pages. The reference pattern is shown in each as sample "C." On the examples shown, enclosed numbers (#) are used to reference the sections of the standardized report form. Study each example. The patient demographics, specimen information, and quantitative results are filled in on each. Study the patient pattern compared to the reference (C) on each example. Review the pattern results that were written by the medical technologist. Then cover the pattern result section on each example and try to answer each for yourself.

UNIVERSITY HOSPITAL - CENTRAL LAB CHEMISTRY
PROTEIN FRACTIONATION BY HIGH RESOLUTION ELECTROPHORESIS

MICOM CHEM FORM.HRESPIKE

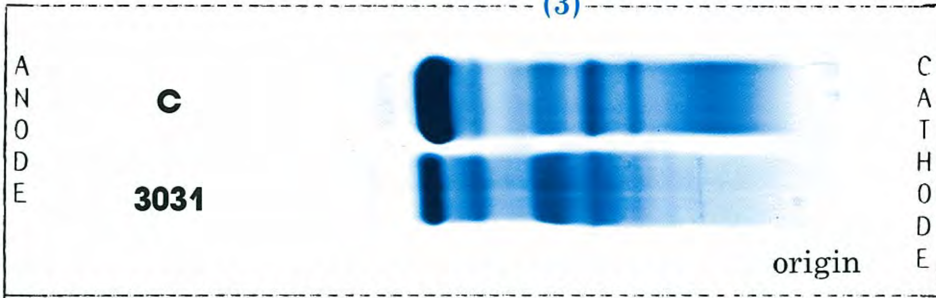
(1)

Date Sample Received 4-5-86 Unit 6E Hospital No. 9045394

(2)

Sample Type: SERUM Access No. 3031 Patient

(3)



Sex F

Age 50 years old

Protein = 3.6 (6.8-8.4 g/dL)

(4)

Albumin = 1.7 (3.7-4.9 g/dL)

ZONES, WITH PROTEINS NORMALLY MIGRATING IN EACH ZONE NOTED *	COMPARED TO A REFERENCE SERUM, THE FOLLOWING DIFFERENCES WERE SEEN
1. **PREALBUMIN ZONE** Prealbumin	
2. **ALBUMIN ZONE** Albumin	Marked decrease
3. **ALBUMIN-ALPHA-1 INTERZONE** Alpha-lipoprotein, (Alpha-fetoprotein)	Increased
4. **ALPHA-1 ZONE** Alpha-1-antitrypsin Alpha-1-acid glycoprotein	Moderate increase
5. **ALPHA-1-ALPHA-2 INTERZONE** Gc-globulin, Inter-alpha-trypsin inh., Alpha-1-antichymotrypsin	Increased
(5) 6. **ALPHA-2 ZONE** Alpha-2-macroglobulin, Haptoglobin	Marked increase
7. **ALPHA-2-BETA-1 INTERZONE** Cold insoluble globulin (Hemoglobin)	Increased
8. **BETA-1 ZONE** Transferrin	Moderate decrease (6)
9. **BETA-1-BETA-2 INTERZONE** Beta lipoprotein	Increased
10. **BETA-2 ZONE** C3	Marked decrease
11. **GAMMA-1 ZONE** IgA, IgM, (Fibrinogen), (Monoclonal Ig's, light chains)	Marked polyclonal decrease Faint band seen
12. **GAMMA-2 ZONE** IgG, (C-reactive protein), (Monoclonal Ig's, light chains)	Marked polyclonal decrease

Estimated total protein in monoclonal spike = ___ g/dl as determined by electrophoretic scan.

* PROTEINS IN ( ) ARE VISIBLE IN AN ABNORMAL PATTERN [Date Assayed 4-5-86 T.I.

(7)

OVERALL IMPRESSION:

1. Hypoalbuminemia
2. Non-specific changes in alpha-1, beta-1 and beta-2 proteins, consistent with nephrosis
3. Marked polyclonal hypogammaglobulinemia
4. Faint monoclonal band in the gamma-1 zone may represent intact immunoglobulins and/or free light chains

MR 2501.002 (Rev. KC 05/85) Reviewed by

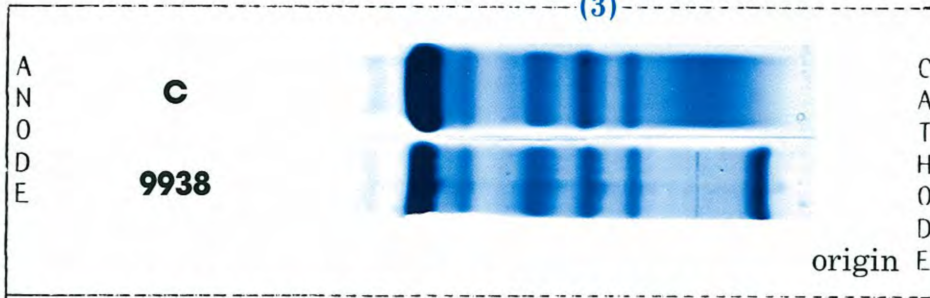
UNIVERSITY HOSPITAL - CENTRAL LAB CHEMISTRY
PROTEIN FRACTIONATION BY HIGH RESOLUTION ELECTROPHORESIS

MICOM CHEM FORM.HRESPIKE

Date Sample Received 3-14-86 Unit CCE (1) Hospital No. 8091449

(2) Sample Type: SERUM Access No. 9938 Patient

(3)



Sex M

Age 67 years old

Protein = 5.4 (6.8-8.4 g/dL)

(4) Albumin = 2.8 (3.7-4.9 g/dL)

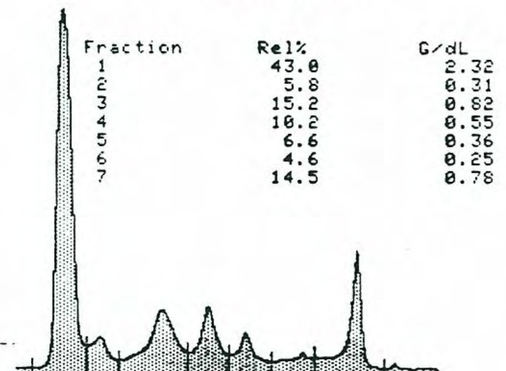
ZONES, WITH PROTEINS NORMALLY MIGRATING IN EACH ZONE NOTED *	COMPARED TO A REFERENCE SERUM, THE FOLLOWING DIFFERENCES WERE SEEN
1. **PREALBUMIN ZONE** Prealbumin	
2. **ALBUMIN ZONE** Albumin	Moderate decrease
3. **ALBUMIN-ALPHA-1 INTERZONE** Alpha-lipoprotein, (Alpha-fetoprotein)	
4. **ALPHA-1 ZONE** Alpha-1-antitrypsin Alpha-1-acid glycoprotein	
5. **ALPHA-1-ALPHA-2 INTERZONE** Gc-globulin, Inter-alpha-trypsin inh., Alpha-1-antichymotrypsin	
6. **ALPHA-2 ZONE** (5) Alpha-2-macroglobulin, Haptoglobin	Slight increase
7. **ALPHA-2-BETA-1 INTERZONE** Cold insoluble globulin (Hemoglobin)	
8. **BETA-1 ZONE** Transferrin	Moderate decrease (6)
9. **BETA-1-BETA-2 INTERZONE** Beta lipoprotein	
10. **BETA-2 ZONE** C3	Slight decrease
11. **GAMMA-1 ZONE** IgA, IgM, (Fibrinogen), (Monoclonal Ig's, light chains)	Marked polyclonal decrease
12. **GAMMA-2 ZONE** IgG, (C-reactive protein), (Monoclonal Ig's, light chains)	Marked polyclonal decrease Monoclonal band seen

Estimated total protein in monoclonal spike = 0.8 g/dl (8)
as determined by electrophoretic scan.

*** PROTEINS IN ( ) ARE VISIBLE IN AN ABNORMAL PATTERN** [Date Assayed 3-14-86 T.I.

(7)
OVERALL IMPRESSION:

Decrease in normal polyclonal immunoglobulins and large slow migrating monoclonal band strongly suggests myeloma. IFE will identify.



Fraction	Rel%	G/dL
1	43.0	2.32
2	5.8	0.31
3	15.2	0.82
4	10.2	0.55
5	6.6	0.36
6	4.6	0.25
7	14.5	0.78

MR 2501.002 (Rev. KC 05/86)

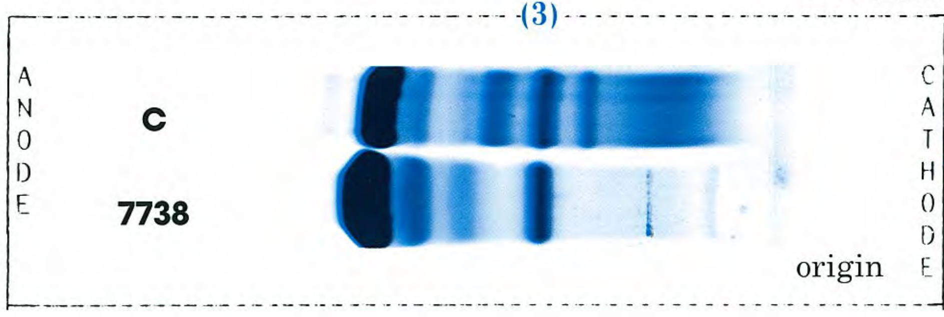
Reviewed by

UNIVERSITY HOSPITAL - CENTRAL LAB CHEMISTRY | MICOM CHEM FORM.HREU
PROTEIN FRACTIONATION BY HIGH RESOLUTION ELECTROPHORESIS

Date Sample Received 4-14-86 Unit CCE (1) Hospital No. 8091449

(2) Sample Type: URINE Access No. 7738 Patient ______

Age 67 years old Sex M



(4)
Total Protein = 339 mg/dL
Collection Period = 24 hrs
Total Volume = 1819 mL
Total Protein Excretion = 6166 mg/day
(0-150 mg/day)

ZONES *	PROTEINS OBSERVED IN URINE
1. PREALBUMIN ZONE	
2. ALBUMIN ZONE Albumin	Present
3. ALBUMIN-ALPHA-1 INTERZONE	
4. ALPHA-1 ZONE (Alpha-1-antitrypsin) (Alpha-1-acid glycoprotein)	Present
5. ALPHA-1-ALPHA-2-INTERZONE (Zn-alpha 2-glycoproteins)	
6. ALPHA-2 ZONE (5) (Alpha-2-microglobulin, seen as 2 bands)	Present
7. ALPHA-2-BETA-1 INTERZONE	(6)
8. BETA-1 ZONE (Transferrin)	Present
9. BETA-1-BETA-2 INTERZONE (Beta-2-microglobulin)	
10. BETA-2 ZONE	
11. GAMMA-ZONE (IgG, IgA, light chains, lysosyme, gamma trace)	Band seen at cathodal end

* PROTEINS IN ( ) ARE VISIBLE IN AN ABNORMAL PATTERN [Date Assayed 4-14-86 T.I.]
(Concentration Factor for Electrophoresis = 2x)

(7)
OVERALL IMPRESSION:

Non-specific proteinuria with a slow gamma band which corresponds to the serum IgG-lambda band.

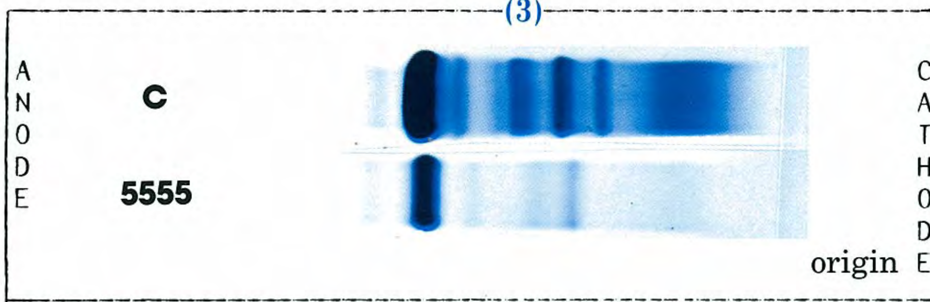
MR 2501.003 (Rev. KC 05/86) Reviewed by ______

UNIVERSITY HOSPITAL - CENTRAL LAB CHEMISTRY MICOM CHEM FORM.HRECSF
PROTEIN FRACTIONATION BY HIGH RESOLUTION ELECTROPHORESIS

Date Sample Received 3-30-86 Unit Lab (1) Hospital No. 76543210

(2) Sample Type: **CSF** Access No. 5555 Patient

(3)



Sex F

Age 46

(4) Protein = 62 (15-45 mg/dl)

ZONES *	PROTEINS OBSERVED IN CSF
1. **PREALBUMIN ZONE**	Present
2. **ALBUMIN ZONE** Albumin	Present
3. **ALBUMIN-ALPHA-1 INTERZONE**	
4. **ALPHA-1 ZONE** Alpha-1-antitrypsin	Present
5. **ALPHA-1-ALPHA-2-INTERZONE** (5)	
6. **ALPHA-2 ZONE**	
7. **ALPHA-2-BETA-1 INTERZONE**	(6)
8. **BETA-1 ZONE** Transferrin	Present
9. **BETA-1-BETA-2 INTERZONE**	
10. **BETA-2 ZONE** Beta-2-transferrin	
11. **GAMMA-ZONE** IgG. (Gamma trace), (oligoclonal bands)	

*** PROTEINS IN ( ) ARE VISIBLE IN AN ABNORMAL PATTERN** [Date Assayed 3-30-86 T.I.]

(7)

OVERALL IMPRESSION:

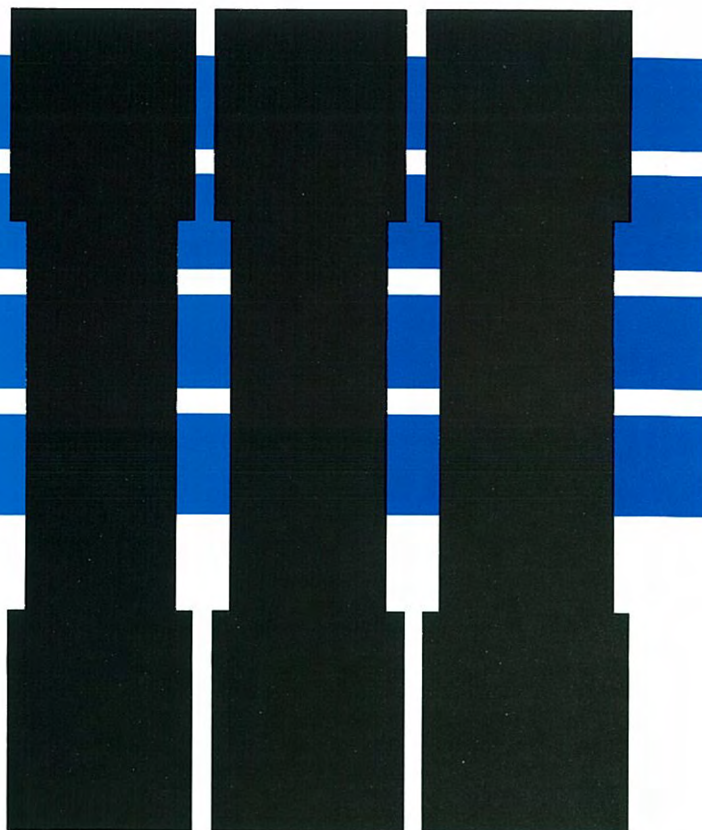
Concentration of sample for HRE - 80x
No oligoclonal bands seen

MR 2501.003 (Rev. KC 05/86) Reviewed by

Notes

SECTION III

Single Protein Changes

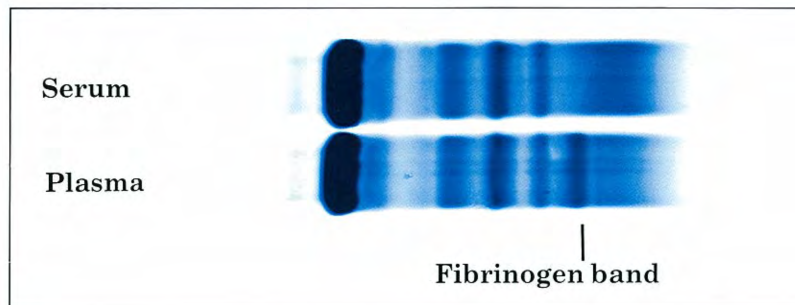


Introduction

The relationships among proteins and in disease states is complex, resulting in a combination of increases and decreases of protein throughout the entire pattern. Rarely is a single change noted in an electrophoretic pattern, since rates of synthesis and catabolism as well as distributional effects contribute to changes in concentration.

In this section, some examples of patterns with single protein changes are shown. The format used with each pattern is 1) a description of what the abnormality is, 2) a summary of how the pattern changes are identified and verified, 3) pertinent report form data, and 4) a summary of the findings in the pattern that is shown. Review each of the examples shown; study the findings and review the descriptions of the abnormalities found in each.

Plasma Specimen



Plasma Specimen (Heparin)

What is it? Fibrinogen is the precursor of the fibrin clot. During coagulation, thrombin splits fibrinogen into a peptide fragment and into insoluble fibrin. Fibrin, in the presence of factor XIII and calcium, forms the network of the clot and so is removed from the serum. In cases where a patient is receiving heparin therapy, the HRE specimen is drawn in a heparinized tube, or the serum is separated from the red cells before completely clotting, fibrinogen will be present and can be seen on the HRE pattern. This is not advantageous as the fibrinogen band can mimic or mask a monoclonal band, and possibly result in an unnecessary IFE run.

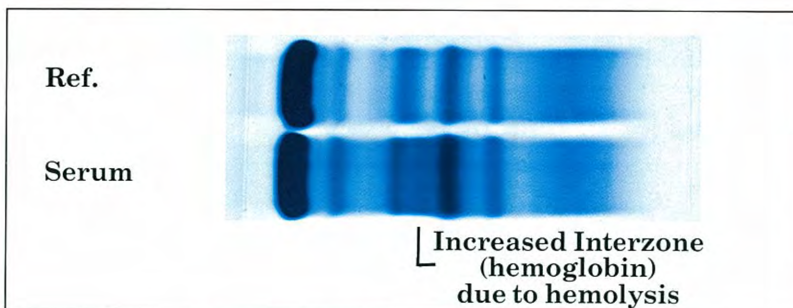
How is it identified and verified? Fibrinogen can be found on the HRE pattern in the mid gamma-1 region as a small band of restricted mobility. It can be identified by: a) locating the original clot tube and noting if it is a heparinized tube, b) precipitating the fibrinogen by adding one part thrombin to one part plasma and repeating the HRE on the supernatant, c) obtaining a fresh specimen in a red topped tube, and/or d) performing an IFE to rule out fibrinogen.

Report form data: The sample was obtained from a 28 year old female. The total protein = 7.1 g/dL (reference range = 6.8-8.4 g/dL); the albumin = 4.0 g/dL (reference range = 3.7-4.9 g/dL).

Pattern results: There was a band noted in the gamma-1 zone and the gamma-2 zone was slightly decreased. The band was thought to be fibrinogen and further investigation showed that the sample had been drawn in a heparinized tube.

Hemolyzed Specimen

What is it? Hemolysis is the presence of free hemoglobin, in vivo or in vitro, in a serum or plasma sample.



Hemolyzed Specimen

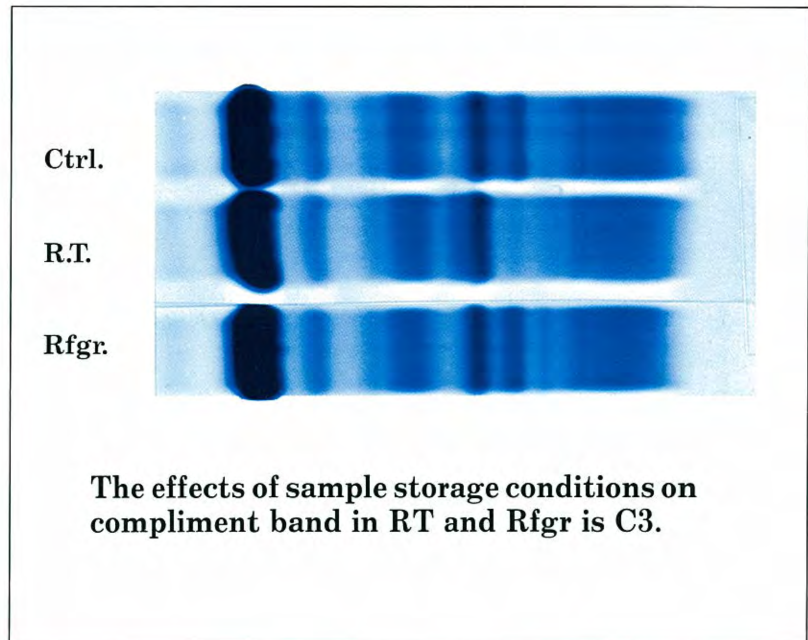
How is it identified and verified? In vitro hemolysis and the formation of haptoglobin-hemoglobin complexes result in a cathodal shift and an increase in the appearance of the haptoglobin fraction of HRE. Intravascular (in vivo) hemolysis results in a decrease in haptoglobin concentration. Occasionally, haptoglobin-hemoglobin complexes can be seen on HRE as a dense protein band in the alpha-2 zone or the alpha-2-beta-1 interzone. Hemolysis can be identified in the sample appearance, as the sample will be reddish in color and not straw colored. Hemolysis can be verified by simply looking at the specimen or by assaying for Plasma Hemoglobin.

Report form data: The sample was obtained from a 28 year old female. The total protein = 7.1 g/dL (reference range = 6.8-8.4 g/dL); the albumin = 4.0 g/dL (reference range = 3.7-4.9 g/dL).

Pattern results: Both alpha-1 and alpha-2 zones are moderately increased; the beta-1 zone is markedly increased; the beta-2 zone is moderately increased; the alpha-2-beta-1 interzone is increased throughout the entire interzone. Visual inspection of the sample showed extreme hemolysis.

Effects of Sample Storage on Complement C3

What is it? The classic path for complement activation involves at least 11 proteins that, when activated by an immune reaction between antigen and antibody, proceed in a cascade fashion to produce molecules that can elicit immune responses such as phagocytosis, chemotaxis, etc. C3 is an intricate part of the complement system. It has the the highest concentration in the complement cascade and is also an acute phase reactant. C3 is very labile and so is sensitive to sample storage conditions, and it will readily disintegrate if it is left in the refrigerator or at room temperature for an extended period of time.



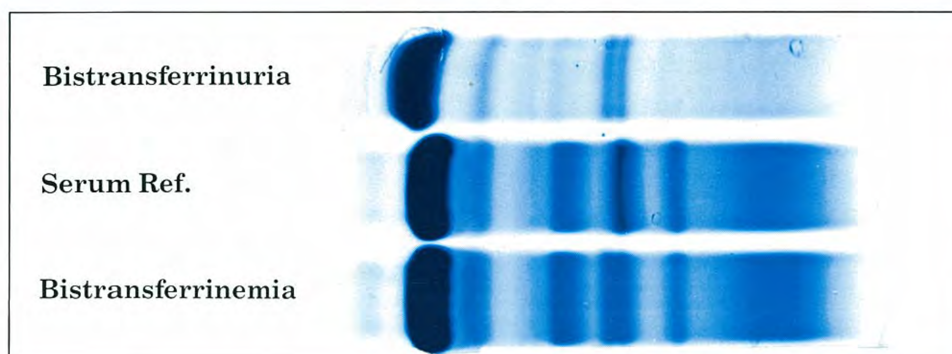
The effects of sample storage conditions on compliment band in RT and Rfgr is C3.

How is it identified and verified? C3 in fresh serum is found in the beta-2 region of the HRE pattern. With C3 that has been allowed to degrade by extended refrigeration (minimum 3 days) the beta-2 region will be slightly to moderately decreased with a faint band seen in the gamma-1 zone. The IFE will be "negative." If the C3 is allowed to degrade at room temperature (minimum 1 day), the beta-2 band will be moderately to markedly decreased and a band can be found at the anodic end of the beta-1 zone. Again an IFE run will show no immunoglobulins.

Report form data: The sample was obtained from a 28 year old female. The total protein = 7.1 g/dL (reference range = 6.8-8.4 g/dL); the albumin = 4.0 g/dL (reference range = 3.7-4.9 g/dL).

Pattern results: In the room temperature (R.T.) sample, the beta-2 band is markedly decreased and a band is present in the alpha-2-beta-1 interzone just anodic to transferrin. In the refrigerated (Rfgr) sample, the beta-2 band is slightly decreased, and there is a band present in the gamma-1 zone. An IFE done on each sample was negative, showing no immunoglobulin bands.

Genetic Variations



Genetic Abnormality

What is it? When evaluating HRE patterns, each fraction should be closely compared with the reference serum for concentration, electrophoretic mobility, and heterogeneity. Heterogeneity indicates hereditary (genetic) alterations, which on protein electrophoresis can be seen as double bands, deficiencies, or changes in protein migration in albumin, alpha-1-antitrypsin, haptoglobin, transferrin, and C3.

How is it identified and verified? Genetic variations can be seen on an HRE pattern as multiple bands, missing bands, or bands with abnormal electrophoretic mobilities. Multiple bands may be very close together and often appear as one "thick" band. When this happens, a repeat HRE with a x2 dilution of the sample often provides a better pattern for evaluation. Since monoclonal immunoglobulins may appear in beta or alpha zones, an IFE should be performed to rule out immunoglobulin involvement whenever abnormal bands are present. A serum HRE pattern that has a missing band where a band of protein is normally found could be due to a genetic deficiency. If hemolysis or age/storage is suspected as the cause of the missing fraction, a fresh, unhemolyzed, sample should be redrawn from the patient and reassayed for HRE. If the fraction is still absent, a specific protein assay should be performed to determine if the protein(s) in question are actually below normal range.

Bands with electrophoretic mobilities that vary from normal in serum HRE patterns are easy to see. Perhaps the most common genetically altered protein is haptoglobin. Its many different phenotypes often cause it to have an altered electrophoretic mobility compared to the reference. Bilirubin, drugs and other substances can bind to various proteins which also alters electrophoretic migration. As a whole, most genetically altered proteins do not cause pathologic conditions unless the protein normally found is not produced at all.

Report form data: The serum sample was obtained from a 74 year old male. The total protein = 6.5 g/dL (reference range = 6.8-8.4 g/dL); the albumin = 3.9 g/dL (reference range = 3.7-4.9 g/dL). The urine sample was obtained from a female, age 82.The urine was concentrated 50x prior to electrophoresis. The urine total protein = 81 mg/dL; the sample represented a random specimen.

Pattern results: The serum HRE (bottom pattern) is normal except for the appearance of 2 bands in the beta-1 zone. The urine HRE (top pattern) shows albumin, alpha-1, alpha-2, and beta-1 proteins present with 2 bands in the beta-1 region. IFE results on both samples were negative. The bands in both samples most likely represent bistransferrin.

Notes

SECTION IV
Multiple
Protein
Changes

SECTION IV: Multiple Protein Changes

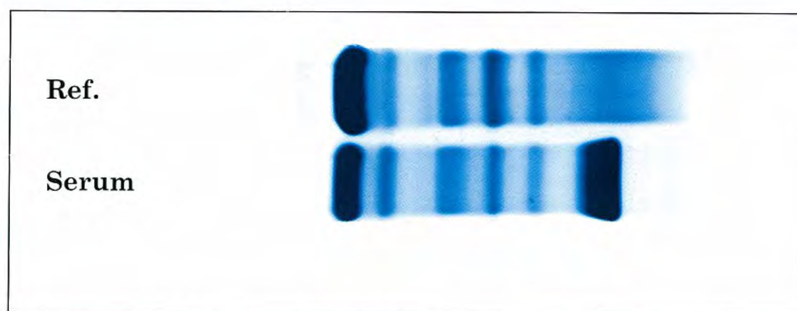
Introduction

The same approach that is used to evaluate patterns with single protein changes can be used to evaluate patterns with complex changes. Each zone is compared to the reference and all differences are noted. Verification of the abnormalities is done by careful analysis of the specimen, confirmatory techniques, such as IFE, and clinical correlation of these and other lab data.

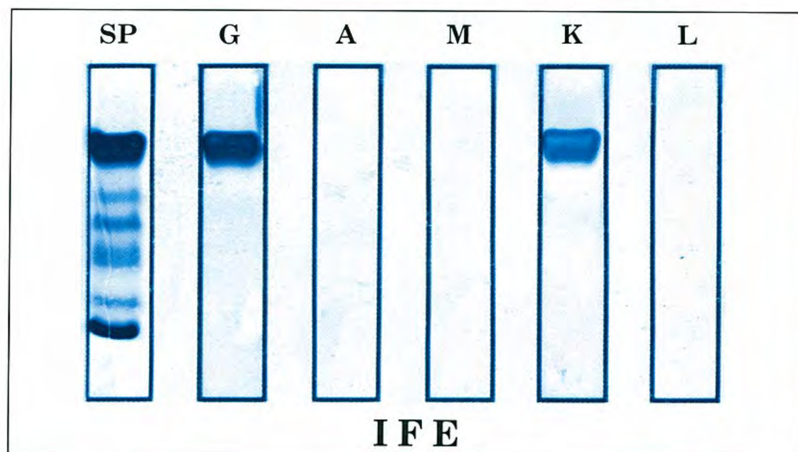
In this section, some patterns with multiple changes are described. The examples focus on both immunoglogulin abnormalities and other commonly seen patterns.

Monoclonal Gammopathy

What is it? Monoclonal Gammopathy is an abnormal proliferation of a single clone of plasma cells that can lead to the synthesis of large amounts of one homogeneous immunoglobulin or immunoglobulin subunit. There are 3 pathogenic signs that define monoclonal gammopathy; 1) excessive proliferation of B-cell clones with no apparent antigenic stimulus, 2) monoclonal band or bands of immunoglobulin origin detected electrophoretically, and 3) frequently there is a decrease in the patient's normal immunglobulin levels. This monoclonal protein is also called a paraprotein or M protein (myeloma protein). In most cases M proteins are indicative of malignancies, such as multiple myeloma or macroglobulinemia.



Monoclonal Gammopathy

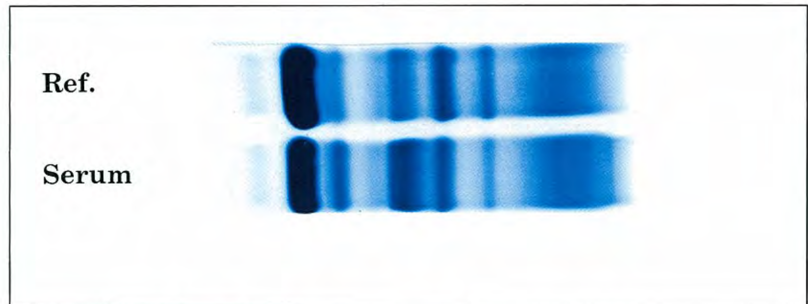


How is it identified and verified? The M protein is identified on the HRE pattern as a dense, highly restricted band that is most often found in the gamma zone but occasionally in the beta and alpha zones, where it may be masked under another fraction. An IgG monoclone tends to migrate in the gamma-2 zone and will often have smooth edges and bulging ends. An IgM monoclone often migrates in the gamma-1 zone and will tend to have sawtooth edges and flat ends. Monoclones of IgA origin, also tend to migrate in the gamma-1 zone but will often be a broad band with blurred edges and bulging ends. Immunologic identification of the M protein is accomplished by Immunofixation Electrophoresis (IFE) or Immunoelectrophoresis (IEP). The amount of M protein is estimated by densitometric scan. Rarely, IgD or IgE monoclonal proteins are present; their presence may be proven with the use of IgD or IgE antisera with IFE or IEP.

Report form data: The sample was obtained from a 44 year old male. The total protein = 12.3 g/dL (reference range = 6.8-8.4 g/dL); the albumin = 2.0 g/dL (reference range = 3.7-4.9 g/dL). The estimate of the grams of monoclonal protein from the densitometric scan = 8.8 g/dL.

Pattern results: Albumin shows a marked decrease; alpha-1 is slightly increased; beta-1 is moderately decreased and beta-2 is slightly decreased. The gamma region draws the most attention in that it is markedly decreased (polyclonal) with a very dense monoclonal band in the gamma-1 region. The band was identified by IFE as IgG/K.

Inflammatory Response



Inflammatory Response

What is it? The inflammatory response pattern (also known as acute phase changes) represents the most common abnormality that we see on high resolution electrophoresis, particularly on hospitalized patients. Response to inflammation is followed by characteristic protein alterations that are reflected on an HRE pattern. The intensity and duration of the protein changes varies with the stage of the inflammatory process.

How is it identified and verified? The pattern changes in an inflammatory (acute phase) pattern are:

Increases in - alpha-1-acid glycoprotein
alpha-1-antitrypsin
haptoglobin
fibrinogen
C-reactive protein

Decreases in - prealbumin
albumin
transferrin
alpha-lipoprotein

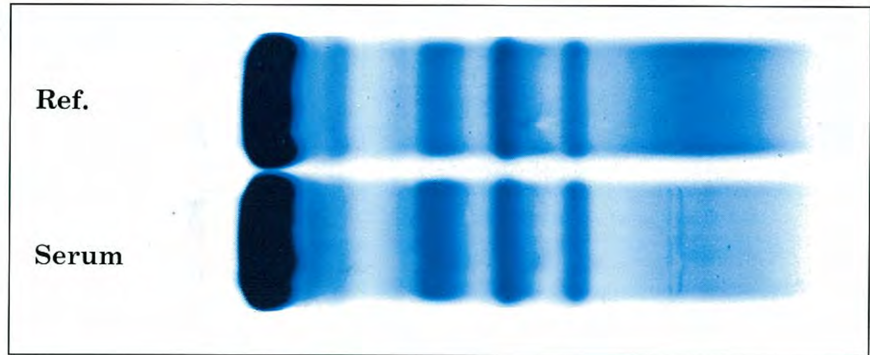
Immunoglobulins may be increased in chronic inflammatory states. Since serum is used for HRE, the fibrinogen level cannot be compared to evaluate its concentration. C-reactive protein will often appear as a band at the cathodal end of the gamma-2 region. Since monoclonal bands of immunoglobulin orgin can also appear in this region, an IFE should be performed to rule this out. An assay for CRP could be done to confirm its increased concentration. Often the alpha-1-acid glycoprotein will appear fuzzy, providing a blurred edge to the alpha-1 band. A decreased transferrin fraction may be masked on HRE by a coexisting iron deficiency in the patient.

Report form data: The sample was obtained from a 44 year old female. The total protein = 5.6 g/dL (reference range = 6.8-8.4 g/dL); the albumin = 2.8 g/dL (reference range = 3.7-4.9 g/dL).

Pattern results: The prealbumin is decreased (not visible on the picture) and the albumin is moderately decreased. The alpha-1 zone is moderately increased and the alpha-2 zone is markedly increased. The beta-1 and the beta-2 zones are both slightly decreased and there is a faint band at the cathodal end of gamma-2. An IFE was performed on this sample and did not identify the band noted in the gamma-2 zone. This pattern seems to indicate an inflammatory response and so the band is most likely C-reactive protein. An assay for CRP should be done to confirm its increased concentration.

Hypogammaglobulinemia

What is it? Hypogammaglobulinemia is a generalized or selective decrease in immunoglobulins. It is a very frequent finding in electrophoresis and may involve all or selective Ig classes. Deficiencies may be caused by 1) defective synthesis 2) pathological loss of immunoglobulins or 3) increased catabolism. Immunodeficiency diseases include x-linked hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, acquired hypogammaglobulinemia, immunodeficiency with hyper-IgM, selective class and subclass deficiencies, and deficiencies associated with drugs and protein-losing states.



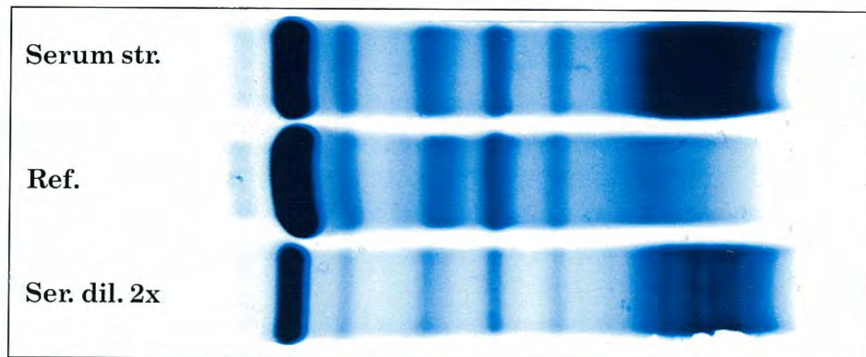
Hypogammaglobulinemia

How is it identified and verified? Hypogammaglobulinemia can be identified by a decrease in density of the generalized staining in the beta and/or gamma regions. Polyclonal IgA decreases appear as decreases in the beta-gamma-1 areas. Quantitative immunoglobulin measurements should be done to verify the decreases seen on the electrophoresis pattern. Hypogammaglobulinemia of infancy must be assessed carefully against a normal reference from the same age group. Urine electrophoresis may also need to be done to rule out light chain disease or to clarify protein-losing conditions.

Report form data: The sample was obtained from a 67 year old male. The total protein = 6.7 g/dL (reference range = 6.8-8.4 g/dL); the albumin = 4.8 g/dL (reference range = 3.7-4.9 g/dL).

Pattern results: The albumin-alpha-1 interzone is increased; the alpha-1 zone is slightly decreased, with a blurred anodal edge. The alpha-2 zone is markedly increased. The most significant finding is a polyclonal decrease in both the gamma-1 and gamma-2 zones. This was verified by quantitative Igs. In this sample, IgA= 61 mg/dL (reference range = 70-312 mg/dL), IgM= 54 mg/dL (reference range = 56-352 mg/dL), and IgG= 481 mg/dL (reference range = 639-1349 mg/dL), all below normal.

Hypergammaglobulinemia



Hypergammaglobulinemia

What is it? Hypergammaglobulinemia or polyclonal gammopathy is a generalized, diffuse elevation of immunoglobulins. It usually involves all classes of immunoglobulins, but may represent the elevation of a single class, e.g. IgG. Marked polyclonal gammopathy is seen in autoimmune or collagen diseases such as lupus erythematosus, rheumatoid arthritis, in liver disease such as hepatitis, and in infections. Frequently, the increased gamma globulin fraction contains one or more narrow or restricted bands. A single restricted band may represent an increase in a single subclass, while multiple bands may indicate the early immune response from several small clones of plasma cells. Oligoclonal bands are frequently seen in viral infections or in diseases with high concentrations of immune complexes.

How is it identified and verified? Hypergammaglobulinemia can be identified by an increase in density of the generalized staining in the beta and/or gamma regions. Polyclonal IgA increases appear as increases in the beta-gamma-1 areas. Increases of single IgG subclasses may appear as an assymetrical polyclonal increase of the gamma zone. Since oligoclonal bands are frequently masked by high increases in polyclonal immunoglobulins, samples with proteins greater than 8 g/dL should be diluted x2 and repeated to further characterize the appearance of the gamma zone. An IFE can be done to identify any bands or restricted zones that might be present. Quantitative immunoglobulin measurements should be done to verify the increases seen on the electrophoresis pattern.

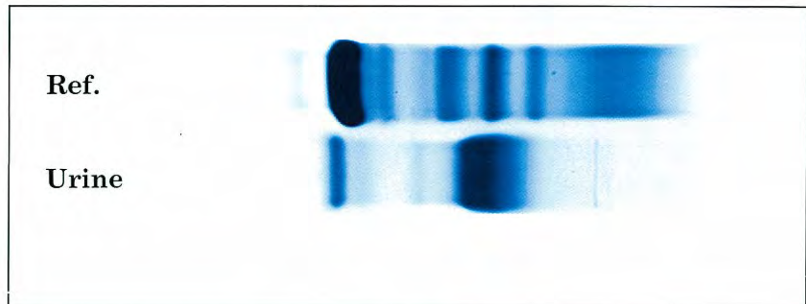
Report form data: The sample was obtained from a 74 year old male. The total protein = 11.0 g/dL (reference range = 6.8-8.4 g/dL); the albumin = 2.7 g/dL (reference range = 3.7-4.9 g/dL).

Pattern results: On the undiluted sample, the albumin zone is moderately decreased and the beta-1 zone is slightly decreased. The most significant finding of the pattern was a marked polyclonal increase in the gamma-2 zone. On the diluted sample (x2 dilution), oligoclonal banding was also seen in the gamma-2 zone. An IFE performed on the specimen showed that the oligoclonal bands consisted of both IgG/K and IgG/L immunoglobulins.

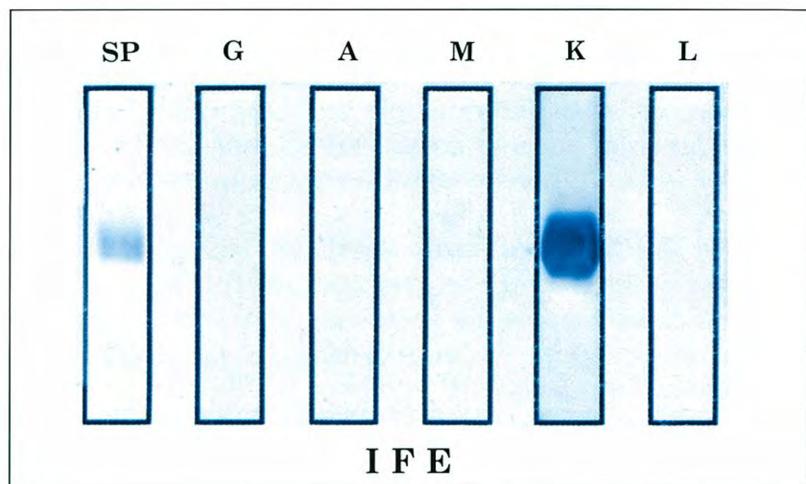
Light Chain Disease

What is it? Light Chain Disease is a monoclonal gammopathy where only kappa or lambda light chains (Bence Jones proteins) are manufactured by an abnormal clone.

How is it identified and verified? Light chains are detected by HRE in the serum and in the urine as dense, highly restricted bands found most often in the gamma zone but occasionally in the beta and alpha zones. The serum HRE will often demonstrate a hypogammaglobulinemia, but the urine concentrated 50X for HRE will show an M spike of light chain origin. Identification of the paraprotein is accomplished by Immunofixation (IFE) or Immunoelectrophoresis (IEP) of the concentrated urine. The amount of paraprotein is estimated by densitometric scan. The amount of light chain that is excreted is calculated by



Light Chain Disease

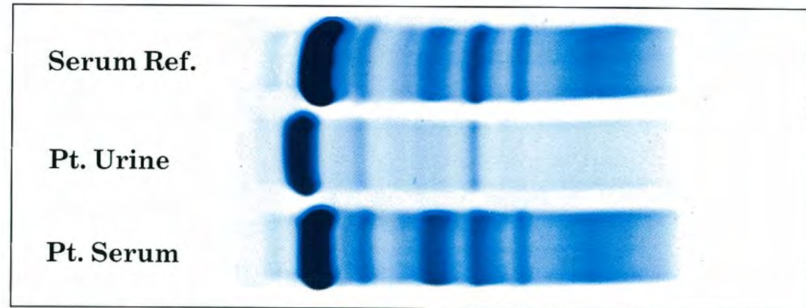


1) calculation of total urine protein excretion per day:
total protein mg/dL x 10 dL/L x n liters excreted/day
2) calculation of light chain excretion:
total protein excreted per day x relative % light chain from densitometric scan.

Report form data: The sample was obtained from a 57 year old male. The urine protein = 138 mg/dL. The sample represented a 24 hr collection with a total volume of 889 mL. On the HRE, the sample was concentrated x50. The urine protein excretion - 1226 mg/day (reference range = 0 - 150 mg/24 hr). The light chain excretion = 972 mg/day.

Pattern results: Evaluation of the urine pattern shows that proteins are present in albumin, and the alpha-1-alpha-2 interzone. The broad band extending from the alpha-2-beta-1 interzone to the beta-1-beta-2 interzone, was identified as kappa light chains by IFE.

Glomerular Proteinuria



Glomerular Proteinuria

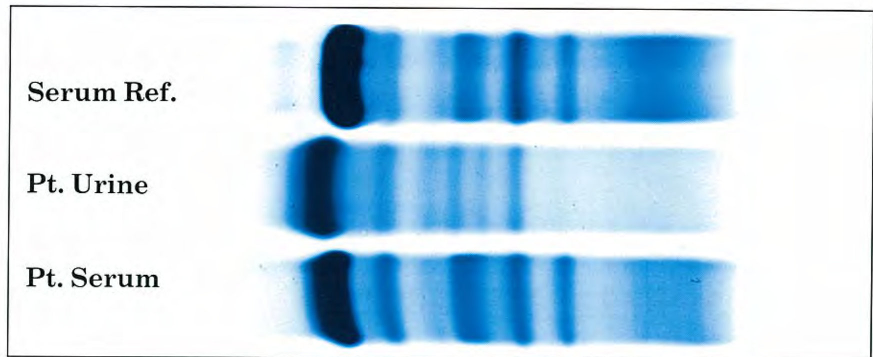
What is it? Glomerular proteinuria results from increased glomerular permeability of proteins such as albumin, transferrin, alpha-1 antitrypsin and alpha-1-acid glycoprotein. The corresponding serum pattern shows decreases in these proteins. Large molecular weight proteins such as alpha-2 macroglobulin and beta-lipoprotein are selectively retained, and may show large increases in the corresponding serum pattern.

How is it identified and verified? Both serum and urine should be done on the patient suspected of glomerular damage. The urine sample should be concentrated x50 before electrophoresis. The total urine protein value and collection parameters is required to calculate the total protein excretion per day. The presence of albumin, alpha-1 proteins, and transferrin in the urine, with the reduction of these proteins in the serum, indicates glomerular damage. If light chain disease is suspected, an IFE of the urine should be performed to rule out light chain presence in the beta or gamma regions.

Report form data: The sample was obtained from a 19 year old male. The serum total protein = 6.1 g/dL(reference range = 6.8-8.4 g/dL); the albumin = 3.2 g/dL (reference range = 3.7-4.9 g/dL). The urine total protein = 52 mg/dL. The sample represented a 24 hour collection of 4,260 mL that was concentrated x50 before the HRE was done. The urine excretion of protein = 2,215 mg/day (reference range = 0 - 150 mg/24 hr).

Pattern results: The urine pattern shows albumin, alpha-1, alpha-2 (very faint), and beta-1 proteins as present. The serum pattern shows slightly decreased albumin and a decreased beta-1 zone. Alpha-1 zone is slightly increased. The alpha-2 zone is moderately increased. Glomerular damage is indicated. The presence of small amounts of alpha-2 microglobulin and Zn-alpha-2 microglobulin indicates that inflammation and some tubular damage has also occurred.

Mixed Proteinuria



Mixed Proteinuria

What is it? Urine patterns frequently show a mixture of proteins, representing some glomerular and some tubular damage, or some glomerular damage plus overflow proteinuria. When significant amounts of albumin, alpha-1 proteins, and transferrin are present, glomerular proteinuria is indicated. When alpha-2 microglobulin (double band) and beta-2 microglobulin are present, tubular damage is indicated. When alpha-1 proteins plus several faint bands in the alpha-2 region (antichymotrypsin and Zn-alpha-2 glycoprotein) are present, inflammation is also suggested. In all cases, the simultaneous electrophoresis of serum and urine plus the correlation with other lab data will help to pinpoint the disease state.

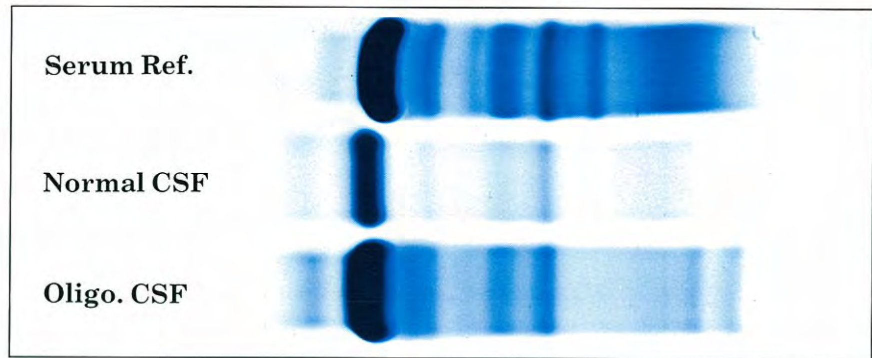
How is it identified and verified? Both serum and urine should be done on the patient suspected of renal damage. The urine sample should be concentrated x50 before electrophoresis. The total urine protein value and collection parameters is required to calculate the total protein excretion per day. The combination of proteins present can be used to determine if glomerular or tubular damage has occurred or if overflow proteinuria exists. If light chain disease is suspected, an IFE of the urine should be performed to rule out light chain presence in the beta or gamma regions.

Report form data: The sample was obtained from a 26 year old female. The serum total protein = 4.9 g/dL (reference range = 6.8-8.4 g/dL); the albumin = 2.5 g/dL (reference range = 3.7-4.9 g/dL). The urine total protein = 186 mg/dL on a 24 hour collection with a total volume of 2262 mL. The urine protein excretion = 4,207 mg/day. (Reference = 0 - 150 mg/24hr). The sample was concentrated x50 before electrophoresis.

Pattern results: Albumin, alpha-1, alpha-2 (many bands), and beta-1 proteins are present in the urine. A faint beta-2 band is also present. The serum shows a decreased prealbumin and slighty decreased albumin and beta-1 zones. The alpha-1 and alpha-2 zones are both moderately increased and the gamma-2 zone is slightly decreased (polyclonal). The albumin in both the serum and urine shows a fast anodal edge. The serum pattern looks like an inflammatory or an acute phase reactant pattern. The urine pattern indicates both glomerular and tubular damage as well as the presence of acute phase reactants.

C S F Oligoclonal Banding

What is it? Oligoclonal bands are multiple, dense, highly restricted bands found in the gamma region on HRE. These bands represent immunoglobulins (mostly IgG) that are produced locally within the central nervous system. Oligoclonal bands are characteristic of multiple myeloma but can be found in viral and bacterial infections of the CNS and in some lymphoproliferative disorders.



C S F

How is it identified and verified? CSF (concentrated 80-100 X) should always be run in conjunction with the patient's serum for electrophoresis. If both the serum and CSF for HRE demonstrate monoclonal or oligoclonal bands they are probably of serum origin. Oligoclonal bands that appear on the CSF HRE but not on the serum HRE are usually indicative of a demyelinating disease (multiple sclerosis) or sometimes a CNS infection. CSF oligoclonal banding appears on HRE as more than one dense band of restricted mobility in the gamma region. Occasionally a faint but discrete band will be noted at the extreme cathode end of the gamma zone. This band is gamma trace protein and is normal and not considered an immunoglobulin. IFE will identify true oligoclonal bands as immunoglobulins and will be "negative" for gamma trace protein. Oligoclonal bands of viral/bacterial nature are transient and will disappear from the CSF HRE pattern with therapy, whereas, oligoclonal bands from a demyelinating disease are more persistent throughout the course of the disease.

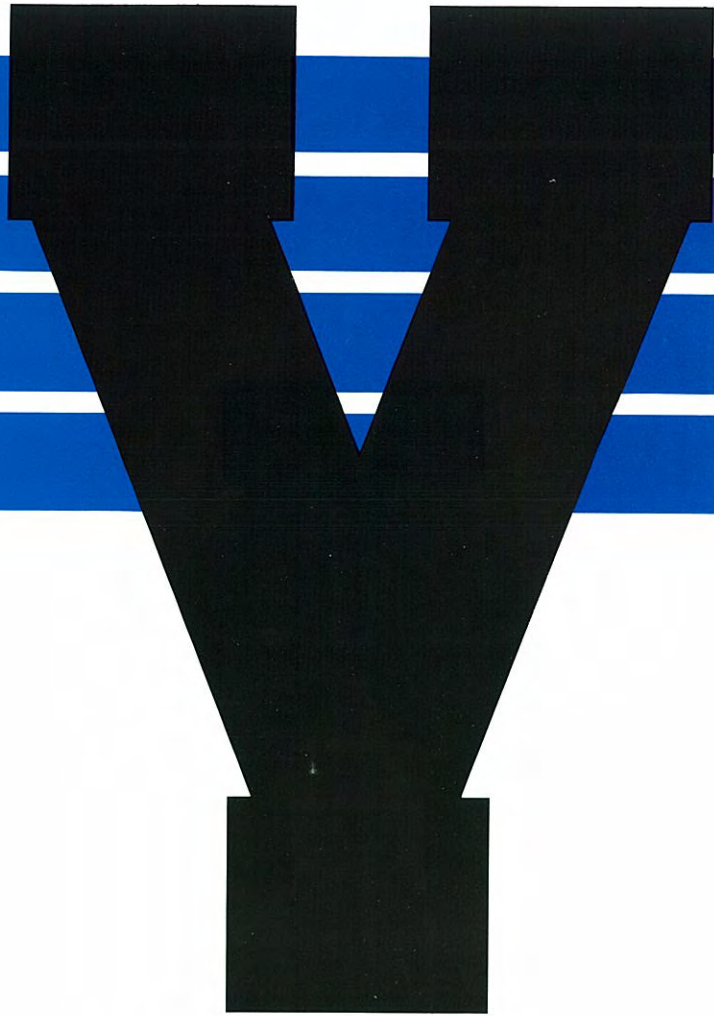
Report form data: The CSF sample is a simulated CSF that has been concentrated x80 before electrophoresis.

Pattern results: The patterns for CSF used here are simulated samples. Please note the prominent pre-albumin and the oligoclonal banding in the gamma region of the oligoclonal sample. In many CSF samples a beta-2 band is visible, not shown on the above patterns.

Notes

SECTION V

Self-Directed Learning Activities



SECTION V: Self-Directed Learning Activities

Instructions

1. Instructions can be found on each lesson. Follow them carefully and answer all of the questions.

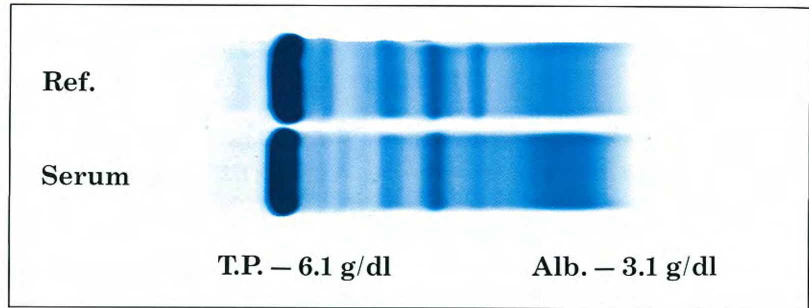
2. Complete each section before proceeding to the next section.
   a. Read the instructions with each pattern.
   b. Answer all of the questions.
   c. Review the answers provided.
   d. If there are discrepancies between your answers and the given answers, go back to the appropriate sections and review the material.
   e. When you are satisfied with your answers, proceed to the next pattern.

3. Providing you have read the LAP, the lessons should be fairly easy. Should you have any questions, comments, or wish to discuss your answers, see your supervisor and/or consult the pertinent references listed in the back.

Pattern A

Instructions:

Please evaluate Pattern A and fill in your evaluations in the appropriate spaces and answer the questions below. The sample is from a female, age 35. The total protein = 6.1 g/dL (reference = 6.8-8.4 g/dL); albumin = 3.1 g/dL (reference = 3.7-4.9 g/dL).



Pattern A

ZONES, WITH PROTEINS NORMALLY MIGRATING IN EACH ZONE NOTED *	COMPARED TO A REFERENCE SERUM, THE FOLLOWING DIFFERENCES WERE SEEN.
1. PREALBUMIN ZONE Prealbumin	
2. ALBUMIN ZONE Albumin	
3. ALBUMIN-ALPHA-1 INTERZONE Alpha-lipoprotein (Alpha-fetoprotein)	
4. ALPHA-1 ZONE Alpha-1-antitrypsin (Alpha-1-acid glycoprotein)	
5. ALPHA-1-ALPHA-2 INTERZONE GC-globulin, Inter-alpha-trypsin inhibitor, Alpha-1-antichymotrypsin	
6. ALPHA-2 ZONE Alpha-2-macroglobulin, Haptoglobin	
7. ALPHA-2-BETA-1 INTERZONE Cold insoluble globulin, (Hemoglobin)	
8. BETA-1 ZONE; Transferrin	
9. BETA-1-BETA-2 INTERZONE Beta-lipoprotein	
10. BETA-2 ZONE; C3	
11. GAMMA-1 ZONE IgA, Fibrinogen, IgM (monoclonal Igs, light chains)	
12. GAMMA-2 ZONE IgG, (C-reactive protein) (monoclonal Igs, light chains)	

1. What are the significant abnormalities in this pattern?

2. What proteins could be found in this (these) zones?

3. What tests would you do to confirm the identity of these proteins?

4. What disease state could you associate this pattern with (primary abnormality)?

Pattern A

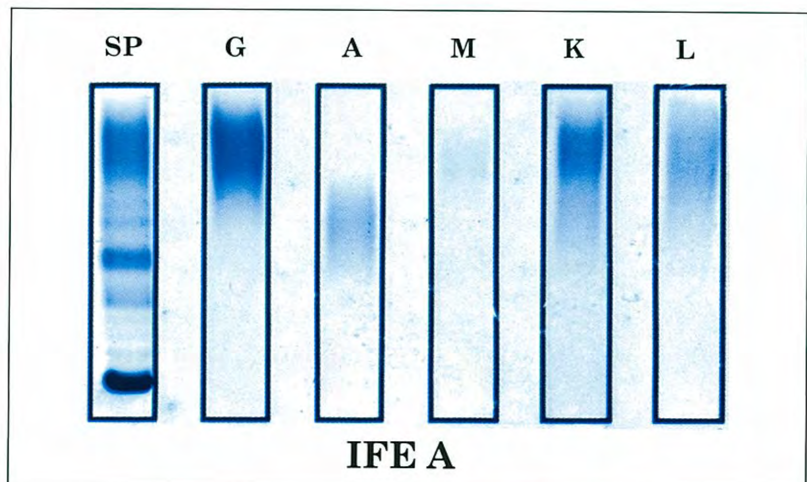
(continued)

The pattern differences seen were:

1. Prealbumin	- note-prealbumin may not be visible on the photo, so do not evaluate zone.
2. Albumin	- slight decrease
3. Albumin-alpha-1 interzone	- decreased
4. Alpha-1 zone	- marked decrease
5. Alpha-1-alpha-2 interzone	- band noted
6. Alpha-2 zone	- slight decrease
7. Alpha-2-beta-1 interzone	- normal
8. Beta-1 zone	- normal
9. Beta-1-beta-2 interzone	- normal
10. Beta-2 zone	- marked decrease
11. Gamma-1 zone	- faint band noted
12. Gamma-2 zone	- marked polyclonal increase

The significant pattern differences are the two bands noted; one in the alpha-1-alpha-2 interzone and one in the gamma-1 zone. The band in the alpha-1-alpha-2 interzone could be of immunoglobulin origin or could be a genetic abnormality of alpha-1. The faint band in the gamma-1 zone could be of immunoglobulin origin, a genetic abnormality of beta-2, a C3 degradation by-product, or fibrinogen. Proteins of immunoglobulin origin can be confirmed or ruled out by performing an IFE. Fibrinogen can be ruled out as the sample was known to be serum and the patient was not receiving heparin therapy. The sample was drawn several days prior to the assay and was not properly stored, so the band in the gamma-1 zone most likely was a C3 degradation by-product. The disease states associated with this pattern could be a monoclonal gammopathy and/or genetically altered proteins.

An IFE was performed on the sample for Pattern A and the results are shown. What are the results of the IFE? What conclusions can or cannot be drawn from the IFE results?



IFE A

IFE Pattern A

(continued)

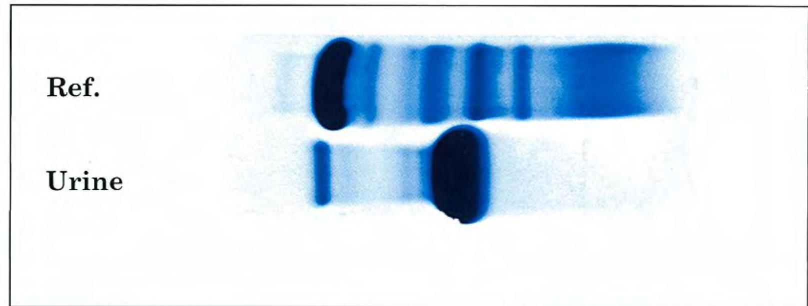
The IFE does not identify either band. Only polyclonal Igs are seen with the IFE. A decomposing C3 is the probable cause of the band in the gamma-1 zone. The band in the alpha-1-alpha-2 interzone is most likely a genetically altered alpha-1 antitrypsin. Specific alpha-1 antitrypsin quantitation or IFE done with anti-alpha-1- antitrypsin would help define the split band further. The split alpha-1 band on this sample was identified through another technique, crossed immunoelectrophoresis, as alpha-1 antitrypsin.

If you are satisfied with your answers, continue with the next pattern. If not, review before continuing further.

Pattern B

Instructions:

Please evaluate Pattern B and fill in your evaluations in the appropriate spaces and answer the questions below. This specimen is a urine obtained from a 51 year old male. The sample was concentrated x25 before the HRE was done. The total urine protein = 649 mg/dL (Reference = 0 - 150 mg/24 hr); the specimen was a 24 hour collection with a total volume of 1155 mL.



Pattern B
T.P. — 649 mg/dl
Concentration factor — 25x

ZONES, WITH PROTEINS NORMALLY MIGRATING IN EACH ZONE NOTED *	COMPARED TO A REFERENCE SERUM, THE FOLLOWING DIFFERENCES WERE SEEN.
1. PREALBUMIN ZONE	
2. ALBUMIN ZONE Albumin	
3. ALBUMIN-ALPHA-1 INTERZONE	
4. ALPHA-1 ZONE (Alpha-1-antitrypsin) (Alpha-1-acid glycoprotein)	
5. ALPHA-1-ALPHA-2 INTERZONE (Zn-alpha-2-glycoprotein)	
6. ALPHA-2 ZONE (Alpha-2-microglobulin, seen as 2 bands)	
7. ALPHA-2-BETA-1 INTERZONE	
8. BETA-1 ZONE; (Transferrin)	
9. BETA-1-BETA-2 INTERZONE (Beta-2-microglobulin)	
10. BETA-2 ZONE	
11. GAMMA ZONE (IgG, IgA, light chains, lysozyme, gamma trace)	

1. What are the significant abnormalities in this pattern?

2. What proteins could be found in this (these) zones?

3. What tests would you do to confirm the identity of these proteins?

4. What is the excretion of protein in mg/day for this sample?

Pattern B

(continued)

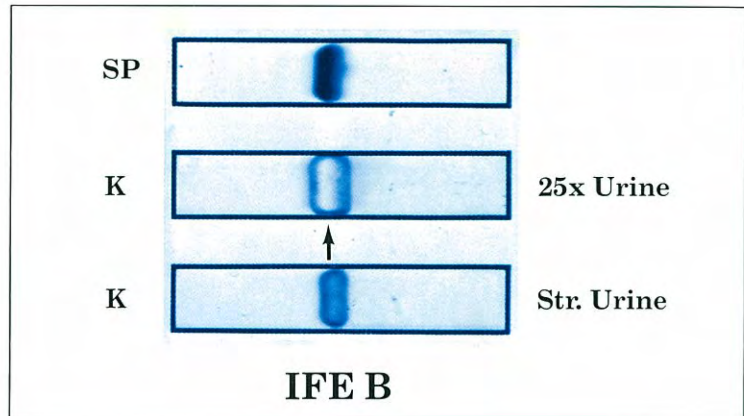
Proteins were present in the following zones:

Albumin
Alpha-1
Alpha-2
Alpha-2-beta-1 interzone; dense monoclonal band noted

The significant abnormality of this urine HRE pattern is the dense monoclonal band in the alpha-2-beta-1 interzone. This protein could be of immunoglobulin origin since alpha-2 microglobulins and transferrin found in this region are not seen as a massive band with bulging sides. Light chains are the more likely cause, so an IFE should be done.

The urine protein excretion per day = 7,496 mg.

An IFE run was performed on the concentrated urine sample for Pattern B. The partial results of that IFE are shown. What are the results of the IFE run? Assume that the Ig's not shown were negative by IFE. What disease state is associated with this finding? What is the phenomenon at the arrow (on the IFE) called?



IFE B

IFE Pattern B

(continued)

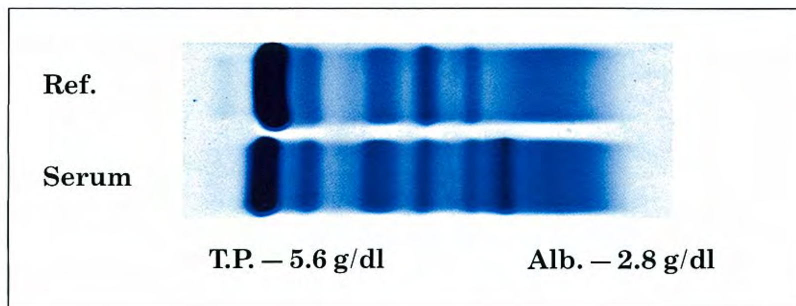
The IFE shows a reaction of the patient's urine with anti-kappa, so the patient is excreting kappa light chains. This finding is consistent with Light Chain Disease (LCD) or Bence Jones Proteinuria. The phenomenon at the arrow is called "prozoning." This occurs when there is an excess of antigen in the antibody/antigen reaction (in this case, the patient's concentrated urine is the antigen in excess). Repeating the IFE using a less concentrated specimen will eliminate the prozoning and provide a better IFE.

If you are satisfied with your answers, continue with the next pattern. If not, review before continuing.

Pattern C

Instructions:

Please evaluate Pattern C and fill in your evaluations in the appropriate spaces and answer the questions below. The serum sample was obtained from a 42 year old female. The total protein on the sample = 5.6 g/dL (reference = 6.8-8.4 g/dL); the albumin = 2.8 g/dL (reference = 3.7-4.9 g/dL).



Pattern C

ZONES, WITH PROTEINS NORMALLY MIGRATING IN EACH ZONE NOTED *	COMPARED TO A REFERENCE SERUM, THE FOLLOWING DIFFERENCES WERE SEEN.
1. PREALBUMIN ZONE Prealbumin	
2. ALBUMIN ZONE Albumin	
3. ALBUMIN-ALPHA-1 INTERZONE Alpha-lipoprotein (Alpha-fetoprotein)	
4. ALPHA-1 ZONE Alpha-1-antitrypsin (Alpha-1-acid glycoprotein)	
5. ALPHA-1-ALPHA-2 INTERZONE GC-globulin, Inter-alpha-trypsin inhibitor, Alpha-1-antichymotrypsin	
6. ALPHA-2 ZONE Alpha-2-macroglobulin, Haptoglobin	
7. ALPHA-2-BETA-1 INTERZONE Cold insoluble globulin, (Hemoglobin)	
8. BETA-1 ZONE; Transferrin	
9. BETA-1-BETA-2 INTERZONE Beta-lipoprotein	
10. BETA-2 ZONE; C3	
11. GAMMA-1 ZONE IgA, Fibrinogen, IgM (monoclonal Igs, light chains)	
12. GAMMA-2 ZONE IgG, (C-reactive protein) (monoclonal Igs, light chains)	

1. What are the significant abnormalities in this pattern?

2. What proteins could be found in this (these) zones?

3. What tests would you do to confirm the identity of these proteins?

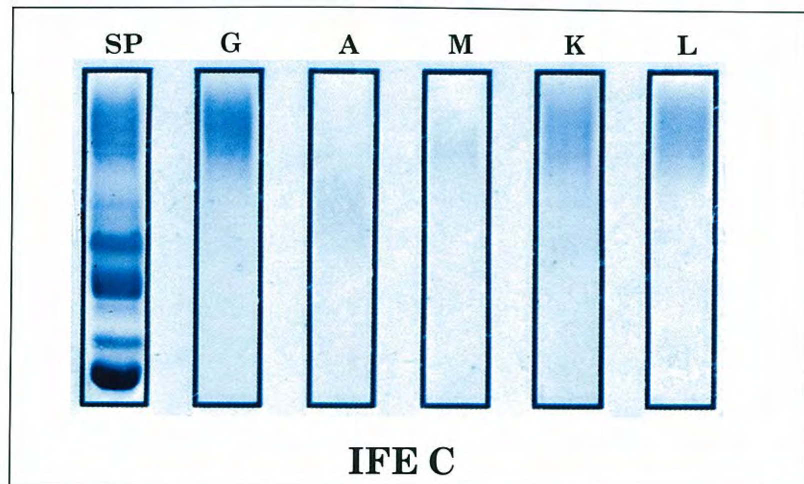
Pattern C

The pattern differences seen were:

1. Prealbumin - note-prealbumin may not be visible on the photo, so do not evaluate zone.
2. Albumin - moderate decrease
3. Albumin-alpha-1 interzone - normal
4. Alpha-1 zone - marked increase
5. Alpha-1-alpha-2 interzone - normal
6. Alpha-2 zone - moderate increase
7. Alpha-2-beta-1 interzone - normal
8. Beta-1 zone - slight decrease
9. Beta-1-beta-2 interzone - normal
10. Beta-2 zone - normal
11. Gamma-1 zone - moderate increase with a marked band noted
12. Gamma-2 zone - slight polyclonal increase

The significant pattern abnormality for this pattern is the marked band noted in the gamma-1 zone. This band could be fibrinogen or it could be of immunoglobulin nature. Fibrinogen can be eliminated as a possibility by verifying that the sample is serum and not plasma, checking if the patient is on heparin therapy, or adding thrombin to the sample and rerunning the HRE. If monoclonal Igs are suspected, an IFE can be performed.

For this sample, the original tube was no longer available so an IFE was performed. The results of the Pattern C IFE are shown. What conclusion(s) can or cannot be made from this IFE run? Are there any tests or procedures that can be run to assist in the HRE/IFE evaluation of this pattern?



IFE C

IFE Pattern C

(continued)

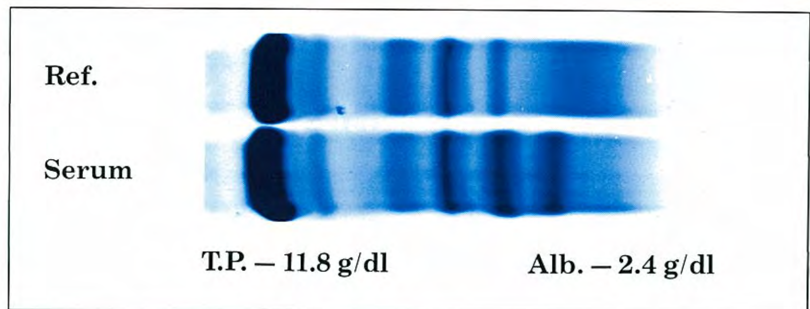
The IFE run did not identify the band in the gamma-1 zone. The band could be IgD or IgE, but this is very rare and not likely. It is more likely that the band is indeed fibrinogen. Thrombin was added to the sample and the HRE was repeated on the resulting supernatant. The band was not present on the reassayed sample; thus, the assumption was made that a plasma sample had been received. If any doubt remains as to the identity of the sample, a fresh specimen should be obtained.

If you are satisfied with your answers, continue with the next pattern. If not, review before continuing.

Pattern D

Instructions:

Please evaluate Pattern D and fill in your evaluations in the appropriate spaces and answer the questions below. The serum sample was obtained from a male, age 71. The total protein = 11.8 g/dL (reference = 6.8-8.4 g/dL); albumin = 2.4 g/dL (reference = 3.7-4.9 g/dL).



Pattern D

ZONES, WITH PROTEINS NORMALLY MIGRATING IN EACH ZONE NOTED *	COMPARED TO A REFERENCE SERUM, THE FOLLOWING DIFFERENCES WERE SEEN.
1. PREALBUMIN ZONE Prealbumin	
2. ALBUMIN ZONE Albumin	
3. ALBUMIN-ALPHA-1 INTERZONE Alpha-lipoprotein (Alpha-fetoprotein)	
4. ALPHA-1 ZONE Alpha-1-antitrypsin (Alpha-1-acid glycoprotein)	
5. ALPHA-1-ALPHA-2 INTERZONE GC-globulin, Inter-alpha-trypsin inhibitor, Alpha-1-antichymotrypsin	
6. ALPHA-2 ZONE Alpha-2-macroglobulin, Haptoglobin	
7. ALPHA-2-BETA-1 INTERZONE Cold insoluble globulin, (Hemoglobin)	
8. BETA-1 ZONE; Transferrin	
9. BETA-1-BETA-2 INTERZONE Beta-lipoprotein	
10. BETA-2 ZONE; C3	
11. GAMMA-1 ZONE IgA, Fibrinogen, IgM (monoclonal Igs, light chains)	
12. GAMMA-2 ZONE IgG, (C-reactive protein) (monoclonal Igs, light chains)	

1. What are the significant abnormalities in this pattern?

2. What proteins could be found in this (these) zones?

3. What tests would you do to confirm the identity of these proteins?

4. What disease state could you associate this pattern with (primary abnormality)?

Pattern D

(continued)

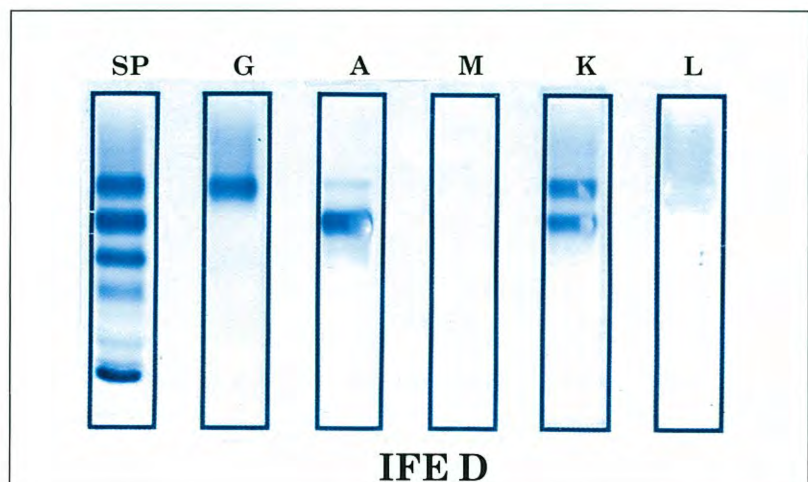
The pattern differences seen were:

1. Prealbumin	- note-prealbumin may not be visible on the photo, so do not evaluate zone.
2. Albumin	- marked decrease (the band is "normal" in appearance but the albumin total is very low)
3. Albumin-alpha-1 interzone	- normal
4. Alpha-1 zone	- normal
5. Alpha-1-alpha-2 interzone	- normal
6. Alpha-2 zone	- slight decrease
7. Alpha-2-beta-1 interzone	- normal
8. Beta-1 zone	- moderate increase
9. Beta-1-beta-2 interzone	- normal
10. Beta-2 zone	- marked increase; band is very broad
11. Gamma-1 zone	- broad marked band near the origin
12. Gamma-2 zone	- moderate polyclonal decrease

The significant pattern abnormalities of this pattern are, the broad beta-2 band and the band in the gamma-1 zone near the origin. The broadening of a band normally found in an HRE pattern can indicate a monoclonal band that is migrating in that zone. In this case the beta-2 band could also contain a monoclonal band in it. The band in the gamma-1 zone could be of immunoglobulin origin or it could be fibrinogen. The sample was drawn in a red stoppered tube, and the patient is not receiving any heparin. This rules out any fibrinogen participation. An IFE could identify the monoclonal band and possibly indicate another band in the beta-2 zone. This patient most likely has a monoclonal gammopathy.

Quantitative immunoglobulins were done on the sample. The IgG= 1,280 mg/dL (reference = 639-1,349 mg/dL); IgA = 1,220 mg/dL (reference = 70-312 mg/dL); IgM = 37 mg/dL (reference = 56-352 mg/dL).

An IFE was performed on the sample for Pattern D and the results of this IFE are shown. What conclusions, if any, can be drawn from this IFE run? What disease state could this indicate?



IFE D

IFE Pattern D

(continued)

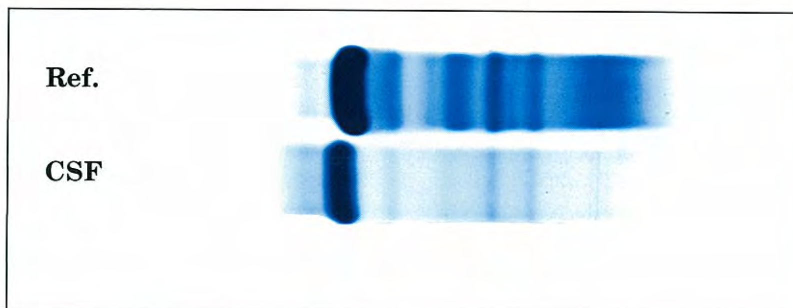
The patient's sera reacted with anti-IgG, anti-IgA, and anti-kappa (in two places on the plate). The patient is demostrating an unusual biclonal gammopathy of IgG-kappa and IgA-kappa. There was, in fact, a monoclonal band in the beta-2 zone. The presence of the biclonal gammopathy is an uncommon finding; it should be verified by repeat analysis or with an IEP.

If you are satisfied with your answers, continue with the next pattern. If not, review before continuing.

Pattern E

Instructions:

Please evaluate Pattern E and fill in your evaluations in the appropriate spaces and answer the questions below. The specimen is a CSF which has been concentrated x80. The protein on the CSF = 63 mg/dL (reference = 17-34 mg/dL). The patient is a female, age 40.



Pattern E
CSF T.P. – 63 mg/dl
Concentration factor – 80x

ZONES, WITH PROTEINS NORMALLY MIGRATING IN EACH ZONE NOTED *	COMPARED TO A REFERENCE SERUM, THE FOLLOWING DIFFERENCES WERE SEEN.
1. PREALBUMIN ZONE Prealbumin	
2. ALBUMIN ZONE Albumin	
3. ALBUMIN-ALPHA-1 INTERZONE	
5. ALPHA-1 ZONE Alpha-1-antitrypsin	
5. ALPHA-1-ALPHA-2 INTERZONE	
6. ALPHA-2 ZONE	
7. ALPHA-2-BETA-1 INTERZONE	
8. BETA-1 ZONE Transferrin	
9. BETA-1-BETA-2 INTERZONE	
10. BETA-2 ZONE Beta-2-transferrin	
11. GAMMA ZONE IgG, (oligoclonal bands, gamma trace)	

1. What are the significant abnormalities in this pattern?

Pattern E

(continued)

Proteins were present in the following zones:

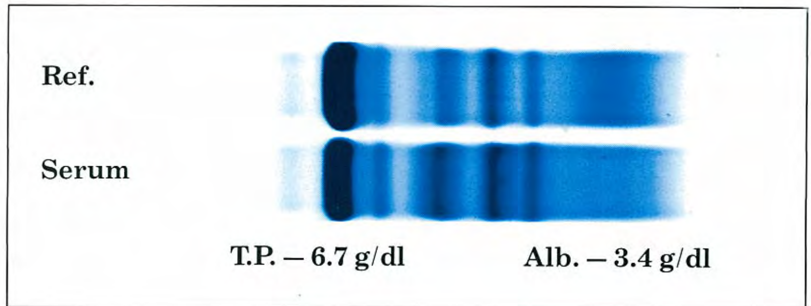
Prealbumin
Albumin
Alpha-1
Alpha-2
Beta-1
Beta-2

There were no significant abnormalities seen in this CSF. No oligoclonal bands were noted.
If you are satisfied with your answer, continue with the next pattern. If not, review before continuing.

Pattern F

Instructions:

Please evaluate Pattern F and fill in your evaluations in the appropriate spaces and answer the questions below. The serum sample was obtained from a female, age 58. The total protein = 6.7 g/dL (reference = 6.8-8.4 g/dL); albumin = 3.4 g/dL (reference = 3.7-4.9 g/dL).



Pattern F

ZONES, WITH PROTEINS NORMALLY MIGRATING IN EACH ZONE NOTED *	COMPARED TO A REFERENCE SERUM, THE FOLLOWING DIFFERENCES WERE SEEN.
1. PREALBUMIN ZONE Prealbumin	
2. ALBUMIN ZONE Albumin	
3. ALBUMIN-ALPHA-1 INTERZONE Alpha-lipoprotein (Alpha-fetoprotein)	
4. ALPHA-1 ZONE Alpha-1-antitrypsin (Alpha-1-acid glycoprotein)	
5. ALPHA-1-ALPHA-2 INTERZONE GC-globulin, Inter-alpha-trypsin inhibitor, Alpha-1-antichymotrypsin	
6. ALPHA-2 ZONE Alpha-2-macroglobulin, Haptoglobin	
7. ALPHA-2-BETA-1 INTERZONE Cold insoluble globulin, (Hemoglobin)	
8. BETA-1 ZONE; Transferrin	
9. BETA-1-BETA-2 INTERZONE	
10. BETA-2 ZONE; C3	
11. GAMMA-1 ZONE IgA, Fibrinogen, IgM (monoclonal Igs, light chains)	
12. GAMMA-2 ZONE IgG, (C-reactive protein) (monoclonal Igs, light chains)	

1. What are the significant abnormalities in this pattern?

2. With what disease state would you associate this pattern?

3. What tests would you do to confirm the identity of the identitiy of the protein in the gamma-1 zone?

Pattern F

(continued)

The proteins observed in the serum sample are

1. Prealbumin	- note-prealbumin may not be visible on the photo, so do not evaluate.
2. Albumin	- slight decrease
3. Albumin-alpha-1 interzone	- normal
4. Alpha-1 zone	- slight increase
5. Alpha-1-alpha-1 interzone	- increased
6. Alpha-2 zone	- marked increase
7. Alpha-2-beta-1 interzone	- increased
8. Beta-1 zone	- marked increase
9. Beta-1-beta-2 interzone	- increased
10. Beta-2 zone	- moderate increase
11. Gamma-1 zone	- slight polyclonal increase
12. Gamma-2 zone	- slight polyclonal decrease

The significant abnormality of this pattern is the decrease in the albumin coupled with the increases in alpha-1 and alpha-2 proteins which indicates an inflammatory response. The increase in the beta-1 zone is most likely due to a concurrent transferrin increase. The increase in the gamma-1 zone was confirmed by quantitative immunoglobulins. They showed that the IgG = 746 mg/dL (reference = 639-1349 mg/dL), IgA = 662 mg/dL (reference = 70-312 mg/dL), and IgM = 159 mg/dL (reference = 56-352 mg/dL). Thus, the increase seen in the gamma-1 zone was due to the IgA increase.

Notes

GLOSSARY

Glossary

ALBUMIN, serum, urine, csf
MOLECULAR WEIGHT: 66,000 daltons.
NORMAL ADULT CONCENTRATION: 3.5-5.0 g/dL(serum).
MIGRATION: Albumin zone.
VARIATIONS: 1. Broad band with diffuse anodal edge usually indicates bilirubin or drugs conjugated with albumin. Fast albumin. 2. Genetic variants- most commonly is chacterized by cathodal broadening; anodal widening also possible. Split (double) albumin band indicates bisalbuminemia.
FUNCTION: Major determinant of oncontic pressure. Transport of cations, anions, pigments, hormones, organic dyes, drugs, fatty acids, bilirubin, bile acids, vitamins, etc. Protein reserve. HIGH: Hyperinfusion, dehydration states, glomerular proteinuria and chyluria. LOW: Subacute and chronic debilitating diseases, chronic inflammatory disease, malabsorption, malnutrition, gastrointestinal loss, liver disease (especially cirrhosis), renal disease (nephrotic syndrome), third degree burns, severe skin disease and dilution by IV fluids.

ALPHA-1-ACID GLYCOPROTEIN, serum, urine
SYNONYMS: Orosomucoid; alpha-1 seromucoid.
MOLECULAR WEIGHT: 39,500 daltons.
NORMAL ADULT CONCENTRATION: 50-150 mg/dL(serum).
MIGRATION: Anodal side of alpha-1-antitrypsin (fuzzy band).
VARIATIONS: Heterogeneous.
FUNCTION: Unknown; may inactivate progesterone. Implicated in the formation of certain membranes and fibers in combination with collagen. HIGH: Inflammation, pregnancy, steroid therapy and decreased glomerular filtration. LOW: Malnutrition, severe hepatic damage, genetic variations and estrogen therapy, severe protein-losing gastroenteropathies.

ALPHA-1-ANTICHYMOTRYPSIN, serum
SYNONYMS: Alpha-1 ACT.
MOLECULAR WEIGHT: 68,000 daltons.
NORMAL ADULT CONCENTRATION: 30-60 mg/dL(serum).
MIGRATION: Alpha-1-Alpha-2 interzone. VARIATIONS: none
FUNCTION: Inhibits chymotrypsin. HIGH: Inflammation.

ALPHA-1-ANTITRYPSIN, serum, urine, csf
SYNONYMS: Alpha-1-AT; AAT
MOLECULAR WEIGHT: 55,000 daltons.
NORMAL ADULT CONCENTRATIONS: 78-200 mg/dL(serum).
MIGRATION: Alpha-1 zone, which is accompanied by a minor adjacent satellite band in the alpha-1- alpha-2 interzone.
VARIATIONS: Inherited and aquired mobility variants are common and may cause broadening, slight displacement or doubling of the alpha-1 band. May complex to M-proteins and retard its mobility.
FUNCTION: Protease inhibitor in plasma (trypsin, chymotrypsin, granulocytic elastase and collagenase) which is produced in the liver. Neutralizes lysosomal elastase upon phagocytosis of particles by polymorphonuclear leukocytes. HIGH: Inflammatory disorders, liver injury, increased estrogen, pregnancy, malignancies and steroid therapy. LOW: Heterozygous deficiency, homozygous deficiency, juvenile pulmonary emphysema and premature infants, severe protein-losing disorders.

Glossary

ALPHA-FETOPROTEIN, serum
SYNONYMS: AFP; Alpha-1-FP; fetoglobulin
MOLECULAR WEIGHT: 69,000 daltons.
NORMAL ADULT CONCENTRATIONS: < 30 ng/mL (serum).
MIGRATION: Albumin-Alpha-1 interzone. Appears as a sharp band when increased.
VARIATIONS: none.
FUNCTION: Unknown; level is measured in maternal serum/amniotic fluid to screen for fetal neural tube defects. Used to monitor liver cancers. HIGH: Primary cancer of liver, embryonic tumors and maternal serum with fetal neural tube defects.

ALPHA-LIPOPROTEIN, serum
SYNONYMS: High density lipoprotein; HDL.
MOLECULAR WEIGHT: 200,000 - 350,000 daltons.
NORMAL ADULT CONCENTRATIONS: 170-325 mg/dL (serum).
MIGRATION: From albumin to the alpha-1 band. VARIATIONS: increased and decreased mobility can be seen: decreased mobility as seen with alcoholism may result in a blurred alpha-1 band.
FUNCTION: Transport of fatty acids, cholesterol, phospholipids, glycerides, etc. HIGH: Chronic alcoholism and pregnancy. LOW: Inflammation, severe liver disease and inherited hypo-alpha-lipoproteinemia.

ALPHA-2-MACROGLOBULIN, serum
SYNONYMS: Alpha-2-M; antiplasmin.
MOLECULAR WEIGHT: 800,000 daltons.
NORMAL ADULT CONCENTRATION: 125-410 mg/dL(serum, male); 175-420 mg/dL(serum, female).
MIGRATION: Alpha-2 zone. VARIATIONS: Electrophoretic appearance varies with age. In children and adolescents the anodal edge of the alpha-2 macroglobulin has a distinctly sharper front. This can also be seen in older individuals.
FUNCTION: Protease inhibitor, hormone binding, transport protein. Inhibitor of trypsin, chymotrypsin, thrombin, elastase and plasmin. HIGH: Liver disease, diabetes mellitus, nephrotic syndrome, neural tube defects, estrogen therapy, pregnancy, newborns and children until puberty, ataxia-telangiectasia. LOW: Protein-losing gastroenteropathies, protein malnutrition, fibrinolytic therapy, DIC (disseminated intravascular coagulation).

ALPHA-2-MICROGLOBULIN, urine
MOLECULAR WEIGHT: < 20,000 daltons
MIGRATION: Alpha-2-zone. Appear as a pair of protein bands.
FUNCTION: Retinol binding protein.

BETA-LIPOPROTEIN, serum
SYNONYMS: Low density lipoprotein; LDL.
MOLECULAR WEIGHT: about 3,000,000 daltons.
NORMAL ADULT CONCENTRATION: 60-155 mg/dL (Apoprotein B, serum).
MIGRATION: Beta-1-beta-2 interzone. Slightly wavy band with a sharper cathodal demarcation.
VARIATIONS: Disintegration of beta-lipoprotein and increase non-esterfied fatty acid content induces fast mobility. Heparin therapy changes migration. Increase in beta-lipoprotein causes slower mobility.
FUNCTION: Transport of lipids, cholesterol and hormones. HIGH: Nephrotic syndrome, obstructive jaundice, uncontrolled diabetes mellitus and type II hypercholesterolemia. LOW: Familial LDL deficiency.

Glossary

BETA-2-MICROGLOBULIN, urine, csf

SYNONYM: B2M
MOLECULAR WEIGHT: 11,800 daltons
NORMAL ADULT CONCENTRATION: 0.03-.37 mg/day (urine); 1.3-1.7 mg/L (csf)
MIGRATION: Beta-1-beta-2 interzone.
FUNCTION: The beta chain of the human leukocyte antigen (HLA) on cell surfaces; HIGH: B-cell malignancies, renal failure, inflammation, decreased renal tubular function with kidney transplant. High in CSF in acute leukemia and lymphoma with CNS involvement.

BETA-2-TRANSFERRIN, csf

SYNONYMS: Transferrin component.
MIGRATION: Beta-2 zone. Carbohydrate deficient transferrin with a slower mobility than transferrin.

C3, serum

SYNONYMS: Beta-1-C-globulin; factor A
MOLECULAR WEIGHT: 180,000 daltons.
NORMAL ADULT CONCENTRATION: 70-150 mg/dL (serum).
MIGRATION: Beta-2 zone. VARIATIONS: Conversion products of complement migrate both slower and faster than C3.
FUNCTION: Complement is activated by antigen-antibody complexes through the classical or alternative pathways leading to destruction of viruses and bacteria. HIGH: Chronic inflammatory response, biliary obstruction, amyloidosis, severe protein malnutrition. LOW: Complement activation, immune complex diseases, active innumological diseases, aged samples and frozen sera, bacteremia and genetic deficiencies.

COLD INSOLUBLE GLOBULIN, serum

SYNONYMS: Fibronectin, Fibrinolignin.
MOLECULAR WEIGHT: 440,000 daltons(dimer).
NORMAL ADULT CONCENTRATION: 15-30 mg/dL (serum).
MIGRATION: Alpha-2-beta-1 interzone.
FUNCTION: Promotes cell-to-cell adhesion and fibrin clot retraction by cross-linking. Binds to C1q and is part of some immune complexes. HIGH: Pregnancy. LOW: Disseminated intravascular coagulation, inflammation, shock and trauma.

CRP, serum

SYNONYMS: C-reactive protein.
MOLECULAR WEIGHT: about 120,000 daltons.
NORMAL ADULT CONCENTRATION: < 0.8 mg/dL (serum)
MIGRATION: Gamma-2 zone. Often appears as a narrow band at the cathodal end of the gamma-2 zone.
FUNCTION: Acute phase protein; binds polysaccharides on bacteria, fungi, etc., activating classical complement pathway; binds and clears from blood toxic substances released from damaged tissue. HIGH: Acute inflammatory conditions (myocardial infarction,stress,trauma, infection, inflammation, surgery). LOW: Viral infections (exclusive of hepatitis), corticosteroids and other anti-inflammatory agents.

Glossary

FIBRINOGEN, plasma
SYNONYMS: Factor I.
MOLECULAR WEIGHT: 340,000 daltons.
NORMAL ADULT CONCENTRATION: 200-400 mg/dL (plasma).
MIGRATION: Gamma-1 zone. Sharp band with blurred anodal edge.
FUNCTION: Precursor of the fibrin clot; during coagulation, fibrinogen is split by thrombin, forming fibrin. HIGH: Inflammation. LOW: Liver disease, hyperfibrinolysis, malignancies and afibrinogenemia.

GAMMA TRACE, urine, csf
MOLECULAR WEIGHT: < 20,00 daltons.
MIGRATION: Faint but discrete band sometimes seen at the cathodal end of the gamma zone.

Gc GLOBULIN, serum
SYNONYMS: Group-specific component, vitamin-D binding protein.
MOLECULAR WEIGHT: 59,000 daltons.
NORMAL ADULT CONCENTRATION: 20-55 mg/dL.
MIGRATION: Alpha-1-alpha-2 interzone. VARIATIONS: Three common phenotypes exist. Gc1-1 is obscured in the alpha-2 zone, Gc2-2 is slightly more cathodal and appears as a separate minor band in the alpha-2-beta-1 interzone, Gc2-1 combines equal amounts of Gc2-1, Gc1-1.
FUNCTION: Binds vitamin D. HIGH: With oral contraceptives, third trimester of pregnancy. LOW: severe liver disease, hypoproteinemia.

HAPTOGLOBIN, serum
MOLECULAR WEIGHT: 85,000-1,000,000 daltons.
NORMAL ADULT CONCENTRATION: 30-215 mg/dL (serum).
MIGRATION: Alpha-2 zone. VARIATION: Three common phenotypes; type 1-1 migrates anodal to alpha-2-macroglobulin; type 2-1 migrates on both sides of alpha-2-macroglobulin ; type 2-2 migrates cathodal to alpha-2-macroglobulin. Haptoglobin- hemoglobin complexes are found in the alpha-2 zone or alpha-1-beta-1 interzone.
FUNCTION: Binds free hemoglobin to prevent loss of iron in urine. HIGH: Inflammation, corticosteroid therapy, biliary obstruction, aplastic anemia, diabetic angiopathy, hyperhaptoglobulinemia, pregnancy and estrogens. LOW: Intravascular hemolysis, liver disease, hemolytic anemia, congenital absence, B12 and folic acid deficiency and infancy.

HEMOGLOBIN, serum, urine, csf
SYNONYMS: Hgb, Free hemoglobin.
MOLECULAR WEIGHT: 64,456 daltons.
NORMAL ADULT CONCENTRATION: 1-5 mg/dL (serum).
MIGRATION: Alpha-2-beta-1 interzone. Free hemoglobin (sample contaminant due to hemolysis) migrates in the cathodal part of the interzone. At high levels it overlaps into the beta zone and obscures transferrin.
FUNCTION: Transport of oxygen and carbon dioxide from tissues to lungs. HIGH: Hemolysis in vivo or in vitro.

Glossary

INTER-ALPHA-TRYPSIN INHIBITOR, serum

SYNONYMS: Inter-alpha-TI.
MOLECULAR WEIGHT: 140,000 daltons.
NORMAL ADULT CONCENTRATION: 20-70 mg/dL.
MIGRATION: Alpha-1-alpha-2 interzone.
FUNCTION: Proteinase inhibitor of trypsin, plasmin and chymotrypsin. HIGH: recovery phase from thermal burns. LOW: Acute thermal burns, acute inflammation and nasal mucous membrane infection.

IgA, serum, urine

MOLECULAR WEIGHT: 170,000 daltons.
NORMAL ADULT CONCENTRATION: 40-390 mg/dL(serum).
MIGRATION: polyclonal IgA mobility is from the beta to the gamma-1 zone. VARIATION: Monoclonal IgA mobility may extend into beta (or rarely, alpha) regions.
FUNCTION: antibodies which form the first line of defense in microbial invasions; activates complement via the alternate pathway. HIGH: IgA myeloma, chronic liver disease, chronic infections and autoimmune disorders. LOW: Congenital IgA deficiency and chronic sino-pulmonary syndrome, non-IgA gammopathies.

IgG, serum, urine, csf

MOLECULAR WEIGHT: 160,000 daltons.
NORMAL ADULT CONCENTRATION: 525-1650 mg/dL (serum).
MIGRATION: polyclonal IgG migrates from the gamma-1 to the gamma-2 zones. VARIATIONS: Restricted mobility is found with selected response of plasma cell clones; monoclonal proteins form sharp bands in both the gamma-1 and gamma-2 zones.
FUNCTION: Antibodies which form the secondary immune response; neutralizes toxins in tissue spaces. HIGH: IgG myeloma, chronic liver disease, chronic infections, collagen diseases and abscesses. LOW: Antibody deficiency syndromes, aqued deficiency syndromes and corticosteroids, non-IgG gammopathies.

IgM, serum

MOLECULAR WEIGHT: 900,000 daltons.
NORMAL ADULT CONCENTRATION: 25-310 mg/dL.
MIGRATION: polyclonal IgM migrates in the mid gamma zone. VARIATION: monoclonal IgM mobility may extend into the beta (or rarely, alpha) regions.
FUNCTION: Antibodies which constitute the first line of defense in the body; activates complement. HIGH: Waldenstrom's macroglobulinemia, immune response, malaria, trypanosomiasis, filaria, mycoplasm infections, intrauterine, congenital and neonatal infections, primary biliary cirrhosis, infectious hepatitis (A and B), rubella, CMV and coxackie infections. LOW: Congenital or aquired hypogammaglobulinemia and selective IgM deficiency or agammaglobulinemia, non-IgM gammopathies.

LIGHT CHAINS, serum, urine

MOLECULAR WEIGHT: monomer ~ 25,000 daltons; dimer ~ 50,000 daltons.
NORMAL ADULT CONCENTRATION: < 0.1 mg/dL.
MIGRATION: Gamma-1, gamma-2 zone; sometimes found in the alpha or beta zones, especially if complexed with other proteins.
FUNCTION: Form the light chain portion of immunoglobulin molecules. HIGH: Light chain disease, amyloidosis.

Glossary

LYSOZYME, serum, urine
SYNONYMS: Muramidase.
MOLECULAR WEIGHT: 15,000 daltons.
NORMAL ADULT CONCENTRATION: 0.36-0.78 mg/dL (serum), 1.3 - 3.6 mg/day (urine).
MIGRATION: Faint band at the cathodal end of the gamma-2 zone.
FUNCTION: Bacteriolysis. Catalyzes the hydrolysis of the bacterial wall. HIGH: Monocytic leukemia; in urine, with tubular damage. LOW: Neutropenia with hypoplasia of the bone marrow.

PREALBUMIN, serum, csf
MOLECULAR WEIGHT: 54,400 daltons.
NORMAL ADULT CONCENTRATION: 20-40 mg/dL (serum).
MIGRATION: Prealbumin zone. VARIATION: Band spread and migration may vary with complex formation and genetic polymorphism.
FUNCTION: Binds and transports thyroxine and triiodothyronine. HIGH: Alcoholism, steroids and acromegaly. LOW: Inflammatory reactions, malnutrition, early cirrhosis, hyperestrogenism and thyrotoxicosis.

TRANSFERRIN, serum, urine, csf
SYNONYMS: TRF, siderophilin
MOLECULAR WEIGHT: 76,500 daltons.
NORMAL ADULT CONCENTRATION: 200-350 mg/dL (serum).
MIGRATION: Beta-1 zone. VARIATION: Double transferrin (genetic variant).
FUNCTION: Binds iron and transports to storage sites in the liver and reticuloendothelial system. HIGH: Chronic iron deficiency anemias, hypothyroidism and pregnancy. LOW: Malnutrition, Hypoalbuminemia, inflammation, nephrotic syndrome and genetic deficiencies and liver disease.

Zn-ALPHA-2-GLYCOPROTEIN, urine
MOLECULAR WEIGHT: 41,000 daltons.
NORMAL ADULT CONCENTRATION: 0.05-0.21 mg/dL (urine).
MIGRATION: Alpha-1 alpha-2 interzone.
FUNCTION: Acute phase reactant.

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Materials and Methods

Protein separations illustrated herein were performed using the following kits available commercially from Helena Laboratories:

High Resolution Protein Electrophoresis (HRE)
TITAN GEL High Resolution Protein Kit
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70 assays

Immunofixation (IFE)
TITAN GEL Immunofix Kit
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Immunoelectrophoresis (IEP)
TITAN GEL IEPlate Kit
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