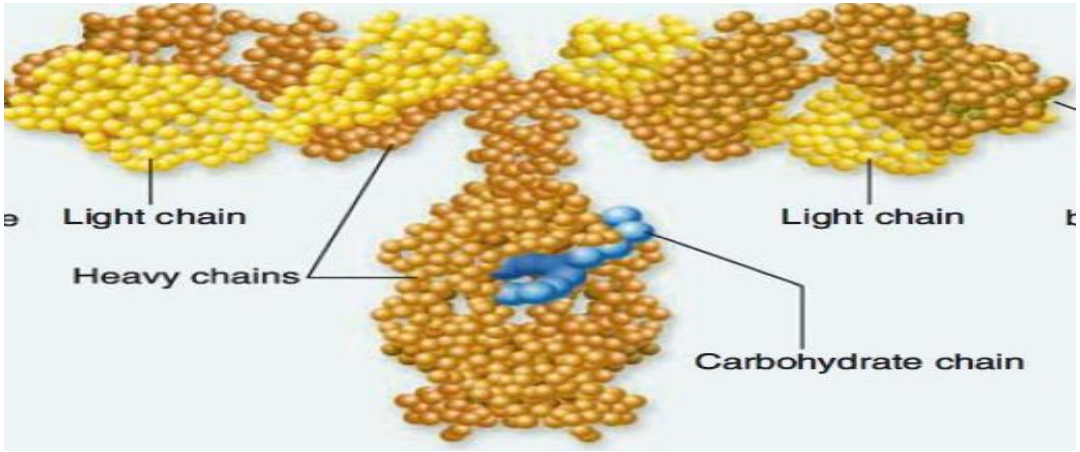
PRINCIPLES AND INTERPRETATION OF SERUM PROTEIN ELECTROPHORESIS AND SERUM FREE LIGHT CHAINS



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OUTLINE

- Objectives
- Introduction/Definition
- Principles of electrophoresis/Factors affecting electrophoretic mobility.
- Forms and types of electrophoresis [FOR CHEMPATH RESIDENTS ONLY]
- Available methods for serum protein electrophoresis in our environment.
- Free light chains and their assay methods
- Patterns of serum protein electrophoretogram (Normal and diseases)

- Salient points in the interpretation of SPE results
- Available standardisation effort at reporting SPE (AACB)
- Quality assurance issues in SPE evaluation
- Prototypes of SPE reports from AACB
- Conclusion
- Acknowledgement
- References.

OBJECTIVES

- At the end of this presentation, participants should be able:
- *To describe vividly what SPE and its principle are all about.*
- *To recognise normal and abnormal serum protein electrophoretogram.*
- *To understand the rudiments in the interpretation of SPE and the role of quality assurance in delivering a reliable SPE report*

Introduction

- Electrophoresis is a technique that separates molecules in their liquid state, based on their ability to move in an electric field.
- The various forms and types of electrophoresis have become the leading methods of the analysis of biomolecules in biochemistry and molecular biology, including genetic materials such as *DNA or RNA, proteins and polysaccharides.*

INTRODUCTION

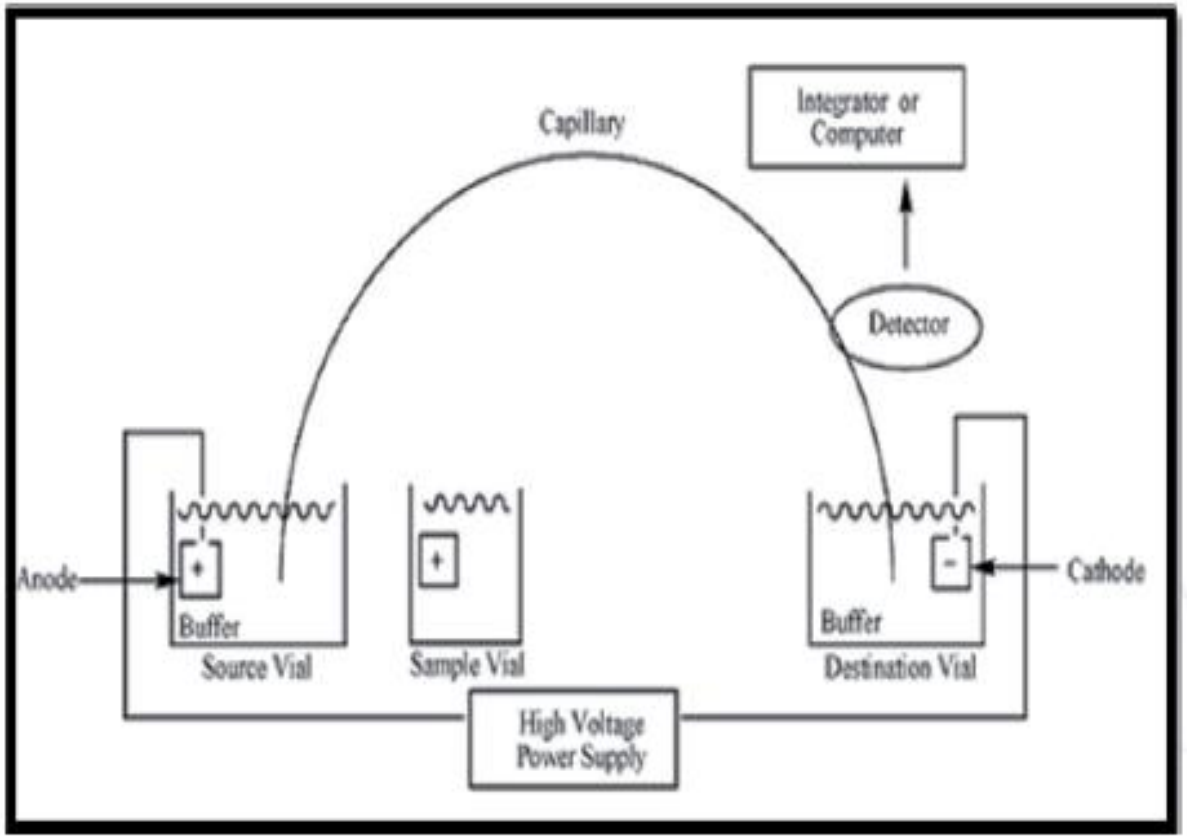
- Serum protein electrophoresis is an electrophoretic method of separating proteins present in the serum to various fractions based on their molecular weight and electric charges.
- Electrophoresis had been widely used in clinical medicine for aiding in diagnosis of various clinical conditions like acute and chronic inflammations, monoclonal gammopathies, nephropathy, liver diseases

Principles of electrophoresis

- Electrophoresis is based on the phenomenon that most biomolecules exist as electrically-charged particles, possessing ionizable functional groups. Biomolecules in a solution at a given pH will exist as either positively or negatively charged ions.
- When subjected to an electric field, ionized biomolecules will migrate at a different pace, depending on the mass and the net charge of each particle in the solution—
- negatively-charged particle, *anions*, will migrate towards a positively charged electrode, or an*ode*, and
- *cations,* or positively-charged particles, will be pulled towards a negatively-charged electrode called cath*ode*.

- The differences in the speed and the direction of each charged particle will result in a migration pattern that is unique to its individual property, leading to the isolation of components of the biomolecules that possess similar characteristics

CE DIAGRAM


Capillary
Integrator or Computer
Detector
Anode
Buffer
Source Vial
Sample Vial
Buffer
Destination Vial
Cathode
High Voltage Power Supply

Factors affecting protein migration

- Their rate of migration through the electrical field, depends on the
- ***strength of the field,***
- ***on the net charge,***
- ***size, and shape of the molecules,***
- ***the ionic strength, viscosity, and temperature of the medium in which the molecules are moving.***

- SPE is a semiquantitative investigation which involves technical expertise to recognize the specific electrophoretic patterns and associate with various clinical conditions.
- This requires a laboratory practice integrated across various divisions of laboratory and with respective clinical and ancillary divisions of clinical medicine

Diagnostic clue toward gammopathy through an increased serum total protein level (greater than 8 g/dl) and an altered serum albumin globulin (AG) ratio (which is usually altered in gammopathy).

A normal AG ratio ranges between 1.2 and 1.8, while there is a significant reduction in the ratio in patients with gammopathy.

This becomes an incidental finding which leads to a concept of “reflex” testing for multiple myeloma work-up including SPE, upon consent from the treating clinician and the patient.

- The separation of proteins by electrophoresis is based on the fact that charged molecules usually migrate through a matrix/medium upon application of an electrical field.
- The rate at which proteins move in an electric field is determined by a number of factors of the electrophoretic system and the nature of proteins itself.

- Some factors to mention are the strength of the electric field, temperature of the system, pH of the ions, concentration of buffer etc.
- Proteins vary in their size and shape and have the charges determined by the dissociation contents of their amino acids.

- Smaller proteins usually migrate faster, and larger proteins take a longer time.
- This physical property of proteins is exploited for its separation by employing the electrophoretic technique.

- There are several support mediums available for separation of serum proteins including agarose, cellulose acetate, capillary medium etc.; when a capillary medium is used, the technique is known as capillary zone electrophoresis (CZE).
- Capillary electrophoresis is the preferred method when compared to its competitors including agarose gel electrophoresis due to the following reasons. CZE provides an improved resolution due to the following factors:

FORMS OF ELECTROPHORESIS

- Based on the type of buffer solution and its effect on the mobility of the charged particles, electrophoresis can be broadly divided into **four** forms :
- ***MOVING BOUNDARY***
- ***ZONE***
- ***ISOTACHOPHORESIS***
- ***ISOELECTRIC FOCUSING***

Moving boundary electrophoresis

- Moving boundary electrophoresis is considered the original form of electrophoresis.
- Samples for separation **are performed in free solution, in tubes or capillary tubes, under constant pH value throughout the separation process.**

- A major advantage of electrophoresis in free solution is *the* ***ability to measure the mobilization of separating particles without other intervening factors unrelated to the separating particles.***
- Nonetheless, this electrophoresis format **is *vulnerable to convection current and the resolution of separation is low* due to the mixing of samples in the solution buffer that can result in the overlapping of components or particles that possess similar characteristics**

Zone electrophoresis

- Its similar to moving boundary electrophoresis in that electrophoretic ***separation takes place in a homogenous buffer system.***
- ***This format often makes use of a support medium or a matrix to subdue convection current and prevent uncontrolled sample diffusion***.
- The matrix, in most cases, also provides an additional *sieving* effect that exerts an influence on electrophoretic separation

- Gel electrophoresis is an example of ZE that uses a polymer-sieving matrix as a support media.
- The technique is widely used in biochemistry and molecular biology research and routine work due to its simplicity and versatility

Isotachophoresis ITP

- ITP is a form of electrophoresis, in which ***all ions migrate at equal velocity (v).***
- In ITP, the ***samples are placed between two non-homogeneous solution buffer, composing of a leading electrolyte at one electrode, and a terminating electrolyte at the other end .***
- Both electrolytes possess the same charge species as that of the particle of interest in the sample.
- When an electric current is applied, the leading electrolyte will have the highest mobility, followed by the charged particles in the sample and the terminating electrolytes, respectively.

- As ITP continues, charged particles in the sample will be displaced, based on its electrical mobility *(μ)* and concentration, in order of decreasing mobility, resulting in a continuous region of charged particles with similar characteristics, sandwiched by regions where the leading and terminating ions are occupied

Isoelectric focusing IEF

- IEF is electrophoresis that is ***performed in a pH gradient, which runs from low to high – from the anode to cathode.***
- IEF is ***only applicable to amphoteric molecules because they can donate and receive protons, acting as acid and base.***
- ***Examples of amphoteric biomolecules are peptides and proteins, which possess the amine and carboxylic acid groups .***
- Once the pH gradient is established, and an electric current is applied, an amphoteric sample will migrate towards either the anode or the cathode, depending on the net charge of the sample.

- **At the *isoelectric point (pI)*, where the net charge of the sample is zero, the velocity *(v)* and the electrical mobility *(μ)* of the amphoteric molecule become zero, stopping the migration .**
- All the four formats of electrophoresis can be performed in both one- and two-dimensions (2D).

- Two-dimensional electrophoresis is performed by conducting the first electrophoresis.
- This is followed by the second electrophoretic separation in a direction perpendicular to the first dimension.
- 2D-electrophoresis can offer more information and resolution.
- This is particularly useful for clinical or field samples, which often requires intensive analysis and characterization but is given only in a limited amount .

TYPES OF ELECTROPHORESIS

- Gel electrophoresis is a form of ZE that uses gel, a non-fluid cross-linked polymer network, as a support medium to maintain stable pH value in the solution buffer, acting as an anti-convective stabilizer.
- It also serves as a separation matrix, due to its porous nature that filters large particles and hinders smaller ones during electrophoretic separation.

- The gel is cast into strips or slabs with slots or sample *wells*.
- Once it is completely polymerized, the gel is submerged in an electrophoresis solution buffer, and samples are loaded into each well before the electric current is applied to initiate electrophoretic separation.
- At the end of the gel electrophoresis, components in the samples will be separated based on their mass.
- GEL;SDS-PAGE & AGAROSE

- As mentioned earlier, *gel electrophoresis is one of the most used types of electrophoresis in research and routine diagnosis due to their ease-of-use and their versatility.*
- It can be adapted to separate a variety of biomolecules by changing the type of polymers used to cast the gel and by adjusting the composition of the polymer, altering the pore size of the gel.

Pulsed-field Gel Electrophoresis

- (PFGE) is a ***variation of gel electrophoresis, in which two electrical fields are periodically applied, in rotation, to the gel electrophoresis at different angles.***
- This type of electrophoresis is specifically designed for the separation of chromosomes, which are high-molecular-weight DNA molecules of over 20 kilobases.

Capillary electrophoresis,

- Capillary electrophoresis, also known as High-Performance Capillary Electrophoresis (HPCE), is a type of electrophoresis performed in a narrow capillary immersed in an electrolyte buffer.
- It is the only type of electrophoresis capable of performing all four types of electrophoreses
- The capillary is typically 20-30 centimeters long and possesses a 25-75 micrometer inner diameter.

HELENA V8 NEXUS CE

https://www.helena-biosciences.com/en/clinical-electrophoresis/v8-nexus

- Electrophoretic separation is initiated when a sample is injected into the capillary, either by high voltage or by pressure, and high electric fields are applied across the capillary.
- Components in the sample are separated along the length of the capillary, based on the format of electrophoresis performed.
- Towards the other end of the capillary, separated components are detected at the *detector*, where the time of detection or *retention time* is automatically recorded.

Immunoelectrophoresis

- Immunoelectrophoresis is a type of electrophoresis that separate *antigens*, including proteins and peptides, based on their reaction and specificity to *antibodies*, or *immunoglobulins (Ig)*.
- The binding of antigen to its corresponding antibody at a specific antigen/antibody ratio, or the *equivalent point,* will result in the precipitation of antigen-antibody complex.
- Thus, antigens in a sample of interest can be separated based on their ability to bind to a given antibody

Affinity Electrophoresis

- Affinity Electrophoresis is a type of electrophoresis that separates a biomolecule that interacts with or binds to another molecule for which it has an affinity.
- It makes use of the phenomenon that the electrical mobility *(μ)* changes when a biomolecule, including nucleic acids, proteins, peptides, and polysaccharides, binds to another molecule, and this change in the electrical mobility will be reflected in the electrophoretic pattern.

INSTRUMENTATION

- All types of electrophoresis separate charged particles while they are submerged in a solution buffer.
- All forms of electrophoresis require a power supply and an electrophoresis unit, commonly referred to as an electrophoresis *chamber*.
- The power supply provides the electric current to the chamber that propels the electrophoretic separation.
- The chamber is composed of two opposite electrodes, cathode and anode, and of a buffer solution reservoir, in which the samples and the separation thereof take place (Walker, 2010).

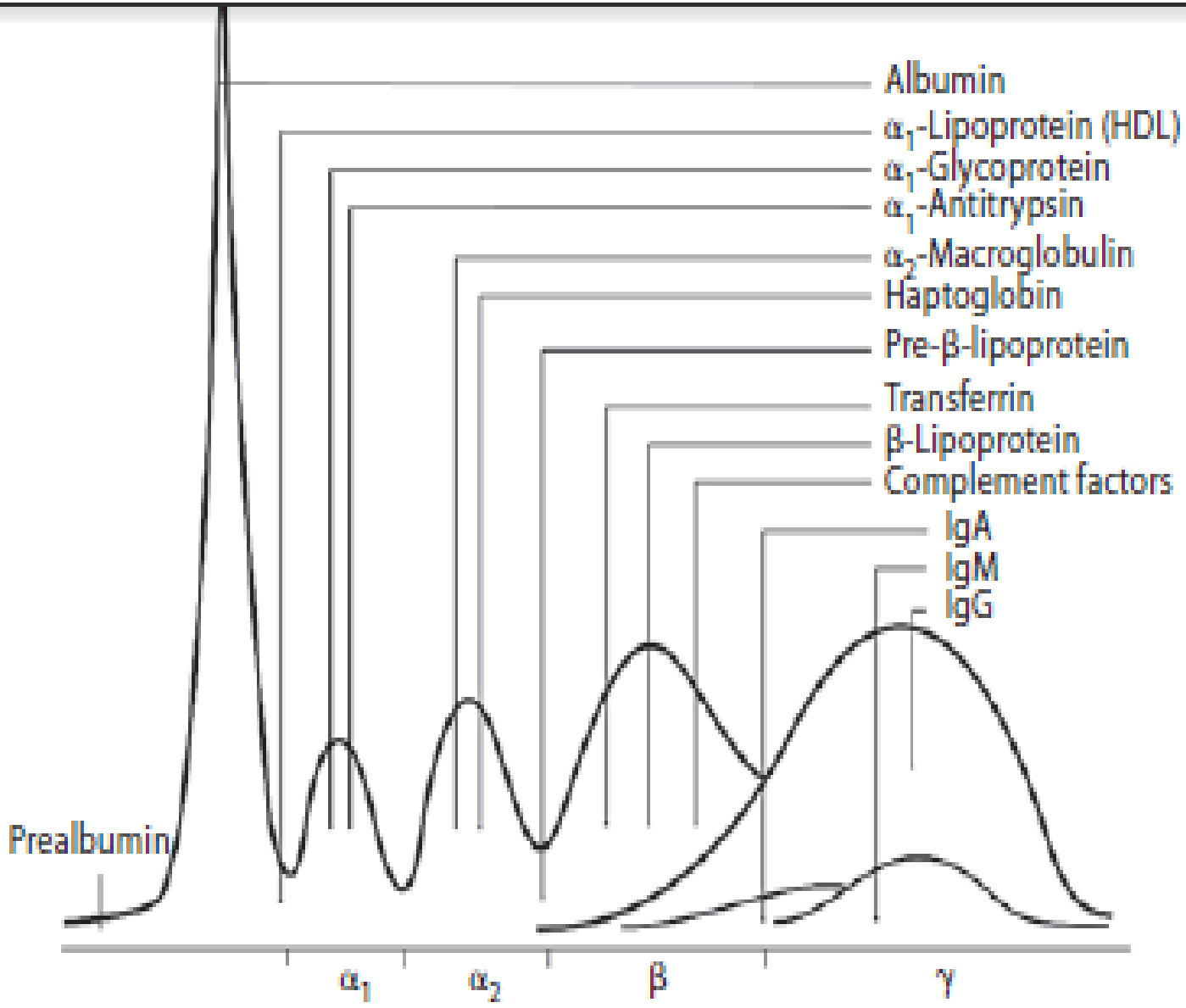
WHAT WE HAVE

- The use of a power supply and an electrophoresis chamber is also required by all types of electrophoresis.
- However, each format necessitates specific equipment for the setting up of the separation process.
- For example, caster trays, glass plates, and combs are prerequisites for gel electrophoresis.
- Capillary electrophoresis requires an internal cooling system that effectively suppresses excessive heating and provides a thermally stable condition during electrophoresis.

- Thus, biomolecules of interest in a sample can be singled out based on their affinity to another molecule—whether or not the biomolecules of interest tend to bind to another molecule more than the other unwanted biomolecule.
- Contemporary settings for affinity electrophoresis are based on either gel electrophoresis or capillary electrophoresis.

Serum proteins

- Serum proteins are a family of albumin and globulins.
- Albumin is the major fraction synthesized from human liver endogenously and available through various dietary sources exogenously including egg, meat, pulses, milk etc.
- Globulins are a group of proteins subclassified into alpha-1, alpha-2, beta-1, beta-2, and gamma globulins based on the electrophoretic mobility.


Albumin
α1-Lipoprotein (HDL)
α1-Glycoprotein
α1-Antitrypsin
α2-Macroglobulin
Haptoglobin
Pre-β-lipoprotein
Transferrin
β-Lipoprotein
Complement factors
IgA
IgM
IgG
Prealbumin
α1
α2
β
γ

INDICATIONS FOR SPE

- **Indications/Applications**
- Unexplained anemia, back pain, bone pain, fatigue.
- Unexplained pathologic fracture or lytic lesions.
- Unexplained peripheral neuropathy.
- Hypercalcemia secondary to possible malignancy.
- Hypergammaglobulinemia.
- Rouleaux formation noted on peripheral blood smear.

- The normal biological interval of serum total proteins in a healthy adult ranges between 6.4 and 8.6g/dl which includes Serum Albumin: 3.5–5.5 g/dl and Globulins: 2.5–3.5 g/dl.

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- Reference ranges are as follows :
- Total protein - 6.4-8.6ġ/dL or 64-86g/L
- Albumin - 3.5-5 g/dL or 35-50 g/L
- Globulin - 2.3-3.4 g/dL
- Alpha-1 globulin - 0.1-0.3 g/dL or 1-3 g/L
- Alpha-2 globulin - 0.6-1 g/dL or 6-10 g/L
- Beta globulin - 0.7-1.1 g/dL or 7-11 g/L

Table 1. Electrophoretic distribution of serum proteins

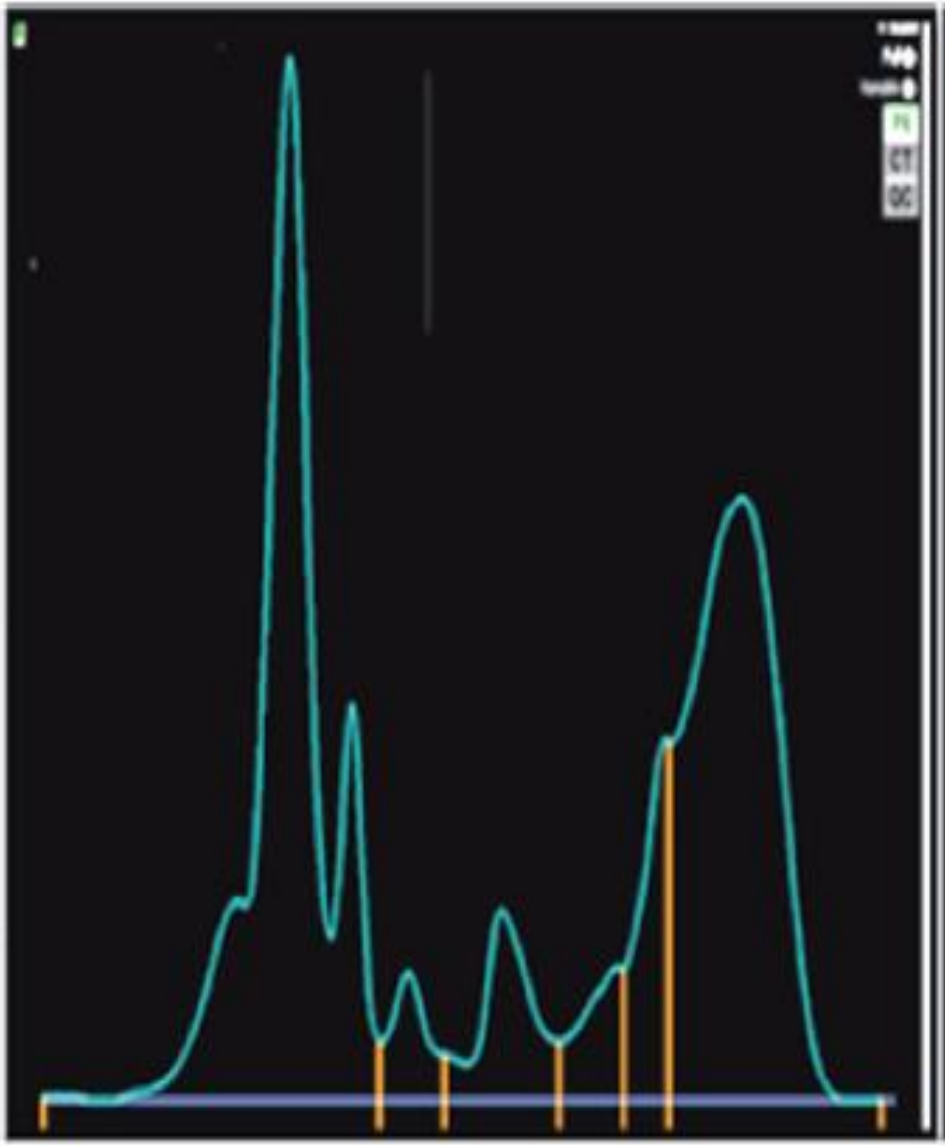
	Distribution %	Absolute amount, g/l
Albumin	55	40
α_1-Globulin	5	4
α_2-Globulin	10	7
β-Globulin	12	9
γ-Globulin	18	13
Total	100	73

Table 2. Changes in serum protein electrophoresis and associated conditions or disorders

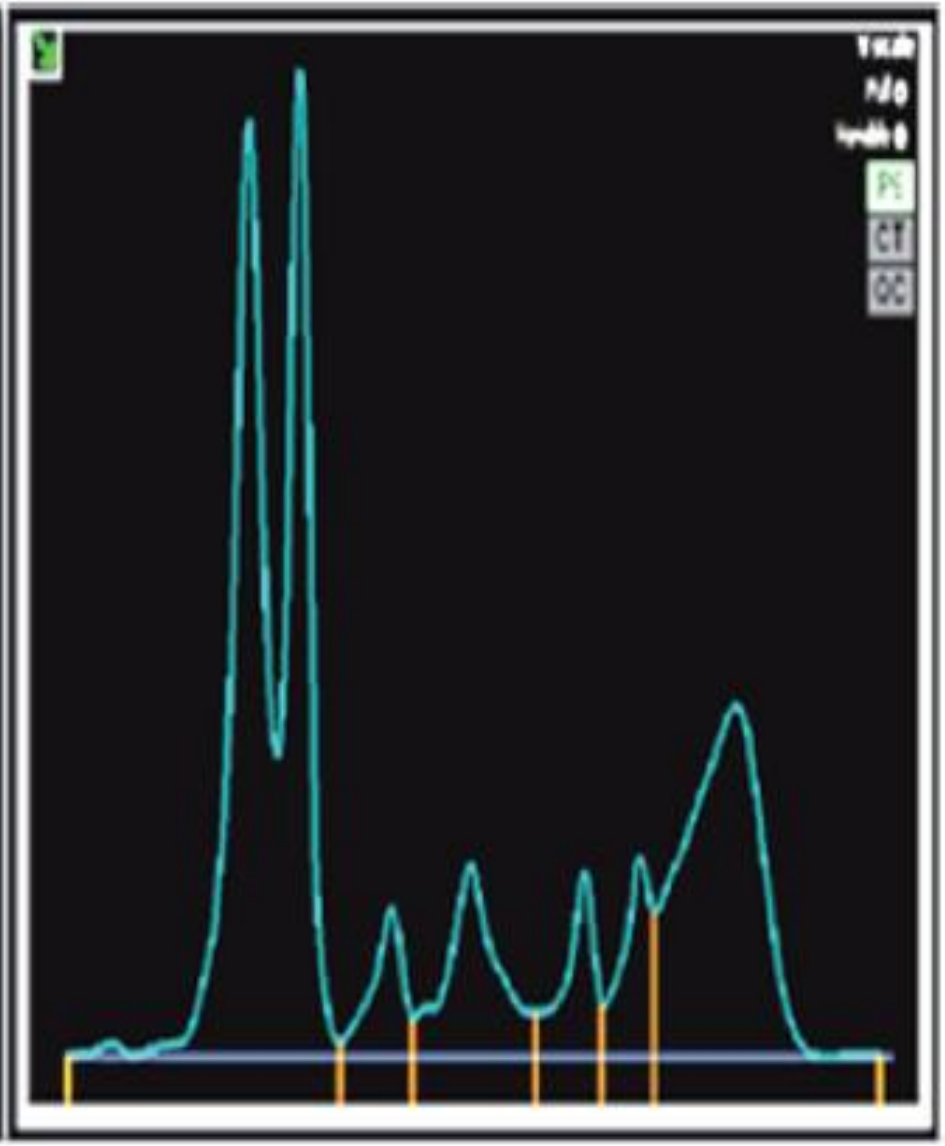
	Increase	Decrease
Albumin	Dehydration	Chronic cachectic or wasting disease Chronic infections Hemorrhage, burns Protein-losing enteropathies Impaired liver function Malnutrition Nephritic syndrome Pregnancy
α_1-Globulins	Pregnancy	α_1-Antitrypsin deficiency
α_2-Globulins	Adrenal insufficiency Adrenocorticosteroid therapy Advanced diabetes mellitus Nephritic syndrome	Malnutrition Megaloblastic anemia Protein-losing enteropathy Severe liver disease Wilson's disease
β-Globulins	Biliary cirrhosis Carcinoma (sometimes) Cushing's disease Diabetes mellitus (sometimes) Hypothyroidism Iron deficiency anemia Malignant hypertension Nephrosis Polyarteritis nodosa Obstructive jaundice Third-trimester pregnancy	Protein malnutrition
γ-Globulins	Amyloidosis Chronic infections (granulomatous disease) Chronic lymphocytic leukemia Cirrhosis Hodgkin's disease Malignant lymphoma Multiple myeloma Rheumatoid and collagen disease	Agammaglobulinemia Hypogammaglobulinemia

Albumin

- is the major fraction in a normal SPEP.
- **A fall of 30% is necessarv before the decrease shows on electrophoresis.**
- Usually a single band is seen.
- Heterozygous individuals may produce bisalbuminemia – two equally staining bands, the product of two genes.
- Some variants give rise to a **wide band or two bands of unequal intensity but none** of these variants is associated with disease.



Bilirubin interference (blunt cathodal peak adjacent to



Bisalbuminemia (bifid peaks in albumin region)

- Increased anodic mobility results from the binding of bilirubin, nonesterified fatty acids, penicillin and acetylsalicylic acid, and occasionally from tryptic digestion in acute pancreatitis.
- Absence of albumin, known as analbuminaemia, is rare.
- A decreased level of albumin, however, is common in many diseases, including liver disease, malnutrition, malabsorption, protein-losing nephropathy and enteropathy

Albumin – alpha-1 interzone

- Even staining in this zone is due to alpha-1 lipoprotein(HDL).
- Decrease occurs in severe inflammation, acute hepatitis, and cirrhosis.
- Also, nephrotic syndrome can lead to decrease in albumin level; due to its loss in the urine through a damaged leaky glomerulus.
-

- An increase appears in severe alcoholics and in women during pregnancy and in puberty.
- The high levels of AFP that may occur in hepatocellular carcinoma may result in a sharp band between the albumin and the alpha-1 zone

Alpha-1 zone

- Orosomucoid and antitrypsin migrate together but orosomucoid stains poorly so alpha 1 antitrypsin (AAT) constitutes most of the alpha-1 band.
- Alpha-1 antitrypsin has an SH group and thiol compounds may be bound to the protein altering their mobility.
- A decreased band is seen in the deficiency state.
- It is decreased in the nephrotic syndrome and absence could indicate possible alpha 1-antitrypsin deficiency.
- This eventually leads to emphysema from unregulated neutrophil elastase activity in the lung tissue.

- The alpha-1 fraction does not disappear in alpha 1-antitrypsin deficiency, however, because other proteins, including alpha-lipoprotein and orosomucoid, also migrate there.
- As a positive acute phase reactant, AAT is increased in acute inflammation.
- Bence Jones protein may bind to and retard the alpha-1 band

Alpha-1 – alpha-2 interzone

- Two faint bands may be seen representing alpha 1-antichymotrypsin and vitamin D binding protein.
- These bands fuse and intensify in early inflammation due to an increase in alpha 1-antichymotrypsin, an acute phase protein

Alpha-2 zone

- This zone consists principally of alpha-2 macroglobulin and haptoglobin.
- There are typically low levels in haemolytic anaemia (haptoglobin is a suicide molecule which binds with free haemoglobin released from red blood cells and these complexes are rapidly removed by phagocytes).
- Haptoglobin is raised as part of the acute phase response, resulting in a typical elevation in the alpha-2 zone during inflammation.
- **A normal alpha-2 and an elevated alpha-1 zone is a typical pattern in hepatic metastasis and cirrhosis.**

- Haptoglobin/haemaglobin complexes migrate more cathodally than haptoglobin as seen in the alpha-2 – beta interzone.
- **This is typically seen as a broadening of the alpha-2 zone.**
- Alpha-2 macroglobulin may be elevated in children and the elderly.
- This is seen as a sharp front to the alpha-2 band.
- **AMG is markedly raised (10-fold increase or greater) in association with glomerular protein loss, as in nephrotic syndrome.**

- Due to its large size, AMG cannot pass through glomeruli, while other lower-molecular weight proteins are lost.
- Enhanced synthesis of AMG accounts for its absolute increase in nephrotic syndrome.
- Increased AMG is also noted in rats with no albumin indicating that this is a response to low albumin rather than nephrotic syndrome itself..
- *AMG is mildly elevated early in the course of diabetic nephropathy*

Alpha-2 - beta interzone

- Cold insoluble globulin forms a band here which is not seen in plasma because it is precipitated by heparin.
- There are low levels in inflammation and high levels in pregnancy.
- **Beta lipoprotein forms an irregular crenated band in this zone.**
- High levels are seen in type II hypercholesterolaemia, hypertriglyceridemia, and in the nephrotic syndrome

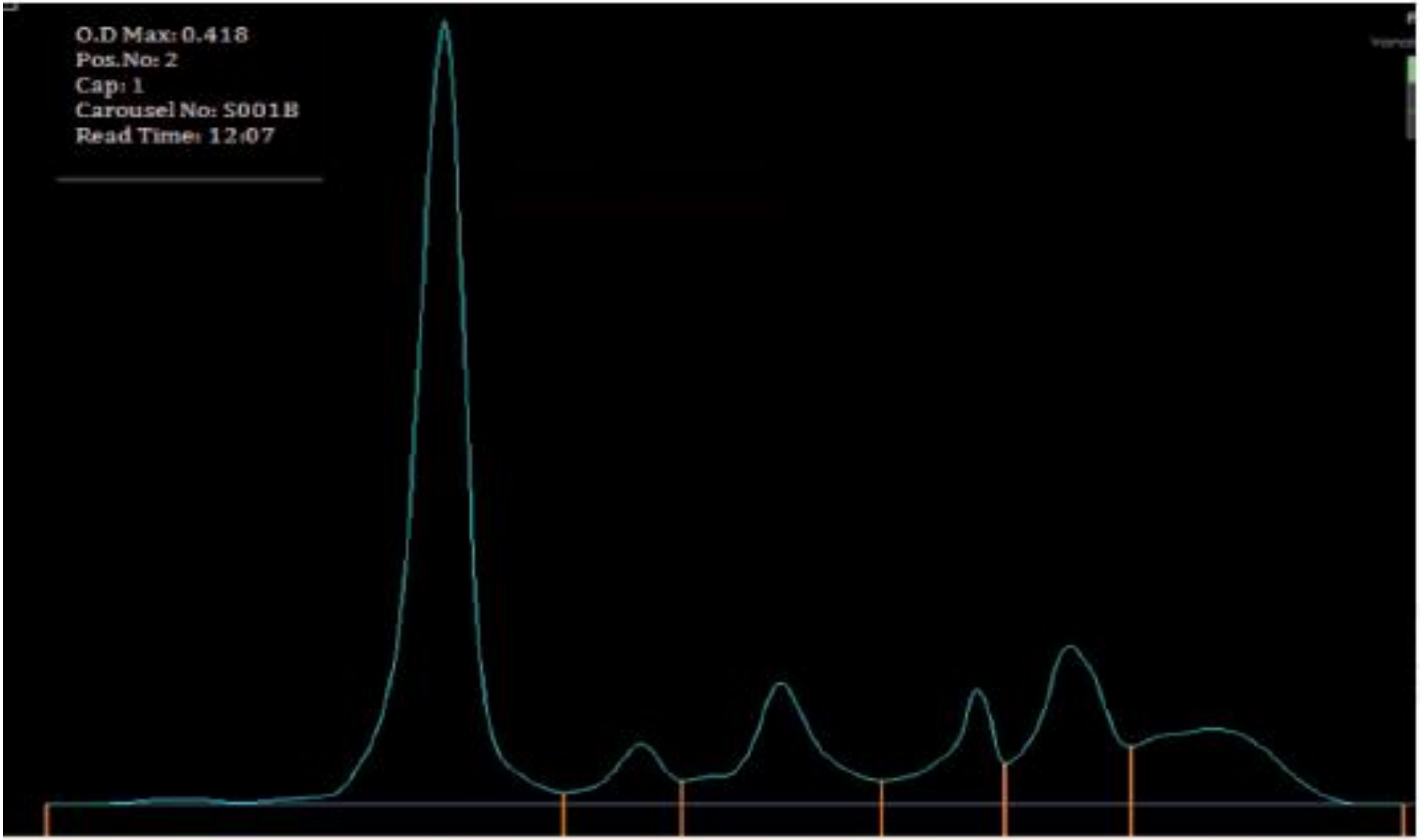
Beta-1 zone

- Transferrin and beta-lipoprotein (LDL) comprises the beta-1.
- **Increased beta-1 protein due to the increased level of free transferrin is typical of iron deficiency anemia, pregnancy, and oestrogen therapy.**
- Increased beta-1 protein due to LDL elevation occurs in hypercholesterolemia.
- Decreased beta-1 protein occurs in acute or chronic inflammation.

Beta-2 zone

- Beta-2 comprises C3 (complement protein 3).
- **It is raised in the acute phase response.**
- Depression of C3 occurs in autoimmune disorders as the complement system is activated and the C3 becomes bound to immune complexes and removed from serum.

- Fibrinogen, a beta-2 protein, is found in normal plasma but absent in normal serum.
- Occasionally, blood drawn from heparinized patients does not fully clot, resulting in a visible fibrinogen band between the beta and gamma globulins.



Fibrinogen producing a peak in beta 2 region(from a plasma sample)

Beta-gamma interzone

- **C-reactive protein is found in between the beta and gamma zones producing beta/gamma fusion.**
- IgA has the most anodal mobility and typically migrates in the region between the beta and gamma zones also causing a beta/gamma fusion in patients with cirrhosis, respiratory infection, skin disease, or rheumatoid arthritis (increased IgA).
- Fibrinogen from plasma samples will be seen in the beta gamma region.

Gamma zone

- The immunoglobulins or antibodies are generally the only proteins present in the normal gamma region.
- Of note, *any protein migrating in the gamma region will be stained and appear on the gel, which may include protein contaminants, artifacts, or certain medications.*
- Depending on whether an agarose or capillary method is used, interferences vary.
- Immunoglobulins consist of heavy chains (IgA, IgM, IgG, IgE and IgD) and light chains (kappa and lambda).

- A normal gamma zone should appear **as a smooth 'blush', or smear, with no asymmetry or sharp peaks**.
- The gamma globulins may be elevated (hypergammaglobulinemia), decreased (hypogammaglobulinaemia), or have an abnormal peak or peaks.
- *Note that immunoglobulins may also be found in other zones; IgA typically migrates in the beta-gamma zone, and in particular, pathogenic immunoglobulins may migrate anywhere, including the alpha regions.*

- Hypogammaglobulinaemia is easily identifiable as a "slump" or decrease in the gamma zone.
- **It is normal in infants.**
- It is found in patients with X-linked agammaglobulinemia.
- IgA deficiency occurs in 1:500 of the population, as is suggested by a pallor in the gamma zone.
- **Of note, hypogammaglobulinema may be seen in the context of MGUS or multiple myeloma.**

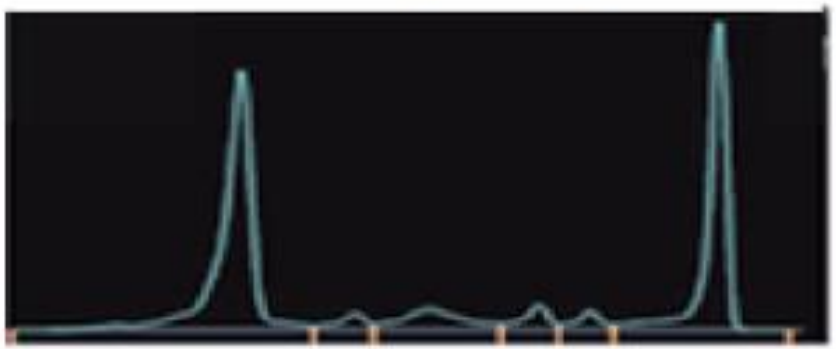
- If the gamma zone shows an increase the first step in interpretation is *to establish if the region is narrow or wide*.
- A broad "swell-like" manner (wide) indicates polyclonal immunoglobulin production.
- If it is elevated in an asymmetric manner or with one or more peaks or narrow "spikes" it could indicate clonal production of one or more immunoglobulins.

Polyclonal gammopathy

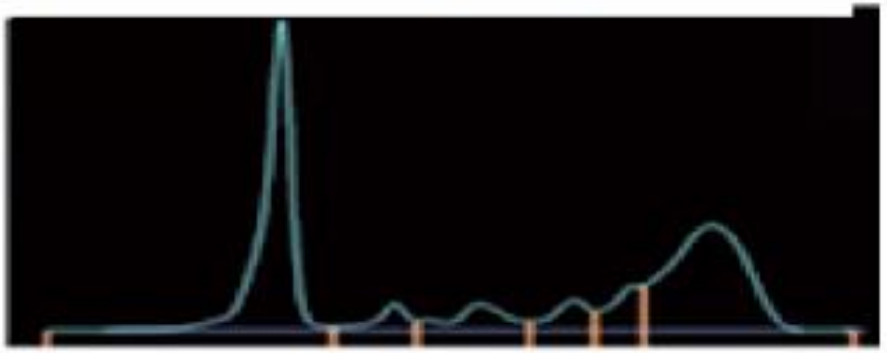
- Polyclonal gammopathy is indicated by a "swell-like" elevation in the gamma zone, which typically indicates a non-neoplastic condition (although is not exclusive to non-neoplastic conditions).
- ***The most common causes of polyclonal hypergammaglobulinaemia detected by electrophoresis are severe infection, chronic liver disease, rheumatoid arthritis, systemic lupus erythematosus and other connective tissue diseases.***

- **A narrow spike is suggestive of a monoclonal gammopathy, also known as a restricted band, or "M-spike".**
- To confirm that the restricted band is an immunoglobulin, follow up testing with immunofixation, or immunodisplacement/immunosubtraction (capillary methods) is performed.
-

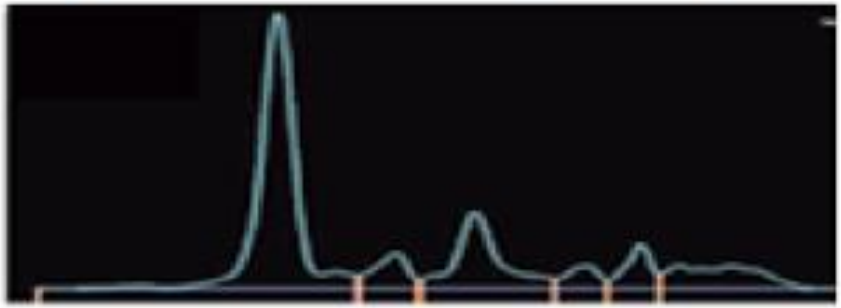
M band in gamma region



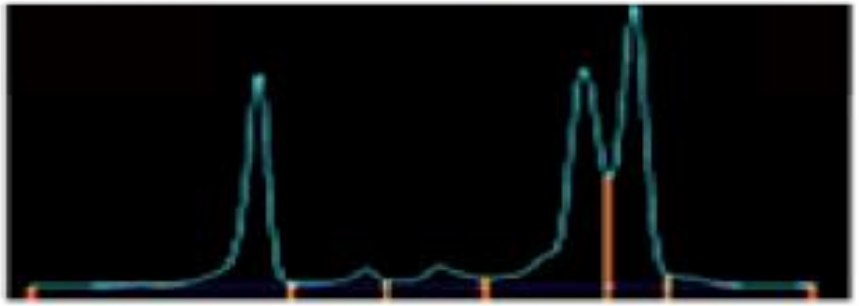
Polyclonal elevation of gamma globulins



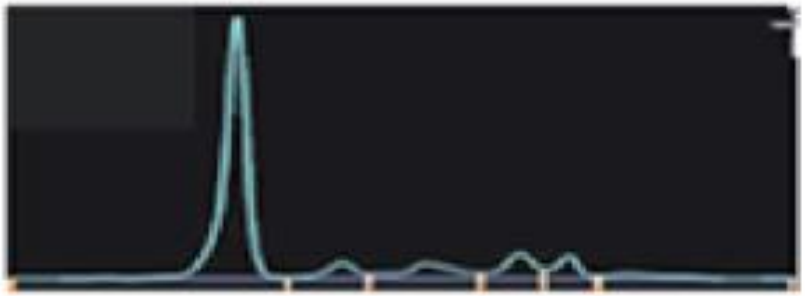
Oligoclonal elevation of gamma globulins



Biclonal elevation of gamma globulins



Hypogammaglobinemia



- Therapeutic monoclonal antibodies (mAb), also migrate in this region and may be misinterpreted as a monoclonal gammopathy.
- **They may also be identified by immunofixation or immunodisplacement/immunosubtraction as they are structurally comparable to human immunoglobulins**

- **The most common cause of a restricted band is an MGUS (monoclonal gammopathy of uncertain significance), which, although a necessary precursor, only rarely progresses to multiple myeloma. (On average, 1%/year.)**

- Typically, a monoclonal gammopathy is malignant or clonal in origin, Myeloma being the most common cause of IgA and IgG spikes.
- Chronic lymphatic leukaemia and lymphosarcoma are not uncommon and usually give rise to IgM paraproteins.

- Note that up to 8% of healthy geriatric patients may have a monoclonal spike.
- Waldenström's macroglobulinaemia (IgM), monoclonal gammopathy of undetermined significance (MGUS),
- amyloidosis,
- plasma cell leukemia and
- solitary plasmacytomas also produce an M-spike.

- Oligoclonal gammopathy is indicated by one or more discrete clones.
- Lysozyme may be seen as a band cathodal to gamma in myelomonocytic leukaemia in which it is released from tumour cells.

Serum Protein Fraction	Increased	Decreased
Albumin	Severe dehydration	Malnutrition, cachexia, liver disease, nephrotic syndrome, protein-losing enteropathies, severe burns
Alpha-1	Inflammatory states, pregnancy	Alpha-1 antitrypsin deficiency
Alpha-2	Inflammatory states, nephrotic syndrome, oral contraceptive use, steroid use, hyperthyroidism	Hemolysis, liver disease
Beta	Hyperlipidemia, iron-deficiency anemia	Hypo-B-lipoproteinemia, malnutrition
Gamma	Polyclonal and Monoclonal Gammopathies	Agammaglobulinemia, hypogammaglobulinemia

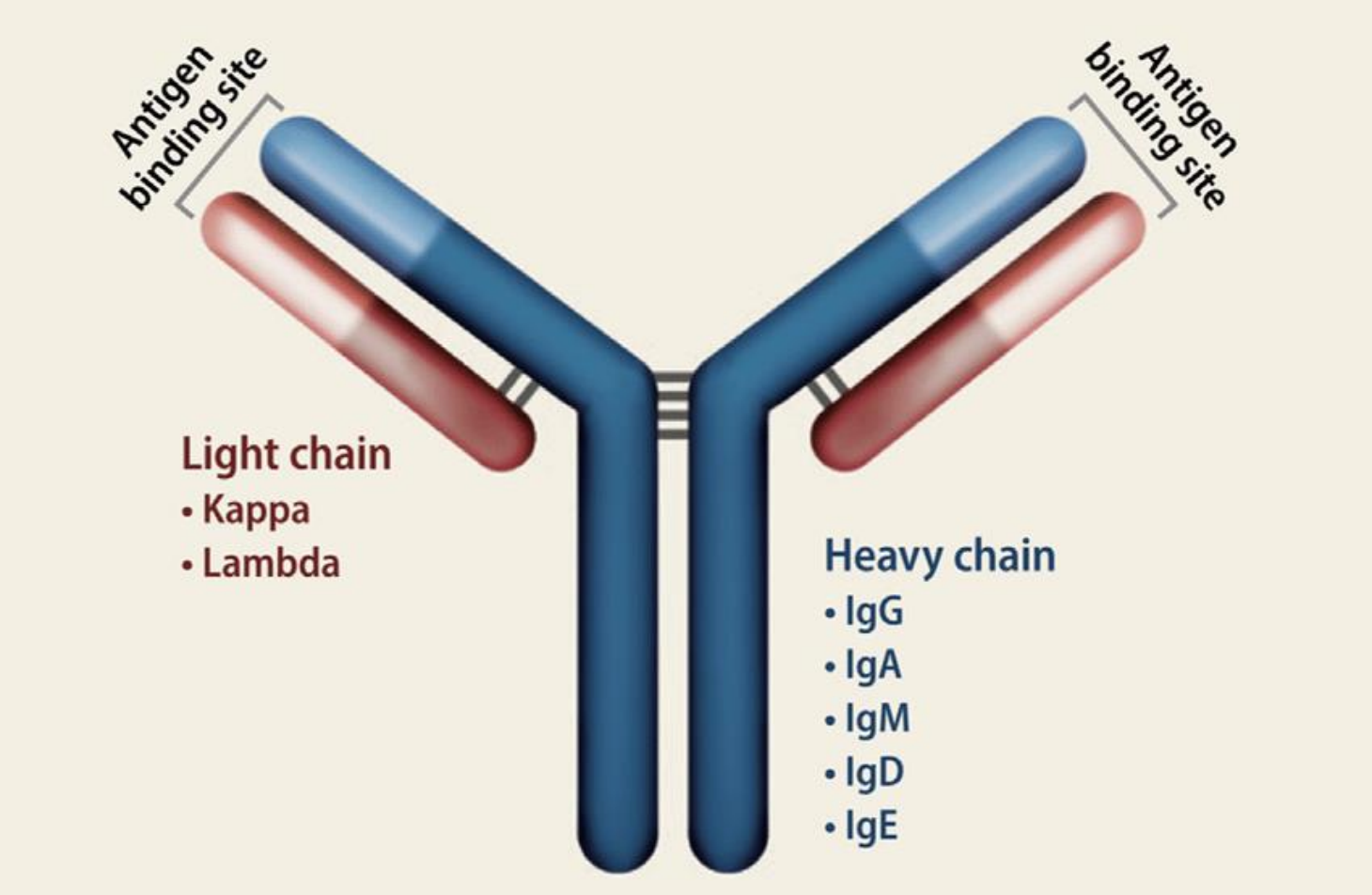
Diseases Associated with a Polyclonal Gammopathy

Condition	Examples
Liver disease	Cirrhosis, autoimmune or viral hepatitis
Connective tissue diseases	Rheumatoid arthritis, systemic lupus erythematosus, scleroderma, Sjogren syndrome
Infection	Bacterial: osteomyelitis, endocarditis, Viral: HIV/AIDS, hepatitis C, Epstein-Barr virus,?covid-19
Hematologic disorders/malignancies	Non-Hodgkin lymphoma, chronic lymphocytic leukemia, thalassemia, sickle cell anemia
Nonhematologic malignancies	Lung, ovarian, gastric malignancies, hepatocellular carcinoma

Free light chains (FLC)

- In the early 2000s, an assay aimed at evaluating serum free light chains (sFLCs) was made available and subsequently tested in different plasma cell disorders.
- Several reports have demonstrated the usefulness of the assay for the diagnosis and monitoring of
- oligosecretory MM,
- nonsecretory MM,
- Bence Jones MM, and
- amyloid light-chain amyloidosis

A reminder of Ig structure



- A normal range for κ and λ chain concentration in the serum of healthy individuals are ***3.3–19.4 mg/L and 5.7–26.6 mg/L for the κ and λ chains, respectively*** .
- Under normal conditions, about 500 mg/day FLCs are produced, with a **κ/λ ratio of about 2/1**.
- As λ LCs are dimeric, their renal clearance is slower compared with κ LCs , leading to a κ/λ ratio in the serum of about 0.58 (range 0.26–1.75).

- FLCs have a serum half-life of 2–6 h as they are rapidly cleared by the glomeruli and metabolized in the proximal tubules.
- When FLCs are produced in excess, the reabsorptive capacity of the tubules can be overwhelmed, thus leading to an accumulation of FLCs in the serum .

FLC ACCUMULATION IN DISEASES

- This can occur in a number of clinical conditions including
- inflammation,
- immunological disorders,
- renal failure, and
- plasma cell neoplasms, ***only in this latter case, due to the overproduction of a monoclonal LC, the κ/λ ratio is abnormal, thus rendering this parameter potentially useful for the diagnosis and monitoring of these hematological disorders.***

sFLC κ/λ ratio

- A prognostic role for an abnormal sFLC κ/λ ratio has been observed in the case of :
- monoclonal gammopathy of unknown significance,
- smoldering MM,
- solitary plasmacytomas, and in
- newly diagnosed symptomatic MM secreting intact monoclonal immunoglobulins.

Free light chains (FLC) Assay

- Immunoassay is used.
- A commonly encountered phenomenon with laboratory testing of FLC includes “prozone” effect or “hook” effect which occurs due to antigen excess and requires appropriate dilution to obtain reliable results.

- A few limitations in the assay have been identified, namely
- A slight to substantial and inter-instrument variability and
- The overestimation of LCs when tested in urine, so that ***urine FLC assay is not recommended for monitoring patients with monoclonal gammopathies***

Bence-Jones protein estimation is OLD

- Bence-Jones protein estimation in urine is an antique piece of laboratory evidence toward multiple myeloma, which is characterized by detection of light chains in urine.
- But since the methodology of testing is manual and does not provide standardization, ***this has been replaced by urine FLC analysis in laboratories practicing good clinical laboratory practices (GCLP).***

Quality assurance in SPE

- Quality assurance in SPE is an essential prerequisite to ensure reliability of an SPE result.
- There are two major aspects of analytical quality including precision (measure of precision) and accuracy (measure of trueness).

- Good clinical laboratory practices demand ***processing of an***
- ***internal quality control (IQC) for assessment of precision and***
- ***external quality assurance (EQA)/proficiency testing (PT testing) for accuracy assessment.***

- IQC is a material which can be prepared in house (patient sample) or available commercially and is to be processed before a patient sample is taken up for processing.

- The clinical laboratory has its responsibility to select and use an IQC which has a matrix comparable to patient sample, preferably covering the clinical decision point (cut off value that differentiates between a normal and abnormal result).

- EQA is an external assessment of the analytical quality wherein the ***laboratory processes a blinded sample and the results are compared against a reference method and/or against the consensus value of other participant laboratories for that specific sample.***

Australasian Association of Clinical Biochemists standardized format of reporting SPE.

- Reporting SPE requires interpretation of the electrophoretic pattern which is followed by comments of such an interpretation along with the piece of advice to the clinician if indicated.
- **There is a big lacuna in the format of reporting of SPE, each laboratorian using his/her own means of interpreting and communicating.**

- It is the need of the hour to have a standardized format of reporting SPE for ensuring patient safety and clinician follow-up.(AACB)
- There are no international guidelines, though the working party on standardized reporting of protein electrophoresis which is an initiative of the Australasian Association of Clinical Biochemists(AACB) has come out with a standardized format of reporting SPE.

Table 3 General interpretive commenting recommendations: all specimens

Pattern	Minimal information to be provided in the interpretive comment
Normal pattern	Normal pattern. Paraprotein not detected
Normal pattern (and clinical context suggests suspicion of plasma cell dyscrasia)	Normal pattern. Paraprotein not detected. Suggest urine protein electrophoresis and immunofixation, and/or serum free light chains if clinically indicated (if not already done/ordered)
Decreased alpha-1 globulins	Decreased alpha-1 globulins. Suggest alpha-1 antitrypsin quatitation and genotyping/ phenotyping if clinically indicated
Decreased albumin and increased alpha-2 and beta globulins	Pattern is consistent with nephrotic syndrome (if corroborated by serum lipid results)
Increased alpha-1 and alpha-2 and/or gammaglobulins	Pattern is consistent with an acute inflammatory process
Increased beta-1 globulin (if IFE performed and paraprotein excluded)	Paraprotein not detected. If indicated, suggest iron studies
Polyclonal hypergammaglobulinaemia	A polyclonal increase in immunoglobulins is present
Polyclonal hypergammaglobulinaemia and acute phase pattern	Pattern is consistent with a chronic inflammatory process
Beta–gamma bridging	Beta–gamma bridging is present due to raised IgA or sometimes IgM. Causes may include cirrhosis, mucosal or cutaneous inflammation
Hypogammaglobulinaemia (first presentation)	Hypogammaglobulinaemia is present. Suggest serum immunofixation and urine protein electrophoresis including immunofixation (or serum free light chains) together with quantitation of total serum immunoglobulins (if not already done/ordered)
Hypogammaglobulinaemia (subsequent presentation)	Hypogammaglobulinaemia is present
Fibrinogen present	Fibrinogen present. Please send repeat serum specimen. (No clinical comment is required if laboratory can run a repeat serum specimen, otherwise needs IFE to ensure small band is fibrinogen and there is no underlying paraprotein; optimally needs repeat serum specimen as a small paraprotein cannot be quantitated by agarose gel SPEP when masked by the presence of fibrinogen)

Table 4 General interpretive commenting recommendations: specimens with a paraprotein and/or small abnormal band

Pattern	Minimal information to be provided in the interpretive comment*
First detection of a paraprotein	Suggest total serum immunoglobulins and urine protein electrophoresis and immunofixation (if not already done/ordered) [Typing and numerical quantitation, e.g. 'An IgG kappa paraprotein was detected in the gamma region']
Follow-up of a known paraprotein which is still present	Nil required [A comment should be made on the original band and its current status, e.g. 'The previously reported IgG kappa paraprotein was detected']
Paraprotein detected only by immunofixation electrophoresis	The previously reported IgG kappa paraprotein is now only visible by immunofixation
If paraprotein has disappeared	A comment is required to confirm the absence of the previously detected paraprotein, e.g. 'The previously reported IgG kappa paraprotein was not detected by immunofixation'
New, small abnormal band with different electrophoretic mobility from the original paraprotein in a patient with a known paraprotein	There is a small (type: e.g. IgG kappa) band approximately (amount: e.g. 1 g/L) on a background of a polyclonal and/or oligoclonal pattern. This band is different from the original paraprotein. Its clinical significance is uncertain
First presentation of small abnormal band (and no known paraprotein)	There is a small (type: e.g. IgG kappa) band approximately (amount: e.g. 1 g/L). Its clinical significance is uncertain. Suggest urine protein electrophoresis and immunofixation (or serum free light chains) and repeat serum protein electrophoresis in 3–6 months if clinically indicated
First presentation of small abnormal bands in polyclonal/oligoclonal background (and no known paraprotein)	There is a small (type: e.g. IgG kappa) band approximately (amount: e.g. 1 g/L) on a background of a polyclonal and/or oligoclonal pattern. Its clinical significance is uncertain but may reflect an inflammatory/reactive process. Suggest urine protein electrophoresis and immunofixation (or serum free light chains) and repeat serum protein electrophoresis in 3–6 months if clinically indicated

*Comments in square brackets refer to reporting formats where the quantitation field label does not specify the paraprotein type (e.g. see worked example 1A compared with 1B shown in the Appendix)

Recommendations for detection system for protein electrophoresis:

- **Gel-based methods and CZE are suitable methods for protein electrophoresis;**
- The electrophoretic system ***should be of high resolution and be able to detect small monoclonal bands that may co-migrate with normal proteins particularly in the beta region. Low-resolution electrophoresis on cellulose acetate is not suitable for protein electrophoresis;***
- **Clinicians should be encouraged to monitor the paraprotein concentration in individual patients using the same method** (used by the same laboratory or laboratory network), hence ensuring analysts have access to the cumulative reports of the paraprotein delineation on the densitometric/CZE scan;
- **Specimens requiring IEF should be referred to a reference laboratory.**

Recommendations for serum protein and albumin quantification:

- ***To facilitate the early recognition of a paraprotein disorder, it is recommended that both albumin and total protein measurements together with calculation of globulin are performed for older adult patients in response to a request for routine liver function tests;***
- **Total protein and albumin quantification as determined by an automated analyser be available for assessment of the protein electrophoresis;**
- **Serum albumin quantification by BCP or CZE is preferable to quantification by BCG although all are acceptable;**
- **Providing the same albumin result on the SPEP report as on the General Chemistry report is preferable but may not be possible depending upon the available Laboratory Information System;**
- **Whatever albumin value is used, the reporting of albumin on an SPEP report requires a consistent approach;**
- Laboratories should investigate major discrepancies between the chemical and electrophoretic albumin quantifications;
- Total protein and albumin should be quantified in g/L to the nearest whole number.

Recommendations for quantitative reporting of SPEP fractions:

- The minimal quantitative fields to be reported are total protein, albumin and, if present, the paraprotein(s);
- The quantitative reporting of all SPEP fractions is optional;
- Protein fractions should be quantified in g/L to the nearest whole number;
- Laboratories should determine their own reference intervals or validate published reference intervals;
- In view of the less common occurrence of plasma cell dyscrasias with multiple paraproteins, up to three quantitative fields should be available for reporting abnormal bands;
- Paraprotein(s) should be consistently reported in the same quantitative field to facilitate long-term cumulative review of the progress of a patient's disease and avoid misinterpretation of results.

Recommendations for urine paraprotein separation and quantification:

- **First voided urine is suitable for screening UPEP;**
- **A 24-h urine specimen is preferred for staging and monitoring of the plasma cell dyscrasias, although first voided specimens are acceptable if a 24-h specimen is not available or practical;**
- **Laboratories should be able to detect BJP at a level of 10 mg/L with levels <10 mg/L reported as 'trace';**
- **As well as reporting the urine total protein, it is recommended that there be an indication as to whether the urine specimen has glomerular and/or tubular proteinuria, and a comment as to whether BJP is detected or not. Any intact monoclonal immunoglobulin should also be quantified and reported;**
- **Creatinine should be performed on first voided urine specimen and the serum**

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