

Understanding **Protein Electrophoresis**

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Introduction

You received this booklet to learn more about a type of laboratory test called Protein Electrophoresis (PEP). After reading this booklet, you should be able to answer the following questions:

- What is protein electrophoresis?
- Why are the values of this test important for someone with multiple myeloma?
- How does this test help with diagnosis and monitoring response to treatment of multiple myeloma?

This booklet is intended to provide you with general information only. It is not meant to replace the advice of your doctor or nurse who can answer questions related to your specific treatment plan. The definitions of all words in **bold** print are found in the glossary at the end of the booklet.

Why is Protein Electrophoresis Important?

Why have a booklet about protein **electrophoresis**? The main reason is that production of a single, **monoclonal** protein (**M-protein**) is a characteristic feature of multiple myeloma. The test used to measure the amount of monoclonal protein in the blood or urine is protein electrophoresis.

This M-protein is manufactured (synthesized) by malignant plasma cells (or myeloma

cells). The amount of protein produced and released into the serum (the liquid part of the blood that is left after the blood cells are removed), and sometimes into the urine, reflects the amount of myeloma present in the body at any given time. This protein, which is released into the serum or urine, is called a serum or urine **tumor marker**.

Only a very few cancers have this type of a marker which, in this case, makes it possible to assess the amount of myeloma at the time of initial diagnosis and track the amount of myeloma throughout the course of the disease. One can look at **response** to treatment, depth of **remission**, and, if necessary, the patient's **relapse** using exact numbers, which is a unique advantage. For example, we can determine if a response is: Partial (PR)=50% reduction of M-protein; very good partial (VGPR)=90% reduction of M-protein; or complete (CR)=no protein detected. We can also identify a $\geq 25\%$ increase in protein level, which we call relapse.

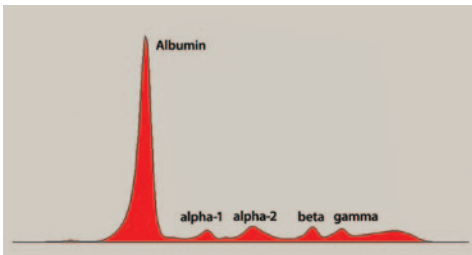


Figure 1. Representation of a normal Serum Protein Electrophoresis result

Measurement of M-protein with Electrophoresis

If the M-protein, which is characteristic of myeloma, is present in serum, the electrophoresis test for it is called **Serum Protein ElectroPhoresis (SPEP)**. Likewise, when monoclonal protein is found in urine, the test for it is called **Urine Protein ElectroPhoresis (UPEP)**.

To measure the amount of the monoclonal or M-protein one needs two pieces of information:

- What is the total amount of protein in the serum or urine?
- What percentage of the total is the M-protein?

The crucial information comes from SPEP and/or UPEP. By calculating the size of the "spike", which depicts the amount of monoclonal protein (done by measuring the area between the top of the spike and the baseline of the graph), one gets the percentage of the total protein that represents the M-protein. For example:

- Spike is 60% of total protein
- Total protein = 12 grams/deciliter (g/dL)
- Spike level = 7.2 g/dL (60% of 12)

Both the total protein and percentage of monoclonal protein change over time. With response to treatment, the spike can drop to 40%, and total protein to 9 g/dL, for example. This gives a spike level of 3.6 g/dL, which is a 50% drop in M-protein, or a partial response.



This type of serial measurement and assessment is key to all myeloma disease monitoring. This is why SPEP and UPEP are so important.

An additional test, called **immunofixation**, is used to determine the type of M-protein. This is important at the time of baseline diagnosis and at the point of maximum response with treatment. If there is a complete response (CR), the immunofixation test is completely negative*; that is, no monoclonal protein can be identified.

What Is Monoclonal Protein?

Monoclonal proteins are **immunoglobulin** (abbreviated as “Ig”) molecules or parts

* How response is defined has been addressed by the IMF’s International Myeloma Working Group (IMWG) and their publication of Uniform Response Criteria. Please refer to the IMF publication Concise Review of the Disease and Treatment Options, Table 7, for further information.

of an immunoglobulin molecule. Normal **plasma cells** produce immunoglobulins, which are the **antibodies** necessary to fight infection. The abnormal plasma cells – “myeloma cells” – present in myeloma patients do not produce antibodies in response to infection. Instead, they produce a monoclonal immunoglobulin molecule that cannot function as an antibody. This immunoglobulin can be comprised of: two heavy chains and two light chains (see Figure 2); light chains only; or fragments/combinations of this immunoglobulin molecule that are unique to each particular myeloma patient.

Immunoglobulins can be formed from one of five possible types of heavy chains (referred to as **IgG**, **IgA**, **IgM**, **IgD** and **IgE**) and two types of light chains (kappa, κ , and lambda, λ). Therefore, there are ten possible combinations of heavy and light chains: IgG kappa, IgG lambda, IgA kappa, IgA lambda, IgM kappa, and so on. Protein electrophoresis is able to detect all of them.

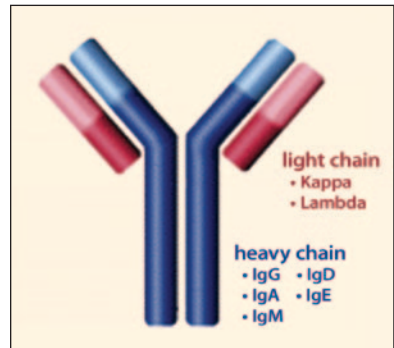


Figure 2. Immunoglobulin structure

Plasma cells produce heavy chains and light chains separately, and these chains are later assembled to form an intact immunoglobulin. For some reason, plasma cells usually produce a higher number of light chains than of heavy chains. Thus, once the intact immunoglobulins are formed, there still will be some light chains remaining. These light chains are called “**free light chains**” (the light chains that participate in the formation of intact immunoglobulins are called “bound light chains,” because they are bound to heavy chains).

The excess of free light chains enters the bloodstream and is subsequently filtered by the kidneys. The kidneys are then able to resorb these free light chains and recycle the amino acids (building blocks that make up all proteins in the body). But when a monoclonal protein is present in the serum, the amount of monoclonal free light chains can become too high for the kidneys to resorb. In this case, monoclonal free light chains are filtered through the kidneys, and those that are not resorbed can be found in the urine; they are then called **Bence Jones protein** (named after the physician who first described them).



What Is Protein Electrophoresis?

Serum Protein Electrophoresis

Serum contains a variety of different proteins that will be separated by electrophoresis into five or six fractions (according to the method used by the laboratory). These fractions (also referred to as “zones” or “regions”) are called Albumin, Alpha 1, Alpha 2, Beta (which can be separated into Beta 1 and Beta 2), and Gamma. Polyclonal (normal) immunoglobulins are found mostly in the Gamma zone. Hence, they are known as gamma globulins.

The diverse, normal immunoglobulins present in serum differ slightly from each other in their structure and electrical charge. For that reason, when they are subjected to electrophoresis, they form a large zone, which is spread out and symmetrical, without any visible deformation.

Monoclonal proteins are produced by one clone of plasma cells, and thus all the molecules are identical and have the same electrical charge. That is why on electrophoresis a monoclonal protein will migrate as a narrow spike (this spike appears most often in the gamma zone, but sometimes can be present in Beta 2 or Beta 1, or even the Alpha 2 zone, although the latter is very rare).

Serum electrophoresis can be used to search for a monoclonal protein as well as to monitor the amount of monoclonal protein.

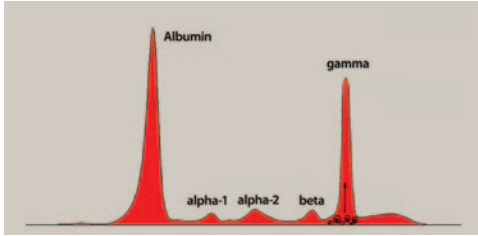


Figure 2. Representation of an abnormal Serum Protein Electrophoresis result, with myeloma cells producing the M-protein, creating an M-spike in the gamma zone.

Urine Protein Electrophoresis

The kidney acts as a filter, eliminating only a few molecules and leaving most of the proteins in the bloodstream. Although some small proteins do pass through the kidney filter, they are later resorbed and recycled into amino acids. Thus, normally urine contains only traces of proteins.

When monoclonal protein is present in serum, often the excess of free light chains will be found in the urine as Bence Jones protein (it will look like a narrow spike, usually in the Gamma or Beta zone).

Urine electrophoresis is used to search for Bence Jones protein and to monitor its concentration. It can also help to assess kidney damage (which is a common complication of multiple myeloma).

Serum and Urine Immunofixation

Once a narrow spike of protein is detected by protein electrophoresis, the presence of

monoclonal protein may be suspected. It is necessary then to confirm its presence and to determine its type by identifying which types of heavy chains and light chains are involved in its structure. Knowing the type of M-protein is important in establishing a diagnosis and in monitoring the patient.

To do this, another method of electrophoresis, called immunofixation (or IFE, for ImmunoFixation Electrophoresis), will be used. Immunofixation methods are more sensitive to the presence of faint monoclonal proteins and may detect them even if electrophoresis does not show any visible



abnormality. But immunofixation does not determine how much M-protein is present. Therefore, both methods are used together: electrophoresis to detect the monoclonal protein and to quantify it, and immunofixation to identify its type.

How is Monoclonal Protein Detected and Measured?

As with other tests, the values calculated by electrophoresis are then compared to the laboratory's reference values (values that a laboratory has determined by analyzing samples coming from healthy people). Electrophoresis fractions are also analyzed



visually. A monoclonal protein is suspected when the electrophoresis pattern exhibits an additional spike, or when a normal fraction presents an abnormal shape or is unusually increased. These abnormalities are usually detected in the Gamma or Beta regions.

To confirm the presence of the monoclonal protein and to identify its heavy and light chains, the serum is further analyzed by immunofixation. Once the presence of a monoclonal protein is confirmed, its amount will be quantified using the electrophoresis curve. To do this, the laboratory will mark the beginning and the end of the monoclonal peak on the curve, and calculate what percentage of the total area under the curve the peak occupies. Using this value and the results of another biochemical test, total protein concentration, the amount of monoclonal protein in g/dL can be calculated. (See the example on p. 7.)

This value will be used by your doctor, in conjunction with other data, to establish a diagnosis. Later, it will be compared with the values obtained by subsequent electrophoresis results to monitor the evolution of the disease and the response to treatment, as recommended by International Myeloma Working Group guidelines.

According to these guidelines, when a monoclonal protein can be detected and measured, by electrophoresis, monitoring should be performed using serum and/or urine electrophoresis. When the monoclonal

protein is undetectable by electrophoresis or is too small to be measured your doctor will use another test, the free light chain assay, to monitor your condition.*

How Can Protein Electrophoresis Help with Treatment Decisions?

As previously mentioned, the ability to precisely measure the amount of monoclonal protein by SPEP and/or UPEP and to know the exact level of the myeloma tumor burden is a huge advantage. Disease monitoring in the absence of a spike on SPEP or UPEP can be quite difficult.

The level of M-component on SPEP and UPEP reflects the amount of myeloma present. However, it is very important to realize that each patient is different. At the time of diagnosis, some patients have, for example, a very high spike in the serum but not such a high level of myeloma in the bone marrow or bones. And the opposite is also true: some patients can have a low spike, but a lot of myeloma cells. Thus, at diagnosis, it is very important to correlate the spike level with the amount of myeloma in an individual patient. If the spike is low, this can be especially important, since small changes can be more

important in terms of response to treatment as well as potential progression or relapse.

The ways in which SPEP and UPEP help are:

1. Baseline assessment of the amount and type of the myeloma.
2. Serial monitoring to document the speed and level of response.
3. Assessment of possible disease progression or relapse.

*Note: Progressive Disease (PD) = $\geq 25\%$ increase** (from low point). PD can also be confirmed by occurrence of any one of the “CRAB” features***.*

Practical Recommendations for use of SPEP and UPEP

When new myeloma treatment is started, the M-protein level in blood (serum) and/or urine should be measured each month (or each cycle of treatment, normally at 3–6 week intervals) using SPEP and/or UPEP. Regular testing is required to assess the impact of treatment. If the treatment is not working well, this will be evident within 2–3 months: the SPEP/UPEP M-protein levels would drop by $< 25\%$ or increase by $\geq 25\%$ (progressive disease [PD]). At this point a change in treatment may be required. This is an important

* For further guidance on disease monitoring, refer to the following IMWG publications. All are available at myeloma.org. IMWG Uniform Response Criteria, IMWG Guidelines for Serum Free Light Chain Analysis (update in progress), and IMWG Guidelines for the Diagnostic Work-up of Myeloma. Additionally, refer to the 2011/2012 edition of the IMF publication Concise Review of the Disease and Treatment Options.

**Two consecutive readings are required to confirm progression or relapse.

***See other IMF publications and the IMF web site at myeloma.org, or call the IMF hotline at 800-452-2873 (in the U.S. and Canada) for an explanation of “CRAB” features and additional details.

time to discuss the results and treatment options with the doctor.

If the treatment is working well, frequently there will have been substantial improvement (reduction) in the M-protein levels on SPEP/UPEP within the first 2–3 months. As noted above, the level of response can be quantified as $\geq 50\%$ (partial response [PR]); $\geq 90\%$ (very good partial response [VGPR]); 100% (complete response [CR]). The classification of CR also requires that no M-protein is detectable using immunofixation (IFE) and that a bone marrow test shows no evidence of myeloma. Achieving the higher levels of response such as VGPR and CR will typically take at least another 2–3 months of treatment (total of 4–6 months at that point). Several additional months of therapy may be required to achieve maximum response.

Further decisions are required with respect to additional treatment options, such as an autologous stem cell transplant (ASCT) or consolidation therapy to maximally improve the response. Thereafter, there can be a decision to begin maintenance therapy, and this is best discussed with your physician.

Once a stable level of response is reached, the frequency of monitoring can be reduced to every 2–3 months, which can continue on an ongoing basis unless new issues emerge. It is most helpful if direct discussions with your doctor occur about the SPEP/UPEP results as they become available. The IMF offers the free *Myeloma Manager*[™]

computer program, which enables one to create tables, graphs, and flow charts of test results over time. This facilitates patient-doctor discussions and planning.

Will Insurance Cover the Cost of Protein Electrophoresis?

Protein electrophoresis is a standard, accepted test which is normally covered by Medicare and most insurance programs.

However, like all testing, it may be that insurance covers only part (for example, 80%) of the cost. It is important to be aware of what patient cost is incurred with each test.



If cost is an issue, discuss the urgency and/or needed frequency of testing with your treating physician. If the myeloma is stable, less frequent testing may be acceptable.

Additional Questions

We suggest the discussion of the following questions with your doctor to provide a better understanding of your electrophoresis results:

- What are the normal ranges for immunoglobulin levels on SPEP at this lab, and how do my results compare?
- How much variation in monthly SPEP and/or UPEP results is considered within normal limits? At what point is an increase or decrease considered significant?
- If there is no detectable monoclonal protein by electrophoresis, does that always mean I am in complete response?
- Is it necessary to do both serum and urine electrophoresis?
- Should patients with MGUS be monitored with serum and urine electrophoresis? If so, how often?

About the IMF

*“One person can make a difference,
Two can make a miracle.”*

Brian D. Novis
IMF Founder

Myeloma is a little-known, complex, and often misdiagnosed bone marrow cancer that attacks and destroys bone. Myeloma affects approximately 75,000 to 100,000 people in the United States, with approximately 20,000 new cases diagnosed each year. Although there is presently no known cure for myeloma, doctors have many approaches to help myeloma patients live better and longer.

The International Myeloma Foundation (IMF) was founded in 1990 by Brian and Susie Novis shortly after Brian’s myeloma diagnosis at the age of 33. It was Brian’s dream that future patients would have easy access to medical information and emotional support throughout their battle with myeloma. He established the IMF with the 3 goals of treatment, education, and research. He sought to provide a broad spectrum of services for patients, their families, friends, and health care providers. Although Brian died 4 years after his initial diagnosis, his dream didn’t. Today, the IMF reaches out to an international membership of more than 195,000. The IMF was the first organization dedicated solely to myeloma, and today it remains the largest.

The IMF provides programs and services to aid in the research, diagnosis, treatment, and management of myeloma. The IMF ensures that no one must brave the myeloma battle alone.

We care for patients today, while working toward tomorrow's cure.

How Can the IMF Help?

PATIENT EDUCATION

INFORMATION PACKAGE

Our free IMF InfoPack provides comprehensive information about myeloma, treatment options, disease management, and IMF services. It includes our acclaimed *Patient Handbook*.

INTERNET ACCESS

Log on to myeloma.org for 24-hour access to information about myeloma, the IMF, education, and support programs.

ONLINE MYELOMA FORUM

Join the IMF Internet Discussion Group at www.myeloma.org/listserve.html to share your thoughts and experiences.

MYELOMA MINUTE

Subscribe to this free weekly email newsletter for up-to-the-minute information about myeloma.

PATIENT & FAMILY SEMINARS

Meet with leading experts in myeloma treatment to learn more about recent advances in therapy and research.

MYELOMA MATRIX

On our website and in print, this document is a comprehensive guide to drugs in development for myeloma.

MYELOMA TODAY NEWSLETTER

Our quarterly newsletter is available free of charge by subscription.

SUPPORT

MYELOMA HOTLINE: 800-452-CURE (2873)

Toll-free throughout the United States and Canada, the IMF Hotline is staffed by trained information specialists and is in frequent interaction with members of our Scientific Advisory Board.

SUPPORT GROUPS

A worldwide network of more than 100 myeloma support groups holds regular meetings for members of the myeloma community. The IMF conducts annual retreats for myeloma support group leaders.

RESEARCH

BANK ON A CURE®

This DNA bank will provide genetic data for research in new drug development.

INTERNATIONAL MYELOMA WORKING GROUP (IMWG)

IMF's International Myeloma Working Group consists of 150 leading myeloma researchers from around the world who collaborate on a broad range of myeloma research projects. With a goal to improve myeloma treatment options and diagnostic systems, their work focuses on protocols to provide a more durable remission for myeloma patients while improving quality of life, addressing the needs of both myeloma patients and the physicians who treat them.

THE INTERNATIONAL STAGING SYSTEM (ISS)

This updated staging system for myeloma will enhance physicians' ability to select the most appropriate treatment for each patient.

RESEARCH GRANTS

Leading the world in collaborative research and achieving extraordinary results, the IMF Grant Program supports both junior and senior researchers working on a broad spectrum of projects. The IMF has attracted many young investigators into the field of myeloma who remain in the field and actively pursue a cure for the disease.

Glossary

Antibody: A protein produced by certain white blood cells (plasma cells) to fight infection and disease in the form of antigens such as bacteria, viruses, toxins, or tumors. Each antibody can bind only to a specific antigen. The purpose of this binding is to help destroy the antigen.

Antibodies can work in several ways, depending on the nature of the antigen. Some antibodies disable antigens directly. Others make the antigen more vulnerable to destruction by other white blood cells.

Bence Jones protein: A myeloma monoclonal protein present in urine. The amount of Bence Jones protein is expressed in terms of grams per 24 hours. Normally a very small amount of protein (<0.1 g/24 h) can be present in the urine, but this is albumin rather than Bence Jones protein.

The presence of any Bence Jones protein is abnormal.

CRAB: **C** = elevated Calcium, **R**= Renal (kidney) dysfunction, **A** = Anemia, **B** = Bone disease

Electrophoresis: A laboratory test in which a patient's serum (blood) or urine molecules are subjected to separation according to their size and electrical charge. For myeloma patients, electrophoresis of the blood or urine allows both the calculation of the amount of myeloma protein (M-protein) as well as the identification of the specific M-spike characteristic for each patient. Electrophoresis is used as a tool both for diagnosis and for monitoring.

Free light chains: A portion of the monoclonal protein of light molecular weight that can be measured in a sensitive assay, the Freelite® test.

IgG, IgA: The two most common types of myeloma. The G and the A refer to the type of protein produced by the myeloma cells. The myeloma protein, which is an immunoglobulin, consists of two heavy chains,

(for example, a G type) combined with two light chains, which are either kappa or lambda. Therefore, the two most common subtypes of myeloma have identical heavy chains (i.e. IgG kappa and IgG lambda). The terms heavy and light refer to the size or molecular weight of the protein, with the heavy chains being larger than the light chains.

Since the light chains are smaller, they are more likely to leak out into the urine, resulting in urine Bence Jones protein.

IgD, IgE: Two types of myeloma that occur less frequently.

IgM: Usually associated with Waldenström's macroglobulemia. In rare cases it can be a type of myeloma.

Immunofixation: An immunologic test of the serum or urine used to identify proteins in the blood. For myeloma patients, it enables the doctor to identify the M-protein type (usually IgG, IgA, kappa, or lambda). The most sensitive routine immunostaining technique, it identifies the exact heavy and light chain type of M-protein.

Immunoglobulin (Ig): A protein produced by plasma cells; an essential part of the body's immune system. Immunoglobulins attach to foreign substances which have entered the body (antigens such as bacteria, viruses, fungi) and assist in destroying them. The classes of immunoglobulins are IgA, IgG, IgM, IgD, and IgE.

Monoclonal: A clone or duplicate of a single cell. Myeloma develops from a single malignant plasma cell (monoclonone). The type of myeloma protein produced is also monoclonal; a single form rather than many forms (polyclonal). The important practical aspect of a monoclonal protein is that it shows up as a sharp spike (M spike) in the serum electrophoresis test.

M-protein (M-spike): Antibodies or parts of antibodies found in unusually large amounts in the blood or urine of multiple myeloma patients. M-spike refers to the sharp pattern that occurs on protein electrophoresis when an M-protein is present. Synonymous with monoclonal protein and myeloma protein. (See *monoclonal* above.)

Plasma cells: Special white blood cells that produce antibodies. The plasma cell is the malignant cell in myeloma. Normal plasma cells produce antibodies to fight infection. In myeloma, malignant plasma cells produce large amounts of abnormal antibodies that lack the capability to fight infection.

The abnormal antibodies are the monoclonal protein, or M-protein. Plasma cells also produce other chemicals that can cause organ and tissue damage (i.e., anemia, kidney damage, and nerve damage).

Reagent: a chemical substance known to react in a specific way. A reagent is used to detect or synthesize another substance in a chemical reaction.

Relapse: The reappearance of signs and symptoms of a disease after a period of improvement.

Remission or response: Complete or partial disappearance of the signs and symptoms of cancer. Remission and response are used interchangeably.

Tumor marker: A substance in blood or other body fluids that may suggest that a person has cancer. In the case of myeloma, M-protein is a tumor marker; it is an indirect way to gauge the number and activity of myeloma cells.