

Understanding Serum Free Light Chain Assays



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Introduction

You received this booklet to learn more about a type of laboratory test called the Serum Free Light Chain Assays. These tests are also known collectively as Freelite™. After reading this booklet, you should be able to answer the following questions:

- What are free light chains?
- How are free light chains related to multiple myeloma?
- How does the Freelite™ 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** are found in the glossary at the end of the booklet.

Multiple Myeloma and Monoclonal Protein

Myeloma is a cancer of the **plasma cells** in the bone marrow. Myeloma is synonymous with **multiple myeloma**. The function of normal plasma cells is to produce **antibodies**, also known as **immunoglobulins**, which have an important role in fighting infection. Each type of plasma cell produces only one type of immunoglobulin. There are many different types of plasma cells in the body,

and each type of plasma cell produces only one type of immunoglobulin. The result is the production of a wide variety of different immunoglobulins.

In multiple myeloma, one particular plasma cell (a clone) is duplicated a very large number of times, causing excess production of one type of immunoglobulin called a **monoclonal protein** or **M-protein** – also called myeloma protein, paraprotein, or the M-spike. The identification of an M-protein is important for diagnosis, and the measurement of its level is an aid for monitoring the effectiveness of treatment.

What Are Free Light Chains?

Structurally, normal immunoglobulins (abbreviated Ig) are composed of smaller units called heavy chains and light chains, and together they form a large complex (see Figure 1). There are five types of heavy chains, and each type is assigned a specific

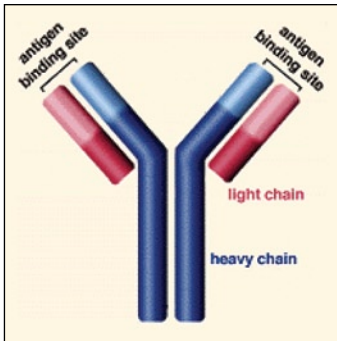


Figure 1. Structure of an immunoglobulin (antibody).

letter. These five types are abbreviated as IgG, IgA, IgM, IgD, and IgE.

There are two types of light chains, and they are referred to as kappa (κ) and lambda (λ or L). Each plasma cell produces only one type of heavy chain and one type of light chain. Altogether, there are 10 subtypes of normal immunoglobulins (see Table 1).

Table 1. Subtypes of Immunoglobulins.

IgG kappa	IgG lambda
IgA kappa	IgA lambda
IgM kappa	IgM lambda
IgD kappa	IgD lambda
IgE kappa	IgE lambda

The heavy and light chains are produced separately within the plasma cells and are assembled to form a whole (“intact”) immunoglobulin. When the light chains are attached to the heavy chains, the light chains are referred to as *bound light chains*. However, when the light chains are not attached to the heavy chains, they are called *free light chains*. For unknown reasons, the plasma cells typically produce more light chains than are required to create the whole immunoglobulins or monoclonal proteins. The excess light chains enter the bloodstream as *free light chains* (that is, not attached to the heavy chains). Thus, both in the normal situation and in individuals with myeloma and related disorders such as monoclonal gammopathy of undetermined significance (MGUS), excess light chains enter the bloodstream



as free light chains. The *amount* of free light chain production is linked to the activity of myeloma or plasma cell growth.

How is Monoclonal Protein Detected and Measured?

Monoclonal proteins may be detected and measured in blood and/or urine. When measurements are taken in blood, all of the cells are removed, leaving only the yellow liquid component that is called serum. If multiple myeloma is suspected, your doctor will evaluate for the presence of an abnormal monoclonal protein (M-protein). Several tests can be ordered to detect the M-protein, including serum protein electrophoresis (SPEP), urine protein electrophoresis (UPEP), and/or the Serum Free Light Chain Assays (Freelite™). If one type of light chain (kappa or lambda) is

produced in excess this is consistent with a monoclonal production.

SERUM AND URINE PROTEIN ELECTROPHORESIS

Two tests that are widely performed to measure M-protein levels and to monitor responses to treatment are SPEP and UPEP. The M-protein is identified as a “spike” on the SPEP or UPEP tracing (see Figure 2). SPEP and UPEP measure the amount of M-protein in a sample, but cannot identify the type of M-protein that is present. That is, the test can not identify the subtype as IgG kappa, IgA lambda, etc (Table 1).

IMMUNOFIXATION ELECTROPHORESIS

A second type of electrophoresis, referred to as immunofixation electrophoresis (IFE) is performed to identify the subtype of M-protein that is being produced by the myeloma cells. The subtype is identified by bands on the IFE (see figure 2). but generally, it can not

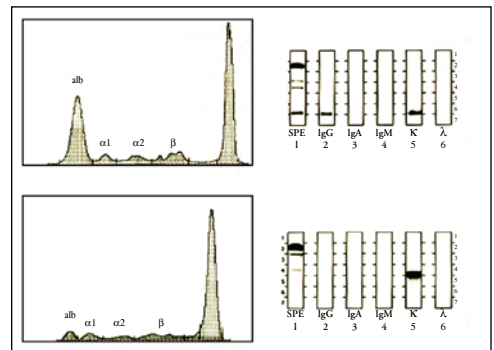
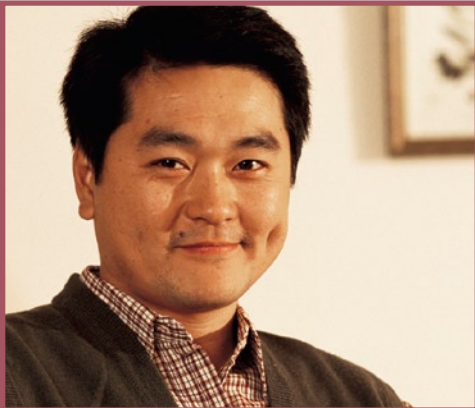


Figure 2. Illustrates SPEP (above left), UPEP (below left), and their respective IFEs (right).

measure the amount of the M-protein subtype that is present in the sample. An SPEP may be performed first to determine if, and how much, of an M-protein is present. If the SPEP demonstrates the presence of an M-protein, an IFE will be done to determine what subtype of M-protein is present.

SPEP, UPEP, and IFE have advantages and disadvantages. Among the disadvantages are that they are relatively insensitive for the detection of free light chains, in that the free light chain level must typically be many times the normal level in order to be detected with SPEP, UPEP, or IFE. For example, the normal level of one type of free light chain in the blood is approximately 10 milligrams per liter (abbreviated mg/L). However, the free light chain level in blood would have to be 50 times the normal level to be detected by SPEP and at least 15 times the normal level to be detected by IFE.

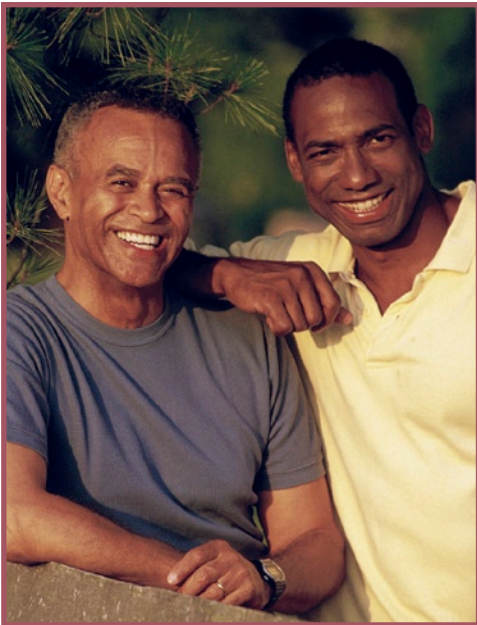


SERUM FREE LIGHT CHAIN ASSAYS

The serum free light chain assays are capable of detecting free light chains at their normal (non-elevated) levels in the blood. Importantly, these assays can detect mildly increased levels of free light chains even when these levels are undetectable by SPEP and IFE. This means that multiple myeloma could be detected earlier than might be possible with either SPEP or IFE and it is particularly useful in instances when only small amounts of light chains are produced by the myeloma.

The free light chain assays are best performed on serum rather than urine because of the filtering effects of the kidneys. Part of the normal function of the kidneys is to prevent protein loss from the body into the urine. As a result, an elevated level of M-protein may be detected in the blood before it is detected in the urine. Hence, the serum free light chain assays may replace the need for urine studies in the initial diagnosis of myeloma and related plasma cell diseases, however, urine studies are still important as part of serial monitoring. Serum free light chain assays are more sensitive in serum, the 24-hour urine sample is difficult to collect and transport, and the specimen is more difficult to store than serum, however, urine studies do show other aspects of myeloma disease, like kidney damage.

Like other tests that detect M-protein, the serum free light chain assays have advantages and disadvantages. As discussed above,



one advantage is greater sensitivity than is available with SPEP, UPEP, and IFE. Another advantage is that the serum free light chain assays are automated and therefore require less time to perform in the laboratory than SPEP, UPEP, and IFE. However, although the serum free light chain assays are excellent for detection of free light chains, they are unable to detect whole immunoglobulins. Some types of myeloma secrete only whole immunoglobulins. Therefore it is often best to perform SPEP or IFE to detect elevated levels of intact immunoglobulins in combination with the serum free light chain assays to detect free light chains.

In people with a myeloma that produces only light chains (Bence Jones myeloma), there is an increased amount of kappa or lambda light chain, depending upon the light chain produced by the myeloma. But excess light chains can also occur to a greater or lesser extent with all types of myeloma, not just light chain or Bence Jones myeloma. Therefore, measurement of free light chains can be used to diagnose and monitor the vast majority of people with myeloma regardless of the subtype of the myeloma.

The Serum Free Light Chain Assays: Normal Versus Abnormal

Normal levels of serum free light chains are*:

- Kappa: 3.3–19.4 mg/L*
- Lambda: 5.71–26.3 mg/L*
- Kappa/lambda ratio: 0.26–1.65

***Note:** *The units here are mg/L; different laboratories use different units. It is important to double-check the units used when comparing numbers in lab values.*

Light chains produced by myeloma cells will be exclusively kappa or lambda, depending upon the type of myeloma. Thus, if the myeloma cells produce kappa light chains, the level of kappa free light chains will increase in the blood. If, on the other hand, the myeloma cells produce lambda light chains, the level of lambda free light chains will increase in the blood. Your doctor will need to interpret the results of the serum free light chain assays together with other

clinical information in order to make a final interpretation of the results. A specialist in hematology/oncology is highly qualified to make this decision.

The Kappa/Lambda Ratio

- The kappa/lambda ratio is as important for diagnosis and monitoring of myeloma as are the levels of kappa and lambda
- When the level of either kappa or lambda is very high and the other chain is normal or low, then the ratio is abnormal and indicates that the myeloma is active
- If levels of *both* kappa and lambda light chains are increased, the ratio may



be within the normal range, and this generally indicates a disease other than myeloma, such as poor kidney function. When the kidneys are not working properly, both types of light chains are retained in the blood and are not removed by the kidneys. The result is increased levels of both kappa and lambda in the blood. In this situation, in general, the abnormally increased levels are not themselves a direct result of currently active myeloma

- If the kappa and lambda levels are both within the normal range, sometimes the ratio may be abnormal. In this situation, there may be a persistent low level of active myeloma with excess production of the abnormal light chains.
- A normal kappa/lambda ratio after treatment is a particularly good remission and is termed a **stringent complete response**. Normalization of the kappa/lambda ratio correlates with possible longer remissions, and studies are in progress to investigate more about the nature of this relationship.

How Can the Serum Free Light Chain Assays Help with Treatment?

Serum free light chain assays can help in several ways:

1. Evaluation of early response and early relapse

Because free light chains are broken down and/or excreted by the kidneys rather quickly (within just a few hours), changes

in blood levels in response to treatment occur rapidly. Thus, with a good response to treatment, myeloma cells will die, they will stop producing free light chains, and the blood levels of the free light chains will decrease within a few hours to days. In this situation, the decrease in free light chains occurs much more quickly than the decrease in IgG or IgA, because these compounds are broken down much more slowly by the body. Decreases in free light chain levels can therefore be a very sensitive indicator of early response. Typically, response to treatment can be detected by serum free light chain assays in a matter of hours to days, whereas it may take one to three weeks to detect response using SPEP and IFE.

At the time of relapse, the sensitivity of the free light chain assays is also very important. Even very small amounts of myeloma that start to grow as part of relapse produce measurable amounts of free light chains in most instances. The serum free light chain levels of either kappa or lambda, depending upon the type of myeloma, often increase before the increases in IgG and IgA and other immunoglobulins can be detected by SPEP or IFE. Imaging tests, such as FDG-PT or CT-PET, are also useful in the assessment of minimal amounts of disease.

2. Monitoring patients with low levels of myeloma protein (M-protein)

Myeloma that produces low levels of M-protein is called non-secretory or hyposecretory disease. Approximately 70% to

80% of people with non-secretory or hyposecretory myeloma have measurable abnormalities of M-protein using the serum free light chain assays. The Freelite™ test has been incorporated into response criteria to assist in assessing effectiveness of treatment in people with hyposecretory myeloma (see Table 2 below).

Table 2. *Response to Treatment Using Freelite™ in Hyposecretory Myeloma*

Stringent Complete Response	Normalized free light chain ratio
Partial Response	≥ 50% decrease in the difference between the light chain produced by the myeloma cells and the other light chain



3. Enrollment in clinical trials

Clinical trials are the only route by which new medicines are made available and a potential cure discovered. People with myeloma may participate in clinical trials to help test the safety and effectiveness of new treatments. In order for a patient with myeloma to be eligible to participate in a trial, there must be a way to monitor their M-protein levels in the blood or urine. People with hyposecretory (formerly “non-secretory”) disease used to be excluded from clinical trials because there was no method to monitor their M-protein levels. With the availability of the serum free light chain assays, the M-protein level can be monitored in the blood of these people. Therefore, people with hyposecretory disease may now be eligible to participate in clinical trials.

4. Indicator of disease activity

A study from the Mayo Clinic showed that patients with monoclonal gammopathy of undetermined significance (MGUS) who also have an abnormal free light chain ratio are more likely to progress and develop active myeloma. Changes in Freelite™ levels are useful for tracking the disease status in almost all people with myeloma, not just those with light chain (Bence Jones) myeloma or non-secretory disease.

5. Assessment of stringent complete response to treatment

One of the goals of myeloma treatment is to reduce the level of M-protein as much

as possible – and sometimes to eliminate it entirely. If the free light chain ratio becomes normal after treatment then this provides a very good and sensitive indication that treatment has been highly effective, and means that the level of light chain paraprotein has been reduced as much as possible. If the free light chain ratio normalizes with treatment, then this result is called stringent complete response. This type of response is the best possible response according to the International Response Criteria in Multiple Myeloma. By definition, a stringent complete response also includes a normal SPEP, a normal UPEP, a normal IFE, and no evidence of myeloma cells in the bone marrow using special tests.



Freelite™ Levels and the Assessment of Response to Treatment

Serum free light chain levels, as measured by the Freelite test, can be used in the same way as the Freelite levels monoclonal protein measurements to assess response to treatment, but they can also be used more frequently in the early weeks of treatment. Some people with myeloma find it helpful to track their own serum free light chain values using tables or spreadsheets – just like people with diabetes track their blood sugar. A table that can be used to follow results of the serum free light assays is located at the end of this booklet.

Specific criteria to assess stringent complete response and complete response have been established by the International Myeloma Working Group and are summarized in Table 3.

Table 3. *Stringent Complete Response and Complete Response*

Stringent Complete Response	Normalization of the free light chain ratio and no evidence of myeloma cells in the bone marrow
Complete Response	Negative immunofixation in the serum and urine, disappearance of any plasmacytoma, and $\leq 5\%$ plasma cells in the bone marrow

In summary, the serum free light chain assays offer several advantages for diagnosis and monitoring of treatment:

- Inclusion of serum free light chain assays can improve the sensitivity of screening protocols for detection and diagnosis of myeloma
- The serum free light chain assays along with other tests can provide valuable information for people with MGUS
- Use of serum free light chain assays to monitor treatment reveals responses to treatment earlier than other laboratory tests such as SPEP
- The improved sensitivity of serum free light chain assays over IFE may allow earlier detection of a relapse of myeloma

Patients Who Benefit the Most From the Serum Free Light Chain Assays Are:

- People with myeloma who have abnormal serum free light chain results at the start of treatment. Monitoring with the serum free light chain assays often allows a rapid assessment of the effectiveness of treatment.
- People with very low levels of light chains with other tests such as SPEP, UPEP, and IFE. These are people who generally have non-secretory (or hypo-secretory) myeloma.

Approximately 80% of people with non-secretory myeloma can have their disease monitored using the serum free light chain assays.

- People with deposits of light chains in the form of AL amyloidosis. People with AL amyloidosis may or may not have active myeloma. Tracking the light chain levels is very helpful to assess the disease status.
- People with light chain only myeloma (Bence Jones myeloma). The major advantages of the serum free light chain assays for these people are:
 - Ease of blood testing versus 24-hour urine collection*
 - The much greater sensitivity of blood testing: mildly increased levels may be detected in the blood but not detected in the urine.

* *It is important to note that periodic 24-hour urine testing is **still recommended** and necessary, both to double-check the light chain excretion level and monitor for any evidence of kidney damage.*

Will Insurance Cover the Cost of Serum Free Light Chain Assays?

In the United States, the serum free light chain assays are reimbursed by Medicare. Please consult with your doctor's office and insurance provider regarding this issue.

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 185,000. 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 You?

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 www.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.

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 plasma cells (a type of white blood cell) that helps fight infection. Also known as an immunoglobulin.

Bone Marrow: A soft, spongy tissue found in most large bones that produces red blood cells, white blood cells, and platelets.

Immunoglobulin: See "Antibody."

Monoclonal protein (M-protein): An abnormal protein produced by myeloma cells which accumulates in and damages bone marrow. A high level of M-protein indicates that myeloma cells are present in large numbers. The M-protein may consist of intact immunoglobulin, free light chains, or both.

Multiple myeloma: A cancer arising from the plasma cells in the bone marrow. The plasma cells form abnormal antibodies, possibly damaging the bone, bone marrow, and other organs.

Plasma cell: A type of white blood cell that produces antibodies.

Plasmacytoma: A tumor made up of cancerous plasma cells.

Protein: A group of compounds that are the main component of a cell.

Stringent complete response: normalization of the free light chain ratio and absence of myeloma cells in the bone marrow following treatment.

White blood cell: A cell made by the bone marrow that helps fight infection and/or disease.

Appointments

<i>Date</i>	<i>Time</i>	<i>Kappa Level</i>	<i>Lambda Level</i>	<i>κ/λ Ratio</i>
<i>Notes</i>				
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