

## Questions and Answers:

### *1. What Are Bence Jones Proteins?*

Immunoglobulin molecules normally consist of pairs of polypeptide chains of unequal size bound together by several disulphide bridges. In each immunoglobulin molecule there are a pair of heavy chains which may be either gamma alpha, mu, delta or epsilon type, and a pair of light chains which may be either kappa or lambda. In some pathological conditions such as Multiple Myeloma, there is a proliferation of one antibody-producing plasm cell leading to excess production of light chains of one specific kind. These free monoclonal light chains can be found in urine and plasma and were first isolated in 1847 by Henry Bence-Jones. Consequently, they are known as Bence-Jones Proteins (BJP).

### *2. How can BJP aid in clinical diagnosis?*

"Bence-Jones proteinuria is the classic example of overflow proteinuria where increased concentrations of proteins of low molecular mass are filtered through the glomerulus and exceed the reabsorptive capacity of tubules in the kidney.

Quantitative measurement of Bence-Jones Proteins and determination that they are monoclonal, aid in the diagnosis of multiple myeloma (malignant proliferation of plasma cells), Waldenstrom's macroglobulinemia (increased production of large immunoglobulins by spleen and bone marrow cells), leukemia (cancer of the blood-forming organs), and lymphoma (cancer of the lymphoid tissue)." The presence of Bence-Jones Proteinuria (BJP, Mr 22,000) in urine is a valuable prognostic indicator for the presence of multiple myeloma (malignant proliferation of white blood cells called plasma cells). Other clinical indicators are increased levels of myeloma cells in bone marrow and the destruction of bone either as osteolytic lesions or as extreme osteoporosis. Average incidence rates of 3 per 100,000 population are common in Europe and USA and reports show that mortality rates from multiple myeloma have been increasing dramatically over the past three decades. Each year nearly 13,000 people in the United States learn that they have multiple myeloma. These people will undergo regular urine tests during follow-up examinations to monitor treatment methods.

### *3. What is Multiple Myeloma?*

Multiple myeloma (MM) or myelomatosis, is a cancer of the plasma cells. Its name is derived from the fact that it causes "multiple" areas of bone lesions in many patients in the original description. It occurs most frequently in people over the age of 60 years. It occurs in approximately 3 in 100,000 people and accounts for 1% of all cases of cancer. In multiple myeloma, an error occurs along the differentiation pathway of B-lymphocytes into normal plasma cells, with a resulting uncontrolled production of large numbers of abnormal plasma cells of a single cell type. These abnormal plasma cells occupy the bone marrow, suppressing the growth and development of normal blood cells. Furthermore, the cancerous plasma cells over-produce dysfunctional and ineffective monoclonal antibodies. It is the cancerous plasma cells and the myeloma proteins they produce that cause the signs and symptoms of multiple myeloma.

### *4. How is the diagnosis of multiple myeloma defined?*

Three major features to diagnose multiple myeloma (otherwise referred to as myelomatosis) are the presence of paraproteins in serum or urine, increased levels of plasma cells in bone marrow and the destruction of bone either as osteolytic lesions or as extreme osteoporosis.

### *5. How should BJP be detected in urine?*

Bence-Jones Proteins are normally detected by urine protein electrophoresis. The sensitivity of the protein stain and the total protein concentration dictates whether the BJP needs to be concentrated. The test for adequate sensitivity of the method is that albumin should be visible in all urines studied. Following electrophoresis that show the presence of protein bands, the typing of the light chains of paraproteins is undertaken by immunofixation electrophoresis.

*6. What is the lowest concentration of BJP I can detect?*

Based on laboratory methods, detection limits for BJP in a urine sample can vary. (e.g. Sebia Immunofixation kit detection limit is 15 – 50 mg/dL corresponds to the lowest concentration.) Many laboratories can detect 0.01 mg/ml BJP in a urine sample.

*7. What is the lowest concentration of BJP I need to detect?*

A value of 0.03-0.05 mg/ml BJP is typically regarded as being clinically significant for early diagnosis of B-cell malignancies. Damacco et al (Damacco, Waldenstrom (1968) Acta Medica Scandinavica, 184, 403-409) quoted 0.06 mg/ml and there are a few well documented reports of concentrations well in excess of these which could not be attributed to B-cell malignancy.

*8. What volume of urine is normally required and how should I store it?*

A 25 ml sample of a 24 hour collection of urine is normally submitted for analyses. The sample should be kept refrigerated to prevent the breakdown of BJP and other proteins by protease enzymes. Samples are typically supplied in 0.1 % sodium azide or 2  $\mu$ l/ml Proclin 150 preservative (Rohm and Haas, US). It is not necessary to adjust the pH of urine before storage.

*9. How can BJP be quantified?*

A value can be obtained by scanning densitometric analysis of the stained electrophoretic separation and relating this to the total urine protein. When BJP is present, without other overt proteinuria, the total protein is an estimate of the BJP. However, this method is generally unreliable. Paraprotein bands can also be quantitated directly from capillary zone electrophoretic (CZE) separations. There is no International Reference Preparation for urinary light chains, and there will be marked variations in reactivity of antisera with monoclonal light chains.

For this reason immunochemical methods of quantitation of BJP are not recommended (Riches PG, Sheldon, J., Smith, AM, Hobbs, JR (1991) *Annals of Clinical Biochemistry*, 28, 253-259). Estimation of the amount of BJP in a 24 hour urine collection may be of value in the monitoring of BJ-only myeloma, however, the variability in its tubular re-absorption and metabolism by the kidney may make these measurements unrepresentative, particularly at low concentrations of Bence-Jones Protein.

*10. How do I perform urine protein electrophoresis (UPE)?  
(process may vary depending on manufacturer – please see your manufacturer sales rep)*

Urine protein electrophoresis (UPE) provides one of the best tools for the general assessment of the human state of health. It is especially useful for detecting proteins of restricted mobility associated with myelomas and other lymphoproliferative disorders. In cases of known monoclonal gammopathy, UPE is an effective tool for monitoring patient therapy. 1. A 25 ml patient urine sample is either filtered through a 0.45  $\mu$ m syringe filter, (Minisart, Sartorius), or

centrifuged at 2,000 g to remove particulates and sediment that would interfere with the analysis. For electrophoretic analysis, the protein concentration of the sample should be above 0.5 mg/ml. Since levels of BJP indicating a pathological condition can be as low as 0.01 mg/ml the urine sample must be concentrated. 5-10 ml of the clarified urine is transferred to a BJP 5 or 10/20 ml solvent absorption concentrator and concentrated 50-100 fold.

2. Concentrated patient urine (5 $\mu$ l) is applied to the centre of a track on a specific, high resolution, gel using an application mask. (process may vary depending on manufacturer – please see your vendor sales rep for additional information.)

3. A voltage is applied and the gel is electrophoresed. Since proteins have different charges they migrate in different directions along the track. Albumin and alpha proteins migrate towards the anode, and beta proteins and immunoglobulins migrate toward the cathode.

4. The gel is now washed, pressed, dried, and stained.

5. Samples are examined for immunoglobulin bands in the Reference track and monospecific immunoglobulin tracks.

### *11. What is immunofixation electrophoresis?*

Immunofixation electrophoresis (IFE) is a two-stage process combining agarose gel electrophoresis, (UPE or SPE), with immuno-precipitation. Proteins are separated electrophoretically on several tracks on a gel. Antisera specific to individual classes of molecules are added to each track. If specific classes of light chain are present, insoluble complexes form with the antisera, which can then be stained and detected.

### *12. How do I perform immunofixation electrophoresis (IFE) of urine? (process may vary depending on manufacturer – please see your manufacturer sales rep)*

1. A 25 ml patient urine sample is either filtered through a 0.45  $\mu\text{m}$  syringe filter or centrifuged at 2,000 g to remove particulates and sediment that would interfere with the analysis. For electrophoretic analysis the protein concentration of the sample should be above 0.5 mg/ml. Since levels of BJP indicating a pathological condition can be as low as 0.01 mg/ml the sample must be concentrated. 5-10 ml of this clear urine is transferred to a BJP 5, 10 or 20 mL

solvent absorption concentrator. The unit is left to stand until the sample has been concentrated 50-100 fold.

2. Concentrated patient urine (5  $\mu\text{l}$ ) is applied to the centre of each track on a specific IFE gel using an application mask.
3. Protein fixative and monospecific antibodies to IgG, IgA, IgM, Kappa and Lambda are applied to the gel.
4. During incubation, insoluble immune complexes are formed between the antisera and where present the immunoglobulins and light chains.
5. The gel is now washed, pressed, dried, and stained.
6. Samples are examined for paraprotein bands.

### *13. How long will a concentration take using a BJP concentrator?*

A typical 50 fold concentration of 5 ml urine using the BJP 5 should take about 45-60 minutes. Using the BJP 20 a 50 fold concentration of 10 ml urine should take about 90-120 minutes. Speed of filtration is affected by several parameters including temperature, pH and protein concentration. Whilst BJP concentrators will provide rapid filtration in most environments, the following suggestions may be helpful in

optimizing speed. Suspended particles will tend to foul the filter element and slow filtration speed. Pre-filtration with a syringe filter will clarify the sample and result in faster filtration speed and improved analytical results following concentration. An acid sample with a pH of less than 5 will take longer to concentrate than a neutral sample. Adjustment to neutral pH will result in faster filtration; however, this may alter slightly the concentration of the Bence-Jones Protein. Initial protein concentration levels will have a significant effect on concentration speed. Whilst a dilute sample will concentrate rapidly, when the macromolecule concentration exceeds 2%, the speed of filtration will rapidly decrease. Concentrations above 5% are not practical with BJP concentrators. BJP concentrators have an impermeable concentrate pocket (dead stop) which prevents the solution from concentrating to dryness concentration to dryness. However, if the concentrate inadvertently remains too long in the concentrator, the remaining solvent will eventually evaporate and the sample may go to dryness. Should this occur, proteins may be returned to solution by pipetting approximately 100 $\mu$ l of buffer solution in and out of the concentrate pocket several times.

*14. I used to use concentrators from another manufacturer, now I use the BJP. Why do I now get more bands in the beta region on a SPE gel?*

"The membrane used in BJP devices is a 7.5 kDa molecular weight cut off, (MWCO) polyethersulfone membrane. Other manufacturers use membranes of up to 15 kDa MWCO. A protein often found in some pathological conditions is b2-Microglobulin, a protein that sheds from dividing cells. In general, the higher the level of b2-Microglobulin found in multiple myeloma, the worse the prognosis. The molecular weight of b2-Microglobulin is 11.8 kDa. This is large enough to be retained by the membrane in the BJP but may pass through larger MWCO membranes. Another reason why multiple bands are sometimes seen is due to incorrect storage of the urine prior to analysis. If the urine sample is a few days old and it has not been refrigerated, the action of the proteases and any microbial growth could produce multiple bands."

*15. What is Hydrasys LC?*

Hydrasys LC is a complete system by Sebia that automatically carries out the different phases of electrophoresis: sample application, migration, incubation, staining, destaining and drying. For more information go to: [www.sebia.com](http://www.sebia.com).

*16. What is Paragon CZE?*

The Paragon CZE 2000 Capillary Electrophoresis System automates clinical serum protein electrophoresis (SPE), urine protein electrophoresis and immunofixation/subtraction electrophoresis (IFE/s) for fast and accurate analysis. For more information go to : [www.beckman.com](http://www.beckman.com)

*17. What is SPIFE?*

SPIFE is an electrophoresis system for IFE, Proteins and Hemoglobins. SPIFE features in-line sample application, automated electrophoretic separation and staining of analytes, multiple stain ports, and a barcode "lockout" feature to assure positive sample ID. For more information go to: [www.Helena.com](http://www.Helena.com)

*18. I have some units that have been on the shelf for a while and they appear yellow - what does this mean?*

Over time the membrane in the units or the glue that attaches the membrane to the plastic may turn yellow - this sometimes occurs but will not affect product performance or the results of testing.