

# SEPARATIONS

Keeping you on track with the latest in EP

## Urine Protein Electrophoresis

### Latest Consensus Statements, Recommendations, and Testing Options

By: Aigars Brants, Ph.D., Scientific Affairs Manager

In kidneys, the glomerular capillary wall is permeable to substances with a molecular weight of 20 kDa or less. Once in filtrate, low molecular weight proteins are reabsorbed and metabolized by the proximal tubule cells. Normal urinary proteins include albumin, serum globulins, and proteins secreted by the nephron. A normal adult excretes up to 150 mg/24 h of protein in urine.

#### What is proteinuria?

Proteinuria is defined as urinary protein excretion of more than 150 mg per day. Microalbuminuria can be a sign of early renal disease, especially in diabetic patients. In microalbuminuria, 30 to 150 mg of protein per day is excreted per day.

#### What are the types of proteinuria?

Proteinuria can be transient (intermittent), orthostatic (relate to sitting/standing), or persistent (always present).

In transient proteinuria, protein in urine disappears when the underlying cause is resolved.

In orthostatic proteinuria, protein excretion is normal when the patient is lying down but is increased when a person is sitting or standing. It occurs in approximately 2 to 5 percent of young people, but is unusual in people over the age of 30.

Persistent proteinuria can be further defined as glomerular, tubular, or overflow. The most common type is glomerular proteinuria with albumin as the primary urinary protein. This type of proteinuria is caused by increased filtration of albumin and other macromolecules across the glomerular basement membrane.

Tubular proteinuria results when malfunctioning tubule cells no longer reabsorb proteins in the filtrate; low-molecular-weight proteins such as  $\beta_2$  microglobulin and immunoglobulin light chains dominate over albumin.

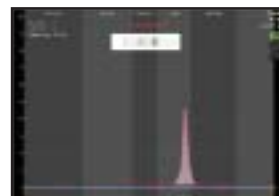
In overflow proteinuria, low-molecular-weight proteins overwhelm the ability of the tubules to reabsorb the filtered proteins. Bence-Jones

proteinuria is the classic example of overflow (and also tubular) proteinuria. Quantitative measurement of Bence Jones proteins, and determination that they are monoclonal, aid in the diagnosis of various disorders including multiple myeloma – the malignant proliferation of plasma cells.

#### What is the utility of urine protein electrophoresis?

Urine protein electrophoresis (UPEP) is utilized to detect monoclonal and other proteins in urine. The test provides information about the location and degree of damage within the nephron. The type of testing, the sensitivity of the protein stain, and the total protein

concentration in the sample dictate whether the urine needs to be concentrated or run neat. Urine immunofixation electrophoresis (UIFE) helps to determine the type of protein detected by UPEP.



Sebia CAPILLARYS Urine Protein result: M-Protein in the beta region

The recent "Guidelines for Standard Investigative Workup: Report of the International Myeloma Workshop\* Consensus Panel 3" states that "both serum and urine should be assessed for monoclonal protein. Agarose gel electrophoresis or capillary zone electrophoresis of serum and

## UPEP: Still Required?

Continued on pg 2

urine is preferred to screen for the presence of monoclonal protein<sup>1</sup>.”

The full consensus guideline is available at the IMW website (<http://mw-delhi09.com/spargoDocs/Consensuspanelthree.pdf>).

<sup>\*</sup>The International Myeloma Workshop (IMW) is a bi-annual meeting of the top physicians and research scientists throughout the world; they attend this meeting to discuss new approaches and treatments exclusively for Multiple Myeloma.

### When to test urine for light chains

The following conditions (to list a few) warrant urine protein electrophoresis: 1) monoclonal protein in serum is >1.5 g/dL, 2) monoclonal free light chains are detected in serum, 3) hypogammaglobulinemia is present in serum; 4) serum electrophoresis shows nephrotic pattern.

### Urine protein electrophoresis or serum FLC: Is UPEP still required?

In short, yes. A 2008 publication in Leukemia entitled International Myeloma Working Group (IMWG)<sup>\*\*</sup> guidelines for serum-free light chain analysis in multiple myeloma and related disorders, discusses the indications for use of the serum free light chain (sFLC) assay in the evaluation and management of multiple myeloma and related clonal plasma cell disorders<sup>2</sup>. The IMWG concluded the following:

- “In the context of **screening**, the serum FLC assay in combination with serum protein electrophoresis (PEL) and immunofixation yields high sensitivity, and negates the need for 24-h urine studies **for diagnoses other than light chain amyloidosis (AL).**”
- “...**once diagnosis of a plasma cell disorder is made, 24-h urine studies are required for all patients.**”
- “For AL screening, however, the urine IFE should still be done in addition to the serum tests including the serum FLC.”
- “**The FLC assay cannot replace the 24-h urine protein electrophoresis for monitoring myeloma patients with measurable urinary M proteins.**”

<sup>\*\*</sup>The International Myeloma Working Group is funded through the International Myeloma Foundation (IMF) and consists of a group of top physicians from around the globe.

### More on light chain amyloidosis (AL)

Recently, through a prospective study, Giovanni et al. proved the importance of urinary studies in patients with suspected AL amyloidosis<sup>3</sup>. The results of the assessment of diagnostic sensitivity of the serum FLC  $\kappa/\lambda$  ratio, a commercial serum and urine agarose gel electrophoresis immunofixation (IFE), and high-resolution agarose gel electrophoresis immunofixation (IFE) were as follows:

- “The diagnostic sensitivity of commercial serum and urine IFE was greater than that of the FLC  $\kappa/\lambda$  ratio (96% vs 76%).”
- “The combination of serum IFE and the FLC assay detected the amyloidogenic light chain in **96%** of patients. The combination of IFE of **both serum and urine** with FLC  $\kappa/\lambda$  ratio had a **100%** sensitivity.”

The authors concluded that “the identification of amyloidogenic light chains cannot rely on a single test and requires the combination of a commercially available FLC assay with immunofixation of **both serum and urine.**” Lastly, the authors state that their “findings underline the complexity of this disease, which still does not allow reliance on only a few diagnostic tests.”

### Should laboratories run urines neat (unconcentrated)?



Sebia HYDRASYS 2

Some labs measure total urine protein concentration and then decide how much to concentrate.

Some others concentrate all samples 50-100x before applying them to the gel, but more and more labs are running urine samples neat.

Recently, an extensive study comparing UPEP and UIFE results from concentrated and unconcentrated urine samples was performed by Roden et al. from the department of laboratory medicine and pathology of Mayo Clinic, Rochester, MN (4). It was concluded that UPEP results had 97% concordance while UIFE results had 98% concordance. Protein concentrations calculated from concentrated and unconcentrated urine protein samples correlated very well ( $r^2=0.99$ , slope 1.04). It was also concluded by the authors that running unconcentrated urine specimens will mean fewer sample rejections because of insufficient volume.

Roden et al. summarized the benefits of running neat urine samples:

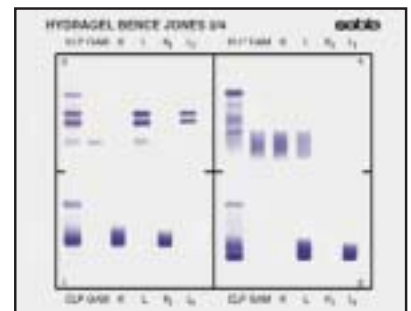
- Decreased potential for errors due to the elimination of multiple tube transfers
- Improved turn-around time (TAT) due to the low-volume urine sample utilized and therefore reduced potential for QNS sample rejection
- Reduced cost with concentrators being eliminated
- Decreased labor for technologists with the elimination of the concentration step

Additional benefits may also be realized such as more accurate representation of the urine proteins contained in the sample; more standardization; and no proteins lost due to the cut-off retention rate of the concentrator.

Sebia offers many approaches for processing neat urine samples on the HYDRASYS<sup>®</sup> and HYDRASYS 2 semi-automated electrophoresis systems. The simplest approach to running UPEP and UIFE assays with neat samples is outlined below.

**UPEP:** 10 $\mu$ L of neat sample is utilized with the HYDRAGEL<sup>®</sup> HR (High Resolution) kit. The gel is processed utilizing the HYDRASYS or HYDRASYS 2 instrument with the **HR3** program and stained using **HR Acid Violet** program. When the HR3 program is utilized, the sensitivity of detection is 1.5 – 2.0 mg/dL for albumin and Bence Jones protein. Although acid violet differs from amido black performance (amido black stain is generally used for quantification purposes, while acid violet is mostly used in qualitative assays since it is more sensitive), the result linearity is equally impressive. Please refer to the HYDRAGEL HR package insert for additional performance data concerning qualitative, semi-quantitative, accuracy, sensitivity and linearity. Note: Sebia's serum controls work well for this method if diluted more than 40 times.

**UIFE:** 10 $\mu$ L of neat sample is utilized with the Sebia HYDRAGEL IF or HYDRAGEL BENCE JONES kits. The gel is processed utilizing the HYDRASYS or HYDRASYS 2 instrument with the **BJ (BENCE JONES)** program and stained using the **IF Acid Violet** program. With the above methodology, Bence Jones protein can generally be detected in urine at 1-2 mg/dL with the free + bound light chain antisera and at >5 mg/dL with free light chain antisera.



In specialized testing for light chain amyloidosis (AL), urine with a total protein <30-50 mg/dL may need to be concentrated up to 50x while using the above methods. For the above methods to be clinically useful, it is important for the interpreter to understand the limit of detection for each method and the detection limit's clinical relevance.

Continued on pg 4



# Sebia Gives Reference Lab a New View of Automation

## CAPILLARYS™ 2 offers flexibility, clearer results, and more



Pictured left to right - *Jamie Darrab, David Odneal, Jennifer Sbafterman, and Kelly Church*

Mention the word “automation” and people often think two things: mega-high-volume output, and employee obsolescence. Yet Physicians Reference Laboratory, LLC, (PRL) had neither in mind when they selected Sebia’s CAPILLARYS™ 2 for hands-free capillary electrophoresis (CE).

In 2006, PRL initiated a major reorganization to improve workflow and boost efficiency of staff technologists. Knowing that automation would be key to these goals, the lab took a close look at what Sebia had to offer. They found they could have a system that provided the desired precision and speed, without completely giving up the best aspects of manual processing.

For example, the CAPILLARYS 2 allows the lab to do small runs in addition to larger runs. “We like the ‘less-batch’ mentality of Sebia’s design,” explains Kelly Church, MT (ASCP), a medical technologist in Immunology. “We can process tests when they need to be processed, rather than waiting for a complete batch as other systems require. **Physicians and patients get their results faster as a result.**” She estimates that the lab performs about 10-20 tests per day on the CAPILLARYS 2, enabling a turnaround time well within 24 hours.



*Eight capillaries function concurrently with a protected horseshoe design.*

Of course, in a lab environment, consistent accuracy is imperative. The CAPILLARYS 2 features a patented capillary horseshoe design that maintains tight temperature control during sample migration—generating consistent reproducible results from analysis-to-analysis and from capillary-to-capillary. The sample remains in a sterile environment for the entire process, which eliminates some of the problems encountered with manual electrophoresis.

“With our previous method, we did staining by hand, and it was difficult to distinguish abnormalities from background ‘noise,’ Church says. She sees a marked difference since converting to the CAPILLARYS 2 and its Protein 6 and Immunotyping assays. **“The process is cleaner and abnormalities are much easier to detect.”**

**The CAPILLARYS 2, a fully automated capillary electrophoresis instrument, enables PRL’s technologists to perform electrophoresis testing in a hands-free, “walk-away” fashion. Up to 80 samples (serum protein) can be processed through the system in an hour; the operator simply places bar-coded primary sample tubes on the instrument and walks away.**

### How it works

- Once samples are loaded, a work list is generated on the monitor, indicating positive sample ID.
- Within the CAPILLARYS 2, a small amount of sample is aspirated from the primary tube and diluted in the dilution segment—no manual pipetting needed.
- The diluted sample is injected into one of eight narrow capillaries charged with electrophoretic buffer of a specific pH.
- Migration and complete separation of the proteins is carried out within the capillary held under constant, high voltage controlled by Peltier effect.
- Direct detection of the proteins occurs at a specific wavelength and the electrophoretic profile appears on the monitor.

A relative quantification of individual zones is automatically determined. With a total protein concentration, the system will calculate each fraction concentration as well. Automatic delimiting of curves and identification of fractions minimizes operator editing.

Clearly this yields significant benefits in terms of accurate diagnosis, operational efficiency and reduced care costs. However, the most positive impact is felt by the patient who won’t experience a “questionable” result that necessitates further, more invasive testing. As Church points out, **“We can screen out a lot more abnormalities the first time around and avoid putting the patient through something much more difficult, such as a bone marrow screening.”**

### When in doubt, a complementary system increases clarity

Unfortunately, medicine isn’t always an exact science, and lab technologists often face the occasional borderline result. PRL uses Sebia’s semi-automated HYDRASYS® system as a back-up for the CAPILLARYS 2 when a second check is desired. The lab also uses the HYDRASYS for urine immunofixation (IFE) and urine protein electrophoresis. The HYDRASYS allows for consistent, reproducible results with a standardized

procedure. (Note: In December 2007, Sebia received FDA clearance for its CAPILLARYS Urine kit, which allows for the separation of urinary proteins with the automated CAPILLARYS 2.)

Dr. Kenneth Cummings, a clinical pathologist at PRL, vouches for the reliability of Sebia’s results. “I am quite conservative when it comes to trying new methodology. We spent a number of months comparing our old electrophoresis system to Sebia’s instrumentation to see how the results matched up. **I have a great amount of confidence in the performance of their technology.**”

Dr. Cummings also likes the fact that the CAPILLARYS neatly interfaces with PRL’s laboratory information system. **“I can view scans on my desktop, in very high resolution, which is extremely helpful to me.”**

### Automation that helps technologists grow

Since PRL has adopted automated electrophoresis, making it less dependent on operator experience, an opportunity has been created for some technologists to move into the electrophoresis side of the lab and increase their skills and knowledge in terms of result interpretation and diagnosis. Thus, rather than eliminating opportunities, technology has actually increased them for some PRL personnel.



*CAPILLARYS 2 - automated capillary electrophoresis*

**“The quality of equipment and the opportunities afforded by it can go a long way in enhancing employee satisfaction,”** notes David Odneal, MT (ASCP) SH, PRL’s Supervisor of Hematology & Immunology. **“Especially in this case, where the technology is so stable and easy to use. Employees are excited about it.”**

**Are you interested in learning more about the benefits of Sebia’s walk-away agarose gel electrophoresis (AGE) technology or completely automated capillary electrophoresis (CE) technology? Simply circle 186 (AGE) or 187 (CE) on the Reader Response Card. Please also visit [www.sebia-usa.com](http://www.sebia-usa.com).**

*About Physicians Reference Laboratory, LLC*

*Based in Overland Park, Kansas, Physicians Reference Laboratory, LLC, (PRL) is a privately owned, full-service diagnostic testing laboratory. For 30 years PRL has been renowned for incorporating new and innovative testing procedures, led by the industry’s most highly qualified professionals who are supported by market leading technologies. PRL strives to deliver accurate diagnostic data that enables the highest quality patient care.*



Sebia CAPILLARYS 2

### Urine testing by capillary electrophoresis technology

Sebia offers a full line of product for analyzing urine. In addition to agarose gel electrophoresis testing on the HYDRASYS and HYDRASYS 2 systems, Sebia offers urine testing utilizing fully automated capillary electrophoresis (CE) technology. The CAPILLARYS Urine kit is utilized for the preparation of urine samples before separation in alkaline buffer (pH 9.9) on the fully automated CAPILLARYS 2 system using CE in free solution. With this technique, charged molecules are separated by their electrophoretic mobility in an alkaline buffer. Separation also occurs according to the electrolyte pH and electroosmotic flow.

The CAPILLARYS 2 has eight capillaries functioning concurrently, allowing 8 simultaneous analyses. The urine sample is dialysed/concentrated prior to processing on the capillary electrophoresis instrument. A high voltage protein separation is then performed on the CAPILLARYS 2 and proteins are detected using 200 nm UV light. Qualitative results are displayed in five zones: albumin, alpha-1, alpha-2, beta, and gamma. Electropherograms are exceptionally clean and do not have any background noise or artificial peaks. If a monoclonal protein is detected or suspected, the same sample can be run using the CAPILLARYS Urine Immunotyping technique for further analysis. In April 2009, Sebia received clearance by the Food and Drug Administration (FDA) to Market the CAPILLARYS Urine Immunotyping assay.



Aigars Brants, Ph.D.,  
Scientific Affairs Manager

Are you interested to learn more about options for urine testing with Sebia products in your laboratory? Please circle number **185** on the Reader Response Card. Also, please visit the Sebia website for more information: <http://www.sebia-usa.com/products/products.html>

### Definitions and Resources

**Multiple Myeloma** is a cancer of the plasma cells in the bone marrow. Malignant plasma cells (myeloma cells) accumulate in the bone marrow; myeloma cells produce and release monoclonal protein (M-Protein) into the blood stream and/or into the urine.

- [www.labtestsonline.org](http://www.labtestsonline.org)
- [www.multiplemyeloma.org](http://www.multiplemyeloma.org)
- [www.myeloma.org](http://www.myeloma.org)

**Light chain amyloidosis (AL)** is a progressive disease caused by the deposition of insoluble fibrils formed by the aggregation of circulating monoclonal light chains produced by a usually small-sized bone marrow plasma cell clone (3). It is a rare disease with approximately 1200 – 3200 new cases reported annually in the United States.

- [www.amyloidosis.org](http://www.amyloidosis.org)

### References

1. Dimopoulos MA, Kyle RA, Jagannath S on behalf of the International Myeloma Workshop Consensus Panel 3. Guidelines for standard investigative workup: Report of the International Myeloma Workshop Consensus Panel 3. XII International Myeloma Workshop 2009.
2. Dispenzieri A, Kyle R, et al. International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. *Leukemia* 2008;1-10.
3. Palladini G, Russo P, et al. Identification of amyloidogenic light chains requires the combination of serum-free light chain assay with immunofixation of serum and urine. *Clinical Chemistry* 2009;55(3):499-504.
4. Roden AC, Lockington KS, Tostrud LJ, Katzmann JA. Urine protein electrophoresis and immunoelectrophoresis using unconcentrated or minimally concentrated urine samples. *American Journal of Clinical Pathology* 2008;130:141-145.

## Sebia supports clinical laboratory organizations, the CLMA and ISLH, at the annual conferences and exhibitions

As in many years past, Sebia supported the Clinical Laboratory Management Association (CLMA) at the annual meeting, CLMA ThinkLab '09, which was held in beautiful downtown Tampa, Florida from May 2 – 5. The CLMA annual conference has always been a conference emphasizing the future of the clinical laboratory industry and growth of laboratory professionals. CLMA, with its 4,000 clinical laboratory professionals, provides leadership and education to its members as well as enhances the image and increases the visibility of the profession. Also in May, Sebia supported the International Society for Laboratory Hematology (ISLH) at the annual meeting, XXII International Symposium on Technological Innovations in Laboratory Hematology, in Las Vegas, Nevada from May 11 – 14.

This annual conference is attended by global leaders in the field of Hematology and focuses on new advances in all aspects of the field. The benefits of Sebia's capillary electrophoresis (CE) technology as an aid in the diagnosis of hemoglobinopathies and thalassemias were discussed during the many hemoglobinopathy sessions and workshops. Sebia now has two CE platforms for performing hemoglobinopathy testing – the CAPILLARYS, and the MINICAP, with a smaller footprint.

Please circle **188** on the Reader Response Card for additional information concerning the CAPILLARYS and/or MINICAP Hemoglobin assays.