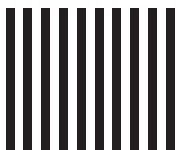




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Raise Your Hgb IQ – cont'd from page 2

Confirmation testing was performed by isoelectric focusing. Results were similar to HPLC findings. Acid hemoglobin electrophoresis was required to determine the final result. Only two bands were detected. One was in the A region and the other in the F region. This proved that the sixth band was the Hemoglobin F-G Philadelphia hybrid which moved to the F region with normal Hemoglobin F. G Philadelphia moved to the A region with Bart's and Hemoglobin A.

In an adult, the pattern would have been totally different. Hemoglobin A₂ would be present, but normal. There would be no large fetal hemoglobin band, probably no Bart's Hemoglobin or Hemoglobin H and no F-G Philadelphia hybrid. The location of the abnormal band would have read G Philadelphia, but the concentration would have been higher than expected. A low MCV would suggest alpha thalassemia, but still leave the possibility of iron deficiency anemia. If iron studies are normal, the final result is concluded.

Since both thalassemia and a hemoglobin variant occurred in the child, the clinician may choose to request a family study on the parents. Each abnormality is inherited separately and may have originated from one or both parents. Before informed counseling can take place, most likely hemoglobin fractionation, CBC and iron studies would be requested to complete the picture on the family. Not all physicians are equally adept at deciphering the entire picture. For that reason, it may be wise to involve a pathologist to review the lab results and speak to the clinician concerning diagnosis and counseling.

Is your laboratory currently performing hemoglobin electrophoresis? Would you like to learn more about Sebia's hemoglobin electrophoretic assays? Please circle 167 on the Reader Response Card.

Are concentrators obsolete? – cont'd from front cover

Urine IF: 10uL of neat sample is utilized with the Sebia IF or Bence Jones kits. The gel is processed utilizing the HYDRASYS instrument with the **Bence Jones** program and staining with the **IF Acid Violet** program. Sebia's sample processor and dilutor, the HydraPLUS, can be used in tandem with the HYDRASYS in order to eliminate both sample and antisera pipetting and achieve positive ID. 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.

Please refer to TABLE 1 for a complete listing of kits and antisera that can be utilized for processing neat urine samples.

In specialized testing for amyloid 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. Sebia also offers a method to concentrate the urines by 33% directly onto the sample application comb utilizing a dry chamber; however, this procedure should be validated by the individual laboratory as it is a variation from the standard package insert procedure.

By running urines neat, a laboratory should be able to save labor, time and money while

TABLE 1

SEBIA PRODUCT	PRODUCT NUMBER
7/15 HR Acid Violet	4102/4122
1/2/4/9 IF	4301/4302/4304/4309
1/2/4 Bence Jones (BJ)	4321/4322/4324
IF Antisera Set	4315
BJ Complete Antisera Set	4335K
Normal Control	4785
Hypergamma Control	4787

SmartCard - Smart Customers.

The Sebia HYDRASYS instrument now has the capability for customer-installed software updates. We would like to thank all of our current customers for your timely responses and taking full advantage of this remarkable new feature. As you all know, the HYDRASYS Programmer & SmartCard allow for timely software updates at your convenience, which contain the latest advancements, improvements, and menu expansions for the HYDRASYS instrument.

Those current customers who responded by February 16, 2005 were eligible to win a \$100 American Express Gift Cheque. *Congratulations go out to Donna Doughty, MT (ASCP); Special Chemistry, University of Kansas Hospital Authority; Kansas City, KS!*

Stay alert to other Sebia mailings as you could be the next winner!

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Clearly Superior...Automatically Better

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Editors: Bonny Champagne, MT (ASCP)
Lydia Dodson-Lehrer, MBA, MT (ASCP)

improving the accuracy, precision, and turn-around time of UPE and IF.

Are you interested in learning more about Sebia's options for utilizing neat urine samples for your electrophoresis testing? Simply circle 165 on the Reader Response Card.

Suggested Reading:

Janik, Borek. Electrophoresis and Immunofixation - The Proteins of Serum, Urine and Cerebrospinal Fluid. Sebia Electrophoresis.

Le Carrer D, Boucraut J. Urine Protein Electrophoresis & Immunofixation Illustrated Interpretations. Sebia Laboratories. 1999.

Morten S, Peter G, Lars N. Simple method for quantification of Bence Jones Proteins. Clin Chem 2002; 48: 2202-2207.

Package Inserts:
SEBIA. HYDRAGEL 7/15 HR
SEBIA. HYDRAGEL 1/2/4/9 IF
SEBIA. HYDRAGEL 1/2/4/9 Bence Jones

The first reference listed above, Dr. Borek Janik's guide, is a value-added electrophoresis reference only available to Sebia customers. In order to obtain the second reference text listed above, by Le Carrer & Boucraut, please circle 166 on the Reader Response card or access the following link to Sebia's website for electronic ordering: <http://www.sebia-usa.com/educationalMaterial/books.html>

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electrophoresis

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SEPARATIONS

Keeping you on track with the latest in EP

To Concentrate or to Not Concentrate...That is the Question

**By: Brade DeBriere, MT (ASCP)
Sebia Regional Sales Manager**

Are concentrators obsolete?

Could your laboratory run urines neat (unconcentrated)? Almost every laboratory I have visited runs urine electrophoresis differently. Some labs measure total urine protein first then decide how much to concentrate, others concentrate all samples 50-100 times before applying them to the gel, but more and more labs are deciding to run neat urine samples.

Who is correct? Could urine electrophoresis be more standardized? The answer to these questions really depends on what you are looking for in the urine sample. Utilizing Sebia's HYDRASYS[®] will result in a limit of detection of approximately 1-2 mg/dL for Bence Jones proteins and other bands, which should satisfy most inquirers.

For those who physically run the urines, most of these benefits of utilizing neat urine samples will seem obvious.

- Less bench work and quicker turn around time due to the elimination of the concentration step.
- Fewer "QNS" worries since much less urine is required for neat sample processing.
- Better visualization and interpretation - samples do not need to sit at room temperature for as long, so proteolytic enzymes from bacteria won't turn protein into "baseline sludge".
- More accurate representation of the urine proteins contained in the sample, no guessing if the sample was concentrated to the target value, and more standardization. Proteins are lost due to the cut-off retention rate of the concentrator. Even if a 10kD concentrator is used, it doesn't mean all protein molecules above 10 kD are retained. (For details, see your specific concentrator package insert for the amount of BJ protein lost.)
- Significant cost savings since purchasing concentrators is not necessary. The average cost of each concentrator is about \$5. How many samples does your lab concentrate each month?

Now let's discuss your options for running neat urine samples. Sebia offers many approaches for processing urine samples on the HYDRASYS semi-automated electrophoresis system. I will narrow down the simplest approach to running urine protein electrophoresis (UPE) and urine Immunofixation (IF) assays with neat samples; however, many variations are possible.

UPE: 10uL of neat sample is utilized with the Sebia High Resolution (HR) kit. The gel is processed utilizing the HYDRASYS instrument with the **HR3** program and stained with the **HR Acid Violet** program. Sebia's sample processor and dilutor, the HydraPLUS[™], can be used in tandem with the HYDRASYS in order to eliminate all sample pipetting and achieve positive ID. According to the Sebia HR package insert, when the HR3 program is utilized the sensitivity of detection is 1.5 - 2.0 mg/dL (for albumin and Bence Jones protein); however, when validated, this will usually exceed 1.0 mg/dL. Although acid violet differs from amido black performance, the linearity of the gel is equally impressive. And, acid violet is a much more sensitive stain. Please refer to the 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.

Continued on back

Raise Your Hgb IQ

By: Katherine K. Freeman



Since I'm the new kid on the block, you should know a little about me. I'm a life-long resident of Alabama who attended the University of Alabama while my hero, Bear Bryant,

was football coach. For all of my life, my professional and personal interests have been many and varied. Professionally, my Baccalaureate training was in the areas of bacteriology, biology and chemistry; while Masters work, in immunology, was performed at the same institution followed by biochemistry research in the Institute for Dental Research. Personal interests include fishing, hunting (with a camera), reading, music, needlework, cooking and trading recipes with good cooks.

My professional journey began when I joined Medical Laboratory Associates in 1973, which eventually became Laboratory Corporation of America. By the mid-1970s our department needed more knowledge of hemoglobinopathy detection, and I was sent to the Centers for Disease Control (CDC) for instruction. This proved to be a turning point in the department and in my life. As supervisor of Special Chemistry, I've served on corporate Special Chemistry and Hepatitis Discipline Committees.

Over the past 31 years, I've had a great time, and having taken early retirement from LabCorp August of 2004, I'm embarking on a new phase of my life. Since having grandchildren, I know why they're called *grand*. One of my greatest pleasures has been writing and illustrating stories for them. Now that they are a little older and I'm retired, we're looking forward to creating tales together. I'm also looking forward to sharing my practical lab experiences with you and answering some of your questions. In fact, in recent months, it has been my honor to share my hemoglobinopathy knowledge with laboratorians at Sebia Area Technical Conferences across the country. I look forward to meeting and talking with more of you while participating in more Sebia conferences in the future.

Hemoglobin Pattern Identification

When a sample arrives in the laboratory for hemoglobin fractionation, the challenge for the technical staff can be intimidating. The clinical significance for the patient is obvious. With hundreds of possible hemoglobin variants and dozens of thalassemia abnormalities, the diagnostic possibilities seem almost endless. The possible effects on the future of the patient can be an awesome responsibility. If genetic counseling becomes necessary, the technical requirement to "get it right" can be the greatest challenge.

In this day of economic considerations and proficiency requirements, it may be necessary to consider these factors when deciding which tests are feasible to offer in the hemoglobin confirmation menu for the laboratory. Patient demographics and other test results may enter into determination of the final reportable result as well. In the southeast, for instance, up to 25% of samples submitted for analysis may show some abnormality. In areas with a less diverse patient population, a lower number may be the rule.

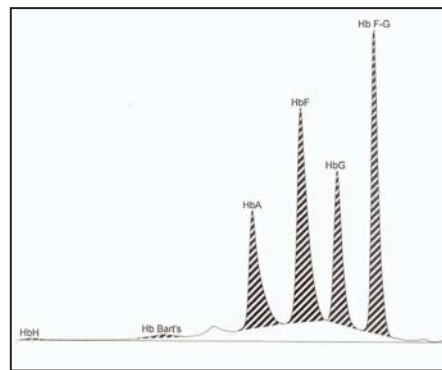
Number of samples received for analysis per day may greatly influence the number and type of confirmation tests offered. If the number of requests is only 2-3/day, for the sake of economy, the lab may hold the samples for 2-3 days in order to get a full gel "run". This decision to delay testing may compromise the final result on a sample containing an unstable hemoglobin or Bart's hemoglobin in small quantity. These findings would, admittedly, be rare, but must be considered when analyzing result data.

If only 10% of samples initially analyzed are found to be abnormal, confirmation testing cannot wait for a full gel, but must be performed right away. If the abnormality appears to be Hemoglobin S on the initial screen electrophoresis, confirmation by Sickie Solubility or Antibody Screen for Sickie Hemoglobin may be used and not wait for acid hemoglobin electrophoresis. If the sample appears to have Hemoglobin S and Hemoglobin C on the alkaline gel, the confirmation tests mentioned may not be adequate. If the sample is positive for sickle hemoglo-

bin, the result may not be SC, but could be Hemoglobin D^{Los Angeles} and the sickling Hemoglobin C^{Harlem}. It is necessary to use alternate means, such as an acid gel to confirm the identities of the abnormal bands.

Laboratories that receive 50 or more samples/day would probably not use alkaline electrophoresis as a screening method. Isoelectric focusing or HPLC can identify many more common abnormal hemoglobin bands on the initial pass to a relatively high level of certainty. It is still a very good idea to confirm the abnormal bands detected by at least one alternate method. A recent case highlights the point. A sample from a newborn was tested by HPLC initially. Six hemoglobin bands were detected. The two fastest were identifiable as Hemoglobin Bart's at 6.2% together. The next fastest was consistent with Hemoglobin F at 31.8%. The fourth band had the exact retention time of Hemoglobin A at 15.3%. Band 5 had the migration characteristics of Hemoglobin G^{Philadelphia} between the A and S regions. The quantity was 13.8%. The last band moved between the S and C regions and was 28.2%. Two possibilities existed. The most likely was the alpha chain variant, Hemoglobin G^{Philadelphia} - Hemoglobin F hybrid. The presence of Bart's Hemoglobin and the high concentration of the variant relative to Hemoglobin A suggested the additional abnormality, alpha thalassemia. The other possibility was contamination from maternal blood for the sixth band.

Continued on back



Newborn sample - Hb F-G^{Philadelphia}
Capillary electrophoresis by Sebia CAPILLARYS



Maryville, Tennessee

Sebia Electrophoresis Speeds Processing Time... And Eases Time Off... At Tennessee Lab

Hydrigel K20 gets a "10" from Blount Memorial

If this were a perfect world, everything would happen according to a nice, neat established plan. Work levels would be well-timed increments. Staffing levels would be like Goldilock's favorite porridge... "just right."

But of course, the real world doesn't operate this way — especially not in healthcare. Medical needs usually aren't anticipated in advance. And it's a 24-hour business run by people with lives to lead outside of work — which means there are always vacations, maternity leaves, sick days and other staffing challenges to work around.

Case in point: the immunology lab at Blount Memorial Hospital, a 300-bed facility outside of Knoxville, Tenn. Once upon a time, the lab had to dedicate one staff person to handle the very hands-on task of immunofixations (IFs). Why? Its manual electrophoresis system could only process one patient per gel, and required constant babysitting — resulting in a workflow as slow as molasses.

Living with the burdens of such a high maintenance electrophoresis system was hard enough on "full staff" days. But whenever the lab found itself short-handed, the pain became almost unbearable — especially since the lab was also responsible for running IFs (on one-day turnaround) for a nearby medical center in addition to all other immunology tests for Blount. "Most of these tests are very hands-on," explains Leigh Black, MT (ASCP). "With only a couple of us here at a time, we really don't have a minute to spare."



Peggy Herron and Leigh Black, MT (ASCP)

So when Sebia showed up at Blount Memorial with the Hydrigel K20, a more efficient solution for electrophoresis, Black and her colleagues took notice. Designed to provide innovative technology in an economical "package" for lower-volume labs, the Hydrigel K20 comprises a num-

ber of components that, working together, yield the highest resolution and performance consistency ever for a manual gel process.

The complete system features: high performance, programmable power supplies (suitable for both routine and research electrophoresis applications); the K20 Applicator; the Electrophoresis Chamber; the Incubator-Dryer IS 80; and the Accessory Kit. And though Blount had specific needs, there's more to the system than just IF — a full assay menu also includes Protein, Hemoglobin, LDL/HDL Cholesterol, Lipoprotein, Isoenzymes and Bence Jones.



The Hydrigel K20 offered several advantages the lab's existing vendor didn't — most notably speed. The Sebia system is set-up to run multiple patients simultaneously: gels come in one- and two-patient sizes, and the system can process any combination of gels together. For example, the lab often runs doubles and singles in one session, enabling odd-numbered runs. And when Blount opens its new Cancer Center in late 2003, processing four IF tests at a time will likely be the norm as volumes increase.

Besides boosting the number of patients processed at a time, the Hydrigel K20 requires less hands-on work than the lab's prior system — and has cut incubation time by a third. "Before, we had to go through this drawn-out three-step washing phase that took full attention and time," Beverly Sinders, MT (ASCP) recalls. "Now we just soak the gel in saline for five minutes — which can be done unattended — then blot it for four minutes and throw it in the dryer.

"Plus, it's much easier to apply the antisera on the Sebia system," she adds. "The antisera easi-

ly travels down each lane through surface tension, whereas before we had to manually tilt the plate to disperse the antisera fully in each well. And Sebia's patented applicator comb means sample application is evenly distributed to the gel...and virtually error-free." Shaving 15 or 20 minutes off one session might not seem like much, but when you consider that one technologist runs multiple sessions a day, it adds up to an hour or more of newfound time per shift. In Blount's case, this was an important benefit as one of the lab's five technologists went on maternity leave not long after the system was implemented.

"Now that we're working with the Hydrigel K20, it isn't as big a deal when someone has to take time off," Sinders says. "The increased efficiency takes the pressure off a bit, and gives us more breathing room."

Something else happened once Sebia came on the scene: each technologist's vision seemed to vastly improve. Then again, maybe it was just the gel quality that got better.

"Our previous vendor's gels were thicker and almost impossible to get clean, even with the decolorant provided," Sinders notes. "We were always having to deal with a pesky blue tint that obscured our ability to clearly read the results. By contrast, **Sebia's gels consistently come out much cleaner and clearer — and that's imperative when you're dealing with a patient's test.**"

Dr. Ernest Fuson, director of the lab agrees. **"We're very pleased with the improvement of quality of the Sebia gel compared to our old system."**

With lab personnel stretched to the max running and interpreting tests, Black points out that the support provided by Sebia's team has been invaluable — particularly when it comes to ensuring timely re-orders. "They keep in regular contact with us," she says, "whereas with our old vendor, there was a long period of time when we didn't even know who our rep was.

"And anytime we have questions, Sebia responds to us immediately," she adds. "We couldn't be happier."

Are you interested in learning more about Sebia's Hydrigel K20 or our automated electrophoresis systems? Simply circle 168 on the Reader Response Card.

We need your feedback

In order to better serve you, we constantly update our Sebia Separations mailing list. Please complete the card and return to us.

Please circle the reader service numbers of those items on which you would like more information.

ARTICLE **165** ARTICLE **166** ARTICLE **167** ARTICLE **168**

Number of electrophoresis tests run per week

Protein _____ Immunofixation _____ Hemoglobin _____

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Check if this is a new address

Comments

Thank you for your assistance.

Is there a hemoglobin issue that has you stumped? We would be pleased to assist you with raising your hemoglobin IQ! Please contact us through the following e-mail address: hemoglobinIQ@sebia-usa.com. Please include your full name, laboratory name & location, and telephone number.