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We Need Your Feedback

Ask Borek (continued)

to separate the products of the two clones.

(ii) One case of biclonal gammopathy was observed where one clone produced IgM/κ and the other clone free λ. All bands appeared to be at the same migration level. The free light chain gave a positive reaction with anti-free lambda antiserum.

(iii) Oligoclonal bands may appear in the presence of a monoclonal band. Typically the monoclonal appears stronger. The monoclonal and one of the oligoclonal components may contain the same type of heavy chain, which is usually M, but different light chains. Rarely all bands may appear to be at the same migration level. Even a high resolution (HR) electrophoresis was unable to separate clearly the components and the pattern was elucidated by inference.

Reference Available

Sebia does offer one of the few electrophoresis texts available today. The book is designed to expand the reader's interpretive skills and to be used as a frequently called upon reference book to assist with difficult clinical cases as discussed above. If you would like to receive information about how to add this text to your reference library, please circle number **120**.

About St. John Health Hospital and Medical Center (continued)

Established in 1952 by the Sisters of St. Joseph in Detroit, St. John Hospital is a regional-referral teaching hospital affiliated with Wayne State University. What began as a single institution five decades ago has evolved into a comprehensive health system with numerous locations and services serving southeastern Michigan.

Today St. John Hospital houses 50 medical and surgical specialties — including the only full-service pediatrics and perinatal center and designated Emergency Trauma Center on the city's east side. Each year thousands of patients visit St. John for renowned expertise in heart disease prevention and treatment, high-risk pregnancy and neonatal intensive care. A state-of-the-art Cancer Center is slated to open in 2001.

In 1999, St. John Health System joined the Daughters of Charity National Health System to create Ascension Health, a collaborative health ministry that includes hundreds of health care facilities in 15 states and the District of Columbia.

Congratulations

Congratulations go out to the lucky BubbleMania winners at both AACC and CLMA. The prize was a vintage bottle of Dom Perignon champagne. All Sebia booth visitors who registered were eligible to win this fantastic prize. Congratulations to the following winners:

CLMA

Ms. Judy Wetzel, Methodist Medical Center of Illinois, Peoria, IL
Ms. Sandra Frommeyer, Compunet Clinical Laboratories, Moraine, OH
Ms. Debbie Jewell, Saint Joseph's Regional Health Center, Bryan, TX

AACC

Ms. Barbara Mervin, Johns Hopkins Hospital, Baltimore, MD
Dr. Barry Elkins, St. Vincent's Hospital, New York, NY
Mr. Thomas Ledue, Rheu Disease Foundation for Blood Research, Scarborough, ME

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sebia® electrophoresis

Separations

BubbleMania hits the AACC and CLMA Scene!!

By: Lydia Dodson-Lehrer, Director of Marketing

There was quite a fuss going on in St. Louis and Chicago this year at CLMA and AACC as Sebia burst onto the scene with the preview of several new products including the CAPILLARYS™ and HydraPLUS.

CAPILLARYS, the new generation of capillary electrophoresis system which will be available early next year, was designed and developed to optimize and completely automate liquid flow electrophoresis in the clinical diagnostic laboratory. The system is a continuous feed capillary system allowing complete walkaway automation, from bar coded primary tube to result.

What makes the CAPILLARYS so special is actually something rather small. It is Sebia's patented "bubble capillary" which has a five times larger diameter at the area of direct protein detection. This innovative design not only enhances protein detection and assay sensitivity, but also allows the most accurate interpretations available today.

The HydraPLUS, also previewed at both national meetings, is a pipetter-dilutor system designed and manufactured by Sebia as a companion product to the highly successful HYDRASYS® Electrophoresis System. Designed and manufactured by Sebia, the HydraPLUS prepares sample dilutions and delivers the prepared dilutions or neat sample directly to Sebia sample applicators effectively reducing repetitive motion injuries, improving testing precision and eliminating any possible pipetting errors.

But there was more, the Hyrys 2 HIT was also introduced. This system is much more than a densitometer as it has an attached scanner which dramatically expands the capabilities of the system allowing up to 10,000 immunofixation results to be imported and stored. Plus the Hyrys 2 HIT has modem and networking capabilities.

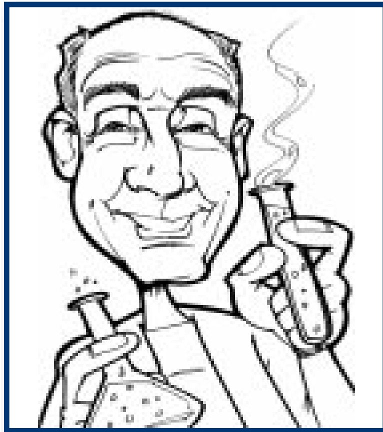
So, if you were unable to see what all the fuss was about in person, we would be glad to send you information on any or all of these systems. Simply circle number **117** for the Capillarys, number **118** for the HydraPLUS, and number **119** for the Hyrys 2 HIT.

Clearly Superior...

AUTOMATICALLY BETTER™

Ask Borek

By: Borek Janik, Ph.D.



"WEAK" AND "WEIRD" BANDS OF IMMUNOFIXATION Part III

In the first two parts of this series of articles on marginal and atypical immunofixation patterns you learned about weak bands and restrictions representing the low concentration extremes of monoclonal components (Part I) and the real and apparent free light chains (Part II). The concluding part of this series will deal with complications certain physico-chemical properties of immunoglobulins can cause. And, of course, there will be a few words about atypical cases that do not fit into any other category.

Polymers, complexes, aggregates and high Ig concentrations

False bands might appear at or close to the point of application (POA) when the concentration of monoclonal Ig is high (≥ 2 g/dL). This is more often observed with a polymerized IgM pentamer or an IgA dimer or trimer, as these can further aggregate, than with a monomeric IgG. At a high concentration and with reduced solubility due to polymerization, the monoclonal Ig that did not react with the nonspecific antiserum often does not wash completely out of the gel and is stained. This results in a false band appearing in each track that is not specific for the monoclonal Ig. Interesting to note is that this false band may be missing in the G track because the sample is twice as dilute as in the remaining tracks. False bands can be easily identified as they are slightly wider than the antisera track itself and therefore wider than true immunofixed bands.

IgM can also trap other Ig's. The trapped Ig forms a complex with its antiserum, but appears at the migration position of IgM. Cryoglobulins, due to their poor solubility, may also cause false bands. When interpretation is ambiguous, repeat the IF after appropriately treating the sample: dilute high levels of Ig, depolymerize polymers or solubilize cryoglobulins.

- At very high concentrations of monoclonal Ig (>3 g/dL) prozoning may occur. This will appear as a band with an unstained hollow in the middle of it similar to an oval doughnut or two close bands.
- Bence Jones Protein (BJP) may bind to other proteins, particularly those with a terminal SH group, e.g., transthyretin, albumin, α-1 antitrypsin, transferrin and enzymes such as lactate dehydrogenase. The band(s) of these complex(es) appear in addition to the major BJP band itself. Treatment with 2ME dissociates the complex. Complexes of α-1 antitrypsin are often seen in patients on chemotherapy.
- Uncommonly, IgM/κ or IgM/λ may complex with polyclonal λ or κ, respectively. Then, one band is present in the M, K and L tracks. These bands are at the same migration distance and the non-monoclonal light chain band is weaker. The same phenomenon has been also observed with monoclonal IgG, but so far, not with IgA. When further analysis is required, the use of Fluidil will dissociate the complex in most cases. When IgM is involved, the sample should be treated with a mixture of Fluidil/2ME before repeating the IF.
- It has been observed in a small number of cases that plasma fibrinogen could complex with polyclonal IgG or polyclonal free light chains. As a result, a faint band can be seen in G, K and L tracks or, in some cases, only in the light chain tracks at the same migration position as the heavy band of fibrinogen in the reference track. Serum contaminated with plasma is unlikely to show this phenomenon.

Other atypical cases

Monoclonal immunoglobulin generally migrates in the same relative position regardless of the procedure used: HYDRAGEL Protein(e), HYDRAGEL β1-β2 and HYDRAGEL 2/4 IF. Rarely, monoclonal IgM has been observed to migrate more anodic on HYDRAGEL 2/4 IF gels than on HYDRAGEL Protein(e) or β1-β2 gels. Two factors are involved: (i) slightly different composition in the additives of the individual gels and (ii) the mobility of monoclonal IgM tends to be concentration dependent, i.e., neat serum is used with the Protein(e) and β1-β2 gels while diluted serum is used for IF analysis.

Several cases have been observed, all including monoclonal IgM, showing a single band in the M, K and L track each that migrated at the same level. It appeared as if one clone of B-cells was producing not only a monoclonal IgM, but also both kappa and lambda light chains - a biological impossibility. Although these were rare cases, they merit further description to illustrate the occasional complexity of monoclonal gammopathies. Three different underlying mechanisms have been identified:

(i) A biclonal gammopathy producing IgM/κ and IgM/λ that happened to co-migrate (in the observed case the heavy chain band was heavier than either of the light chain bands). High Resolution electrophoresis/immunofixation was able

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In the meantime, keep sending in those questions. You may send them to me by mail at Sebia, 400 - 1705 Corporate Drive, Norcross, GA 30093, attn. "Ask Borek", by fax at 770-446-8511 attention "Ask Borek", or by e-mail at bjanik@sebia-usa.com. Whichever method you choose, include your name, laboratory name and phone number should I have questions for you.

Customer Focus

Sebia Electrophoresis Gets High Marks From Teaching Hospital

St. John Hospital and Medical Center, Detroit, MI

There's no such thing as spring, summer or winter break for the core lab of a Wayne State University-affiliated teaching hospital. In fact, the tests keep being piled on.

St. John Hospital and Medical Center, part of Detroit's St. John Health System, had terrific success with marketing efforts to generate additional work for its Rapid Response Laboratory. But there was a down side: this newfound cornucopia put a great deal of strain on an already lean lab staff.

"Our previous (manual) methodology worked fine when we were processing 18 patient samples for serum protein electrophoresis a day," says Edward Bessette, technical specialist for the lab. "But suddenly that number escalated to 35 or 40 — and that's a new ballgame entirely."

> "'Hands-off' gel processing means technologists don't have direct contact with solvents that pose a health hazard."
>
> —Edward Bessette, technical specialist, Rapid Response Laboratory

Through industry contacts, Bessette and his associates were already familiar with a couple of automated electrophoresis systems; they decided it was time to evaluate their options. Sebia's HYDRASYS® system and a competing solution were put to the test — and the HYDRASYS came out ahead.

Designed as a 'walkaway' system, the HYDRASYS expedites the many tedious steps involved in electrophoresis and immunofixation, automatically handling everything from sample application to migration to incubation to staining, destaining and drying.

Sebia's method of sample application scored big points with lab personnel because it relies on a wick instead of the traditional well. "With the HYDRASYS, the sample stays absorbed to the wick, so we don't run the risk of evaporation — which is a common problem with using wells," Bessette explains. "Now we can load one set of 15 samples, put them in the fridge while we load another batch, and not worry about them drying up in the meantime."

Bessette was also impressed with the gel quality of the HYDRASYS — not only for its clarity, but consistency as well. "Because the sample application does not rely on human skill, we get excellent uniformity of patterns from plate to plate, operator to operator," he notes. "Now our people can focus their attention on other tasks instead of processing plates. We're making better use of their time." (And enhancing their safety too, he adds: 'hands-off' gel processing means technologists don't have direct contact with methanol or other solvents that pose a health hazard.) In terms of gel resolution, Sebia clearly exceeded the lab's expectations: having been accustomed to edge effects and poor quality separation between hemoglobin A and hemoglobin F with previous methods, technologists were pleasantly surprised at the crispness of the separation on Sebia's alkaline and acid gels. "Band resolution with Sebia far surpasses our previous system," says St. John staff technologist Gloria Witczak.

> *"…technologists were pleasantly surprised at the crispness of the separation on Sebia's alkaline and acid (hemoglobin) gels."*

Besides making lab techs happy, HYDRASYS also enabled St. John to successfully absorb their huge increase in workload without missing a beat. Now, instead of covering six patients in one run, the lab can test 28 patients per batch. "We can comfortably complete all the day's work without rolling it over to the next shift," Bessette says. "This enhances our service and reliability in the minds of physicians and other customers."

And, as a byproduct of the outstanding clarity and consistency of HYDRASYS results, the lab has decreased the number of repeats — and thereby lowered its material costs. "The instrument allows us to get it right the first time, so we're generating less waste and saving money on gels," Bessette notes.

But in his view, the most important savings a lab can reap from this instrument is the elimination of wasted time. "Tech time is the most valuable commodity we have today…and it's also the most scarce," he says. "Now you have an opportunity to automate a laborious process, conserve manpower and at the same time reduce the chance of human error. Take it."

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We Need Your Feedback

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

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

article 117 *article* 118

article 119 *article* 120

Number of electrophoreesis tests run per week.

Protein____ Immunofixations_____ Hemoglobin_____

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Thank You for Your Assistance