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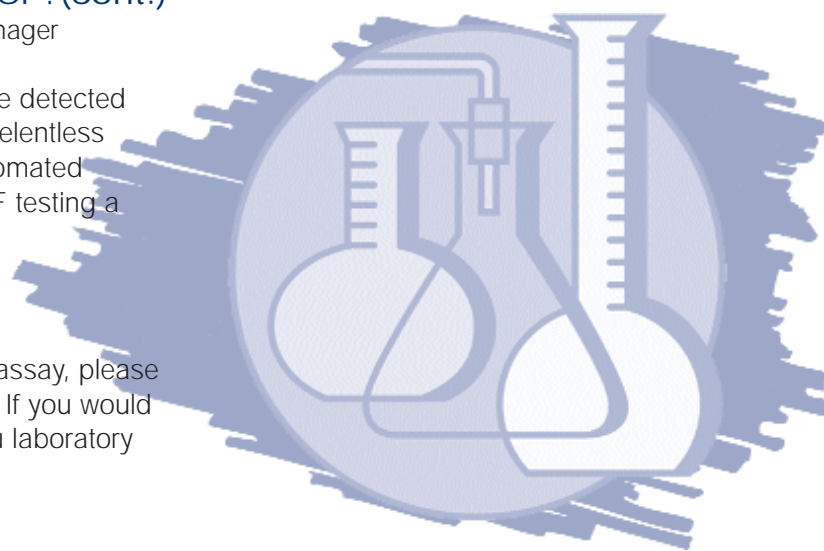
Are Those Really O-Bands In That CSF?(cont.)

By: Paul Mehta, M.D., Technical Support Manager

analysis as concentrations down to 35 mg/dL can be detected with this enzyme enhanced method. Sebia, with its relentless approach to bring innovative, cost effective and automated testing to the field of electrophoresis, has made CSF testing a manageable procedure.

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Separations

Are Those Really O-Bands In That CSF?

By: Paul Mehta, M.D., Technical Support Manager

Various central nervous system pathologies are associated with altered CSF composition. Some of the more common pathologies are Multiple Sclerosis, Neurosyphilis, neoplasms, and AIDS. These conditions have one element in common, the production of oligoclonal bands (O-bands). These bands are a result of the production of intrathecal immunoglobulins and are useful in the confirmation of a diagnosis previously established by other clinical and laboratory methods.



efficiently test CSF samples, Sebia has developed and offers a unique CSF assay that simplifies this process by combining both HR and IF testing into a single assay using unconcentrated spinal fluid. The concentration of CSF is not only time consuming, but concentrators generally are quite expensive. Due to the high sensitivity of Sebia's CSF assay, samples are assayed unconcentrated or even need to be diluted prior to testing.

Up to six unconcentrated CSF specimens can be analyzed simultaneously with their corresponding serums. A high resolution electrophoresis is first performed on paired CSF/serum samples. Assay sensitivity is then enhanced more than one hundred times above traditional immunofixation by utilizing enzyme tagged antisera in the immunofixation portion of the assay. Enzyme tagged anti-IgG is overlaid on each sample pair and visualization is accomplished utilizing a peroxidase reagent. By comparing the patterns of the paired samples, true immunoglobulin based oligoclonal banding can be confirmed or ruled out. In most cases, the production of intrathecal immunoglobulin is IgG in origin, although infrequently IgA or IgM may be observed. These enzyme tagged antisera are also available as well as anti-kappa and lambda for further customized testing.

One additional and unique application of this CSF assay is for the testing for the Tau fraction in nose or ear secretions in injured patients with suspected skull damage to confirm damage to the blood-brain barrier. Again, it is not necessary to concentrate the fluid prior to

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Ask Borek (continued)

Based on the studies performed by the Sebia R&D Team, it has been proven that these faint restrictions are indeed due to a specific reaction of the antiserum with corresponding light chain. These are not false bands and are caused by complete immunoglobulins of the IgG type. Since the IgG is present at a very low concentration only a band in the light chain track might be discernible. The G track restriction might either blend into the relatively dark background of the polyclonal IgG or, due to a greater sample dilution in the G track, might become undetectable. Increasing the sample concentration for the G track is generally not helpful because as the monoclonal Ig concentration increases so does the polyclonal background.

One may still wonder why the lambda restrictions are more frequent than the kappa when the normal ratio of the polyclonal light chains is 2:1 in favor of kappa. The reason is exactly this, more kappa results in higher polyclonal background that can obscure the faint restrictions. A faint single restriction suggests Monoclonal Gammopathy of Undetermined Significance (MGUS) while multiple faint restrictions suggest oligoclonal banding (restricted heterogeneity or reactive patterns), unless of course, the patient's symptoms and clinical diagnosis suggest otherwise. As in any "gray" situation where the presence of monoclonal-like restrictions or bands cannot be explained with certainty, retesting in 6 to 12 months would be a prudent suggestion.

The studies for elucidating the origin of light chain restrictions included (but were not limited to) the following experiments:

I Light chain antisera from at least 5 different suppliers were used. All gave identical patterns in the light chain tracks.

II Light chain antisera produced from different animals (goat, sheep and rabbit) were used. All gave identical patterns in the light chain tracks.

III The G track background was lightened by immunoprecipitating the unwanted light chain from the sample. For example, if the serum showed questionable restriction(s) in the lambda track as described above, all Ig/kappa was removed by immunoprecipitation with anti-kappa antiserum. The gamma track then showed faint restrictions corresponding to those seen in the lambda track.

I will close for now. In the next issue of "Separations" I will discuss polymers, complexes, aggregates and other atypical cases of "weirdo" banding, so stay tuned.

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 **116**.

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

Would you believe that the most frequently asked question on a worldwide basis is "Bitte sehr wo is eine Toilette?" or "¿Dónde está el baño, por favor?" or any international equivalent of this urgent phrase? It might not be so, but Sebia's Technical Support Representatives feel strongly that the runner-up in this contest of popularity must be questions concerning weak and weird bands on immunofixation gels. The weak bands and restrictions and their clinical significance were discussed in the last issue of Separations, Issue 4, Vol. 1. I hope you are now anxious to know what I have to say about the "weirdos". This is quite a lengthy topic so I will again have to break the "weirdos" into two parts.

All unusual bands described here contain a monoclonal Ig component, yet they are atypical based on their migration position, appearance and/or difficulty is experienced in explaining their origin. The deviation from "standard" characteristics is often due to the inherent properties of immunoglobulins, such as their propensity to polymerize, aggregate or form complexes that may result in decreased solubility. Furthermore, the monoclonal immunoglobulin-producing clone can yield excessive amounts of free

light chains or it can produce two types of heavy chains. Dramatic changes in the immunofixation pattern may be also seen in the course of chemotherapy and in post-transplant patients.

The following list of unusual situations is by no means complete or definitive. If you have observed unusual patterns not described here or in the next part of this three part series, please share them with me.

Real and Apparent Free Light Chains

A band could appear in both the free and bound light chain tracks in addition to the band representing a light chain component of the complete monoclonal immunoglobulin. When no corresponding heavy chain component can be detected with any of the heavy chain antisera, one can reasonably assume that such a band represents a free light chain. However, on occasions, the free light chain form cannot be confirmed using free light chain antiserum. Subsequent testing with D and E antisera is also negative. The lack of reaction can be explained by one of the following:

It could be a matter of sensitivity of the free light chain antisera. The latter are generally less sensitive (> 5 mg/dL) compared to free and bound light chain antisera (1 – 2 mg/dL). Therefore, when the free light chain is present at a low concentration (< 5 mg/dL) it might be detected only with the free and bound light chain antiserum. The lower sensitivity results from the manufacturing process. Immunization is performed with the light chain component that is prepared by cleaving it from complete polyclonal immunoglobulins. The antiserum obtained is then purified by immunoadsorption with complete immunoglobulins.

If the presumed free light chain is strong (> 5 mg/dL) yet no reaction with free light chain antiserum is obtained then the free light chain could be present in a polymerized form such as a dimer or larger polymerized form. Polymerization could block the epitopes that normally would react with free light chain antisera. To prove this, de-polymerize prior to electrophoresis.

IFree light chain antisera tend to have a narrower spectrum of specificity than free & bound light chain antisera. For this reason, on rare occasions, the free light chain antiserum might not react with the free light chain that is detectable by the free and bound light chain antiserum.

Occasionally, one or more faint restrictions (bands) may be seen in the lambda track with no apparent counterpart in any of the heavy chain tracks. Similarly, but less frequently, one or more restrictions may be seen in the kappa track. In a few cases, the "free light chain" bands are seen in both lambda and kappa tracks. Such observations could be understandably of great concern as they are suggestive of the presence of free light chains. Although free light chain monoclonal gammopathy is a plausible explanation and multiple bands can be explained as products of polymerization or products of several clones of plasma cells, the frequency at which such patterns are seen would be very alarming, hence highly unlikely. There is no other known causative biological mechanism that can explain the presence and frequency of "free light chains". Therefore, the interpreter may want to resolve this issue by assuming nonspecific reactivity of the antisera; however, this surely should be an unacceptable thought.

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Customer Focus



James Buchanan Duke

Like those nagging issues of efficiency, productivity and cost, for instance. "We're facing the same economic pressures as everyone else," says Mueller MT(ASCP), technical director of outpatient labs for Duke University Hospital in Durham, North Carolina. "Costs are escalating, so we always want to be more productive and maximize our work capacity. Yet staffing is scarce. We needed to find ways to improve our work processes to allow for greater cost- and people-efficiencies."

Given that Duke's Morris Building Clinical Lab had not upgraded its electrophoresis process in ten years, Mueller and her colleagues were enthusiastic about the huge potential afforded by new automation. They compared the Sebia HYDRASYS® to other similar solutions, and the HYDRASYS proved to be the best choice to meet their needs.

The instrument, introduced in 1999, automates the entire electrophoresis process, from sample application to migration to incubation to staining, destaining and drying.

At first sight, it was the superb resolution of the Sebia gels that piqued the team's interest — but what really struck Mueller was the technical knowledge of Sebia's personnel. "In addition to an instrument, we

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—Janet Mueller, MT(ASCP), technical director of outpatient labs



At Duke University Health System, Sebia electrophoresis "Rules" Duke University Hospital in Durham, NC

also needed a partner who could help us advance our lab with new information and insight," she says. "Sebia has proven to be very helpful in this area."

On a day to day level, the HYDRASYS saves technicians time on routine processing — freeing them up for other tasks such as paperwork. "We've been getting a higher number of IFE orders since the opening of our myeloma clinic, and I'm not sure we'd be able to handle the increased volume without this instrument," says Lorri Givens, MT(ASCP), senior medical technologist. She particularly likes the 'walkaway' functionality of the HYDRASYS, which has eliminated tedious manual steps such as blotting and washing.

"Muller was particularly struck by the technical knowledge of Sebia's personnel."

According to medical technologist Michele Jones, MT(ASCP), the HYDRASYS has enabled the lab to handle as many as four times the number of gels that could be done in the manual days. "Before, we could process maybe five gels on a good day," she recalls. "Now we typically process 12...and have done as many as 22 in a single day, which is outstanding."

While the most pressing concern for the lab was to conserve precious tech time, the HYDRASYS also made it possible to combine the phoresing and staining workstations for more effective use of space. Eventually, the lab will combine hemoglobins and protein electrophoresis onto one system, another attractive feature of the instrument.

Mueller's intuition about Sebia hasn't failed her; the company's new state-of-the-art digital imaging system, the Phoresis, will enable the lab to achieve future goals — such as better physician access to results. "This instrument is exciting news because it will eventually allow us to deliver results to doctors via modem for remote interpretation," she explains. "The system offers huge potential." Designed as a flatbed scanner, the Phoresis allows scanning and storage of electrophoresis results from protein, hemoglobins, isoenzymes and all types of immunofixation assays — processing as many as 324 samples in 4 minutes. With it, Duke will be able to store and recall up to 100,000 electrophoresis curves and results and 4,000 immunofixations on a single system.

"Sebia is really committed to the furthering developments in electronic transfer," Mueller says. "This is exactly the kind of thinking that will help us increase our business successfully and profitably in the future."



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Number of electrophoresis tests run per week.

Protein____ Immunofixations____ Hemoglobin____

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Thank You for Your Assistance

