



Type III

These mixed type cryoglobulins are composed of polyclonal RF (typically IgM) and a polyclonal IgG (sometimes IgA). They represent about 50% of cryoglobulinemia cases and are present in low concentrations (<0.1 g/dL). The primary condition for Type III cryoglobulins are lymphoproliferative, auto-immune (rheumatic) diseases and persistent chronic infections with immune complexes (e.g., bacterial endocarditis).

CRYOGLOBULINS

Cryoglobulins are immunoglobulin that can aggregate at temperatures as high as 30°C. In cold (4°C) they become reversibly insoluble and either precipitate or gel, hence they are referred to as cryoprecipitate or cryogel, respectively. This transition may occur at much higher temperatures (below 37°C). Therefore, cryoglobulins can also precipitate in vivo in blood vessels with very serious consequences.

Similar to cryoglobulins are cryofibrinogens. These are fibrin-fibrinogen complexes that have reversible cold precipitability in anticoagulated blood. In true cryofibrinogenemia, the soluble fibrin-fibrinogen complexes precipitate in the presence of fibronectin. They are seen in hematologic and solid malignancies, and thromboembolic diseases.

There is an inherent possibility cryoglobulin will gel or precipitate at room temperatures. A serum containing gelled cryoglobulins may become so viscous that its diffusion into the sample applicator teeth is hindered. The resulting electrophoregrams will be lightly stained and a marked decrease in albumin will be observed. Precipitation of cryoglobulins, often seen as sample turbidity, causes similar effects. A small portion of the cryoprecipitate can be deposited on the gel but it tends to stay at the point of application. Problematic samples have to be treated with Fluidil (Sebia PN 4587). Fluidil, which contains a chaotropic agent, solubilizes both the cryogels and cryoprecipitates. However, when polymerized IgM is involved a reducing agent must be used such as 2-mercaptoethanol.

Type I

These are composed of a single class of monoclonal immunoglobulin; IgM and IgG are most common, IgA and free light chains are less frequent. Type I cryoglobulins account for approximately 25% of cryoglobulinemia cases. The monoclonal protein is present in high concentrations (>0.5 g/dL). They are mostly present in patients with lymphoproliferative diseases, particularly multiple myeloma and Waldenstrom's macroglobulinemia.

Type II

These mixed type cryoglobulins contain a monoclonal component, which always has a rheumatoid factor (RF) activity, and a polyclonal IgG. The monoclonal RF is usually an IgM with kappa light chains. The Type II cryoglobulins are present at much lower concentrations than the Type I. They are most often found in patients with infectious conditions (hepatitis C). Occasionally they are found in autoimmune and lymphoproliferative diseases.

Apart from the cases where the presence of cryoglobulins is obvious, e.g., high serum viscosity or presence of a precipitate, the following may aid in deciding whether or not a sample should be treated and predicting the outcome of electrophoresis of untreated samples:

Monoclonal Type I cryoglobulins generally precipitate within 24 hours at 4°C. Type II and III

mixed cryoglobulins that are often in low concentrations require longer low temperature incubation times (up to 72 hours). Such samples do not require any treatment if the electrophoresis is performed prior to low temperature incubation. Generally, presence of small amounts of cryogel (e.g., with no apparent increase in viscosity) will not adversely affect the results of electrophoresis.

Very small amounts of cryoprecipitate settled at the test tube bottom may become unnoticed. Swirl the test tube. If a small amount of precipitate is noticed, heat the sample to 37°C and perform electrophoresis.

When cryoglobulins are suspected, the evidence of their presence is inconclusive or are present in small amounts, heat the sample to 37°C prior to electrophoresis. In the absence of Fluidil however, gelling or precipitation may possibly take place as the sample diffuses into the applicator teeth.

Sebia immunofixation procedures offer a convenient way for the identification of the immunoglobulin components of cryoglobulin. The identification requires isolation of the cryoprecipitate (cryogel), its solubilization and immunofixation with appropriate antisera.

Store the suspect serum at 4°C (for up to 72 hours before presence of cryoglobulins can be excluded), centrifuge and recover the cryoprecipitate. Wash the precipitate three times with icy saline (centrifuge after each wash). Dissolve the washed precipitate in 100 mL of warm saline (≥ 37°C). Vortex to promote solubilization. If a significant amount of precipitate does not dissolve, add more saline. Add 10 mL Fluidil to 100 mL of the dissolved cryoprecipitate and mix. If the presence of polymerized IgM in the cryoprecipitate is suspected, add 10 mL of 1% BME in Fluidil to 100 mL of the dissolved cryoprecipitate and mix (the temperature of the solution must be <30°C). Perform an immunofixation procedure without any further sample dilution using the Bence Jones migration program for its increased sensitivity. The 2/4 or 9 IF migration program is suitable only when a sufficiently high concentration of the cryoglobulin is obtained (total Ig approximately 100 mg/dL).

Customer Focus



St. Marys

Life is a whole lot easier than it used to be in the chemistry lab at St. Marys Hospital Medical Center.

Since the lab began using the Sebia HYDRASYS® electrophoresis system in January 1999, technologists exert less effort preparing reagents... less time running electrophoresis... and less eyestrain interpreting results.

"When we compared automated electrophoresis solutions, a couple of big pluses set the HYDRASYS apart from the other competitors," explains Eunice Hardy (MT ASCP), Chemistry Technical Specialist at the Madison, Wisconsin-based medical center. "It's a very easy system to operate, and the gel quality is exceedingly good."

The instrument, which is compact enough to fit unobtrusively on the countertop, performs fast agarose gel electrophoresis for protein, immunofixation (IFE), lipoprotein + Lp(a), Hb, Hb A1c, HDL-cholesterol and iso-enzymes. St. Marys currently uses the system to test protein and IFE.

Drilling down to specifics, Hardy attributes the easy operation of the HYDRASYS to two things. First, she and her colleagues find the instrument software to be extremely user-friendly; with just a few touches on an LCD keypad, the HYDRASYS automatically carries out all phases of electrophoresis — from sample application to migration to incubation to staining, destaining and, finally, drying.

"Sebia has made it incredibly easy to move

St. Marys Finds Sebia's Electrophoresis System "Simply Divine"

from test to test on this instrument," Hardy says. "If you can run protein you can run immunofixation without any hassles as far as programming goes."

According to Hardy, simplified reagents are another reason the HYDRASYS is so user-friendly. "Everything comes pre-packaged in one kit — there's nothing to prepare except for the dilution of the Wash, Destain and Stain. That's a real time saver."

"The HYDRASYS allows technologists to batch samples, leading to better resource allocation and significant cost savings."

Looking at gel quality, the St. Marys team likes the crispness of the Sebia gels, particularly for IFEs. The HYDRASYS instrument yields images with sharp and well-separated zones — making for highly accurate readings. "With this system, interpretation is as good or better than the alternatives," Hardy says. "In fact, these plates are more sensitive than what we were accustomed to with our old system, which means we're getting a more complete picture than we did in the past."

As an example, she notes that they are picking up more minimonoclonals as a result of the increased sensitivity of the gels. Has the HYDRASYS helped improve operations at St. Marys? According to Hardy, it has - and the benefits are measurable. For one thing, the instrument allows technologists to batch samples, leading to better resource allocation and significant cost savings. "Our old system didn't give us that capability on every test," she notes.

By relying on the HYDRASYS, the lab has standardized processes among staff - dramatically reducing training time while ensuring accurate results with every test.

"When you have multiple technologists running electrophoresis, consistency becomes a

major concern — unless you have this type of automation," says Hardy. "It's not as 'technique-dependent' as a manual system.

"And not only that, we used to spend many, many hours getting an individual up and running. Now a new employee is ready to go in a matter of hours...which keeps productivity up."

Now that St. Marys has clocked more than a year with the HYDRASYS, are they still pleased with their choice? "Absolutely," Hardy says. "We couldn't be more happy with the HYDRASYS instrument. And our experience with

Sebia's people has been positive too — they are very responsive to our questions and needs, always working to provide answers and solutions quickly so we can keep things moving in our lab. They exemplify everything a partner should be."



About St. Marys Hospital Medical Center

Founded in 1912 to serve the people of South Central Wisconsin, St. Marys Hospital Medical Center offers a full range of inpatient and outpatient treatment and diagnostic services in primary care and nearly all specialties. Special medical/surgical areas of focus include the Center for Cardiovascular Care, Perinatal Center, Pediatrics, Neuroscience Center, Geriatrics and Emergency Services. Since 1972, St. Marys also has been affiliated with the University of Wisconsin School of Medicine's three-year family practice residency program.

St. Marys Hospital Medical Center is a part of St. Louis-based SSM Health Care, owned by the Franciscan Sisters of Mary.



We Need Your Feedback

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- article 108
- article 109
- article 110
- article 111

Number of electrophoresis tests run per week.

Protein _____ Immunofixations _____ Hemoglobin _____

Please include your name and address here.

check here if this is a new address

Comments: _____

Thank You for Your Assistance

"When we compared automated electrophoresis solutions, a couple of big pluses set the HYDRASYS™ apart from the other competitors."

—Eunice Hardy (MT ASCP),
Chemistry Technical Specialist, St. Marys Hospital Medical Center

In the meantime, keep sending in those questions. You may send them to me by mail at Sebia, 190-6611 Bay Circle, Norcross, GA 30071, 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.