
Saint Luke’s Regional Laboratories has completed a molecular diagnostics grant, given by Roche Diagnostics, which will allow the laboratory to become one of the first in the country to offer advanced molecular testing for HIV. Saint Luke’s Regional Laboratories (SLRL) was one of only four Roche Molecular Centers of Excellence in the United States to receive this funding, which amounted to $91,000.

HIV-1 quantitative PCR testing is intended to monitor HIV-1 viral replication, and is also referred to as viral load testing. SLRL has performed HIV-1 quantitative PCR testing in-house since 1995. This research grant gave SLRL access to cutting edge molecular diagnostic equipment designed to monitor HIV-1 more accurately and with improved turn-around-time, compared to earlier assays. The new technology quantitates the virus present in patient samples over a much wider dynamic range than had been previously possible.

SLRL has also been invited to participate in a similar grant later this year for hepatitis C viral load testing, which will support the pre-market evaluation of a new HCV quantitative PCR assay prior to FDA approval.

Immunoglobulin Isotype Switching In Post-Transplant Multiple Myeloma Patients

Oligoclonal bands (OB) and monoclonal bands distinct from the initial presenting paraprotein detected by serum immunofixation electrophoresis (IFE) are reported to be commonly observed in multiple myeloma patients undergoing autologous or allogeneic stem cell transplantation with or without pre-transplantation myeloablative therapy. The appearance of a new single band or OB in myeloma patients after transplantation can be problematic because paraprotein analysis plays a significant role in patient management and assessing the effectiveness of therapy. Several articles have proposed that these abnormal proteins bands (APB) represent either a malignant plasma cell clone producing a different paraprotein, formation of a second malignant clone or the recovery of B-cell function after myeloablative therapy that leads to the temporary production of oligoclonal immunoglobulin. The majority of studies supports the last interpretation that the new bands are likely the result of immune function recovery and represent a benign phenomenon.

In a study of 550 patients receiving high-dose therapy with autologous hematopoietic cell transplantation, IFE was used to analyze the occurrence of APB distinct from the presenting paraprotein in multiple myeloma patients (Blood.1998; 91(9):3518-23). They observed that APB occurred in 55 patients, with 48 occurrences of OB and 23 occurrences of isotype switching (IS). Both OB and IS occurred in 16 cases. A clonal plasma cell isotype switch was not evident in the morphologic and flow cytometric examination of bone marrow in 17 patients with IS by IFE. The study concluded that there was a significant relationship between APB post-transplantation and a positive clinical response as well as event-free and overall survival. The study suggested that OB and IS were transient phenomena associated with recovery of immunoglobulin production rather than emergence of a new malignant plasma cell clone.

A similar study of 72 transplanted multiple myeloma patients showed that APB distinct from the original paraprotein were observed in 31 patients (43%) between 1 and 6 months after their first autologous transplant (Neoplasma 2007; 54(3):225-8). Patients with previous IS who relapsed had the same paraprotein as that observed at the time of initial diagnosis. Like the previous study, the authors of this study found a favorable prognosis associated with the development of APB. They also concluded that APB formation is related to recovery of impaired immunoglobulin production after transplantation in myeloma patients.
A retrospective analysis of 6 post-transplant multiple myeloma patients with serial serum IFE at Saint Luke’s Hospital showed similar findings; 83% (5/6) of patients developed OB or underwent IS (cases 1-5). One patient (case 6) retained their original monoclonal protein but developed an additional IgG band post-transplantation.

<table>
<thead>
<tr>
<th>Case</th>
<th>Pre-transplant Monoclonal</th>
<th>Transplant Date</th>
<th>Post-transplant Monoclonal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IgA kappa (12/01)</td>
<td>1/02</td>
<td>IgG lambda (5/02)</td>
</tr>
<tr>
<td>2</td>
<td>IgA lambda (1/97)</td>
<td>12/97</td>
<td>IgG kappa (11/97)</td>
</tr>
<tr>
<td>3</td>
<td>IgA lambda (12/00)</td>
<td>11/00</td>
<td>IgG kappa (1/02)</td>
</tr>
<tr>
<td>4</td>
<td>Free kappa (11/98)</td>
<td>1/99</td>
<td>IgG kappa &amp; IgA kappa (7/99)</td>
</tr>
<tr>
<td>5</td>
<td>IgG lambda &amp; IgG kappa (5/97)</td>
<td>Between 5/97 &amp; 12/99</td>
<td>IgA kappa (12/00)</td>
</tr>
<tr>
<td>6</td>
<td>IgA lambda (12/01)</td>
<td>3/03 &amp; 7/03</td>
<td>IgA lambda &amp; IgG kappa (5/03)</td>
</tr>
</tbody>
</table>

IS in patients with multiple myeloma should still be closely observed even though the formation of OB and IS has no apparent adverse clinical effect. Lymphoproliferative disorders that complicate immunosuppression and other hematologic disorders are other phenomena that could produce new paraprotein bands.

**New Test - Urine Culture If Indicated**

Appropriate antibiotic therapy for urinary tract infection can be delayed in the time period between receipt of an abnormal urinalysis result by a clinician and ordering of a urine culture. Likewise, unnecessary urine cultures may be requested based on symptoms and prior to receipt of a normal urinalysis result. Effective immediately, physicians have the option of requesting “Urinalysis with culture if indicated.” Urine specimens with this order will have a urine culture performed automatically when either the leukocyte esterase is positive, or an abnormal number of WBC’s is seen on microscopic examination.

The newest guidelines for interpretation of urine cultures have been instituted in Microbiology, so that one or two pathogens in quantities >10,000 cfu/mL will have susceptibility testing performed. Straight cath urines, or surgically obtained urines, such as suprapubic taps or cystoscopy specimens, should be designated as such on the requisition. These specimens are processed differently by Microbiology, and in general, organisms in any quantity have susceptibility testing performed.

Specimen requirement for urinalysis with urine culture is 5 mL of urine in a sterile container, refrigerated for up to 24 hours prior to receipt by the laboratory.

**Mycoplasma & Cold Agglutinin Titers**

Cold agglutinins are nonspecific IgM antibodies which agglutinate red blood cells at cold temperatures between 0 and 30°C. In the past, cold agglutinin titers were often used as a surrogate test for *Mycoplasma pneumoniae*, since mycoplasma infections are often associated with elevated RBC antibodies with anti-I specificity. Numerous conditions besides *Mycoplasma pneumoniae* will give an elevated cold agglutinin titer including: viral infections, hemolytic anemia, liver disease and pregnancy. More specific mycoplasma IgG and IgM immunoassays are preferred to diagnose *Mycoplasma pneumoniae*. Cold agglutinin titers should only be ordered to diagnose cold autoimmune hemolytic anemia.

**Pneumocystis by PCR**

*Pneumocystis jiroveci*, previously known as *P. carinii*, is a causative agent of pneumonia in immunocompromised individuals, especially those infected with HIV. Traditionally, Pneumocystis has been detected in respiratory specimens by means of special staining, either by silver stain (GMS) or fluorescent antibody (DFA). The disadvantages of special stains are lack of sensitivity, expertise required for interpretation, and labor intensiveness of the process.

Recently, Mayo Medical Laboratories has validated a real-time polymerase chain reaction (PCR) assay for detection of Pneumocystis that was found to be 21% more sensitive than special stains. Effective immediately, respiratory specimens with requests for Pneumocystis testing will be forwarded to Mayo Medical Laboratories for rapid PCR testing. Bronchoalveolar lavage fluid (0.5 mL minimum) is the preferred specimen. Induced sputum, bronchial washings, tracheal secretions, and respiratory tissue are also acceptable.