Minimal Residual Disease Testing for B-ALL by Flow Cytometry now Performed at BJH

by: Friederike Kreisel, M.D.

B-lymphoblastic leukemia/lymphoma (B-ALL) is a neoplasm of precursor cells (lymphoblasts) committed to the B-cell lineage and it represents the most common cancer in children with nearly 3000 newly diagnosed patients under the age of 20 each year and a peak incidence at an age of less than 5 years. It is rare in adults, but in patients over 50 years the incidence rises a second time with a peak at the age over 80 years. B-ALL in adults has a higher risk of relapse and lower long-term survival than pediatric B-ALL.

Minimal residual disease (MRD) testing by flow cytometry is a powerful prognostic indicator, representing the strongest indicator for event-free survival in the pediatric population. It can be clinically used to stratify and manage pediatric and adult patients. In the pediatric setting, <0.01% of abnormal lymphoid blasts (of mononuclear cells) in the bone marrow is the Children's Oncology Group (COG) – defined threshold for considering a sample negative for minimal residual disease. Understanding the normal immunophenotypic maturation pathway of hematogones (B-lymphocyte precursors) is crucial in MRD evaluation. Markers most commonly used to distinguish between normal hematogones and neoplastic B-lymphoblasts are CD19, CD20, CD38, CD9, CD45, CD58, CD13/CD33 and CD34. The most common abnormalities in pediatric B-ALL include increased expression of CD10, CD19, and CD58, while CD38 and CD45 are often decreased. Flow cytometry can routinely achieve a sensitivity of 0.01%-0.001%. However, this is dependent on the abnormal blast immunophenotype to be sufficiently different from normal in order to distinguish it from regenerating B-cell populations, and for the sample to lack degenerative changes that could influence interpretation.

The validation process encompassed 60 specimens from 60 patients with an established diagnosis of B-ALL, of which 15 specimens had to reveal minimal residual disease of less than 5%. Our results were compared to aliquots analyzed at one of the two COG approved reference flow cytometry laboratories.

Beginning March 1, 2017, the BJH Flow cytometry laboratory is performing MRD testing in-house. Specimens should be collected in a Sodium Heparin tube (green top) and forwarded to the Flow Cytometry Laboratory in the 4th Floor Institute of Health Care Laboratory. The sample should be stored and transported at room temperature. For assistance please call the laboratory at (314) 362-4628.
A Change in Cytomegalovirus Quantitative Plasma PCR Testing

by: Neil Anderson, M.D.

Monitoring of CMV viral load in peripheral blood is an important part of diagnosing CMV related disease and assessing response to treatment.

On April 10th 2017, the BJH Microbiology Laboratory will be transitioning to a new assay, the COBAS AmpliPrep/COBAS TaqMan CMV assay. This test will no longer be a send out to SLCH. Specimens submitted for testing should consist of at least 2 ml of blood, and must be received in an EDTA tube (purple top). Specimens should also be submitted to the microbiology laboratory no more than 4 hours after collection. Testing will be performed seven days a week and any specimen received by the microbiology laboratory by 1:30 PM will be tested and reported that day.

A significant difference between the COBAS assay and the current assay is the use of plasma rather than whole blood, which can impact the viral load measurement. Only free virus is detected in plasma while free virus and latent virus is detected in whole blood. However, whole blood does not offer significant clinical advantages nor does it allow for better prediction of recurrence of CMV viremia or disease over the use of plasma (1). The prolonged presence of CMV in whole blood may lead to over-treatment with antivirals.

A side by side comparison of the COBAS assay (plasma) to the current assay (whole blood) demonstrated that plasma viral loads are generally 0.5log IU/mL lower on the COBAS assay. Given this difference, we are performing parallel testing of patients for one month. For this period of time the whole blood CMV result will be reported with an addendum containing the plasma result from the COBAS assay (so both results will be visible). This will automatically be performed, provided minimum volume requirements are met and specimens are received by the lab within 4 hours from collection, at no charge to the patient. The comparison period will help physicians follow viral load trends as we convert from whole blood to plasma CMV viral load monitoring.

Testing in parallel began on March 13th and we will transition entirely to the new assay on April 10th. If you have any questions, please contact Neil Anderson, M.D., Assistant Medical Director of Microbiology (362-1307, nanderson@path.wustl.edu).


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Did You Know??

That the BJH Patient-centered Blood Management Program, which was initiated in 2012, is reducing the use of red blood cells?

At its peak, in 2012, 51,042 units of RBCs per year were transfused. In 2016 usage dropped 3.5% to 49,261 units of RBCs per year.
BJH Announces New Laboratory Director

The BJH laboratories are happy to announce that, in February, Joan Rossi MA, CT(ASCP) CM was appointed as the new BJH Director of Laboratory Services. Ms. Rossi has been with BJH for many years and was chosen following a national search for candidates.

Ms. Rossi served as the laboratory manager of BJH Surgical Pathology for 18 years and for the past year has served as the Program manager of the BJH Department of Laboratories. She helped the laboratories shepherd in the new core lab automation in 2016 and is eager to see the blood bank move to its new location in coming months. Ms. Rossi has a deep understanding of the laboratory operations and brings a wealth of valuable experience to her new position.

Clinical & Translational Genomics - Director’s Cut

by: Jon Heusel, M.D., Ph.D.

The Division of Laboratory and Genomic Medicine (LGM) recently announced the formation of a “Unified Genomic Laboratory Services” unit, which is an integration of the Pathology Department’s formally independent clinical genomics and cytogenetics expertise into a single and innovative laboratory; known henceforth as the Clinical and Translational Genomics (CaTG) laboratory.

The CaTG represents a proactive integration of two substantial laboratories performing high-complexity testing in the dynamic field of clinical genomics—the Cytogenetics and Molecular Pathology Laboratory, and Genomics and Pathology Services (GPS), the institution’s clinical Next Generation Sequencing (NGS) laboratory. We recognized the need to consolidate these clinical services from several perspectives including: reimbursement, test ordering practices, increasing applications using NGS technology, and regulatory considerations. Integrating these complementary testing services was a logical next phase of development in the evolution of clinical genomics at WUSM.

Over the last decade, NGS technologies have diversified, and moved into clinical testing applications, driving competition and creating challenging economics for academic institutions to keep pace. Newer high-capacity NGS platforms will lower the cost of sequencing further, and long read instruments will usher in a new wave of applications—particularly for cytogenetics and whole genome assessments. The CaTG represents a new way to manage this avenue toward Precision Medicine, seeking to maximize economics where possible, expertise in disease-focused testing and reporting, and innovation in the design of improved assays coupled with faster, more sophisticated genomics analyses.

The CaTG will also become an important translational research unit, collaborating with existing, well established partners in human genomics, as well as emerging groups in informatics, genetics, and functional genomics at this institution. To support this effort, the CaTG will retain genomics research faculty, with deep experience in building and validating clinical-grade genomics assays, and in variant assessment and reporting. We will partner with institutional efforts to bring forward highly curated genomics knowledge bases and the clinical decision support mechanisms needed to realize the potential of healthcare informed by large data sets and tailored to the unique needs of the individual patient.
Update on Susceptibility Testing for Colistin

by: Carey-Ann Burnham, Ph.D.

Colistin is an antimicrobial of “last resort” for the treatment of patients with infections caused by multidrug-resistant Gram-negative bacteria. Susceptibility testing for colistin is challenging, as the molecule is large and has a propensity to adsorb to testing surfaces. Recent studies evaluating in vitro susceptibility testing and the interpretive breakpoints for colistin have shown that broth microdilution, without surfactant, is the most accurate and reproducible method for colistin susceptibility testing. Based on these data, the current commercially available disk diffusion and agar gradient diffusion methods should not be used, as these methods yield unacceptably high error rates.

Until new commercial methods can be developed, colistin susceptibility testing will be restricted to a reference laboratory setting. Starting March 20, colistin susceptibility testing will not be performed at BJH. If colistin susceptibility information is needed, isolates will be referred to Mayo Medical Laboratories; please call the microbiology laboratory at 314-362-3898 to request this testing. If you have questions about this, please contact Carey-Ann Burnham, Medical Director of Clinical Microbiology, at cburnham@wustl.edu.

New Multiplex Flow Immunoassay Method at BJH

by: Ann M. Gronowski, Ph.D.

February 22, 2017 the BJH Core Laboratory began using the BioRad BioPlex® instrument. The BioPlex utilizes multiplex flow immunoassay (MFI) that allows for simultaneous detection of many antibodies in a single tube. Multi-colored dyed beads are coated with different antigens and each color represents a unique antigen. Patient sample is added and patient antibodies are allowed to bind to the antigens. After a wash cycle, anti-human (IgG or IgM) antibody which is coupled to a phycoerythrin (fluorescent protein), is added. After another wash cycle, the beads are passed through a detector that identifies the color (antigen) of the bead and the amount of antigen captured is determined by the fluorescence of the attached phycoerythrin. The assay is rapid and has increased precision over traditional enzyme immunoassays.

To date, a number of infectious serology tests have been placed on this instrument including Cytomegalovirus, Herpes Simplex Virus 1 & 2, Varicella virus, Measles, Mumps, and Rubella. Within the next few months, EBV and other autoimmune antibody assays will move to this platform, including extractable nuclear antigen (ENA), cyclic citrullinated peptide, celiac tests (tissue transglutaminase and deamminated gliadin), and antiphospholipid screening (cardiolipin and beta-2 glycoprotein).

In coming months we will provide updates on new assays as they come into use.