From the Clinical Laboratories of the
Cancer & Blood Diseases Institute

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IN THIS ISSUE:

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NEW ASSAYS

Interferon-α and β testing (IFN-alpha, IFN-beta)

Interferons are a family of cytokines originally characterized for their antiviral properties. Type I interferons, such as IFN-alpha and IFN-beta, have been demonstrated to possess proinflammatory functions which contribute to the pathogenesis of diseases such as system lupus erythematosus, rheumatoid arthritis, and psoriasis [1,2,3,4].

Rare type I interferonopathies such as SAVI, CANDLE, and SPENCD are inheritable disorders linked to abnormal type I interferon regulation [4]. Even though the current diagnostic tool for these diseases is molecular analysis confirmation, diagnosis can be difficult. The measurement of type I interferons such as IFN-alpha and IFN-beta, may become useful screening or prognostic biomarkers for diagnosis and targeted therapies of type I interferonopathies [3] (see Figure 1).

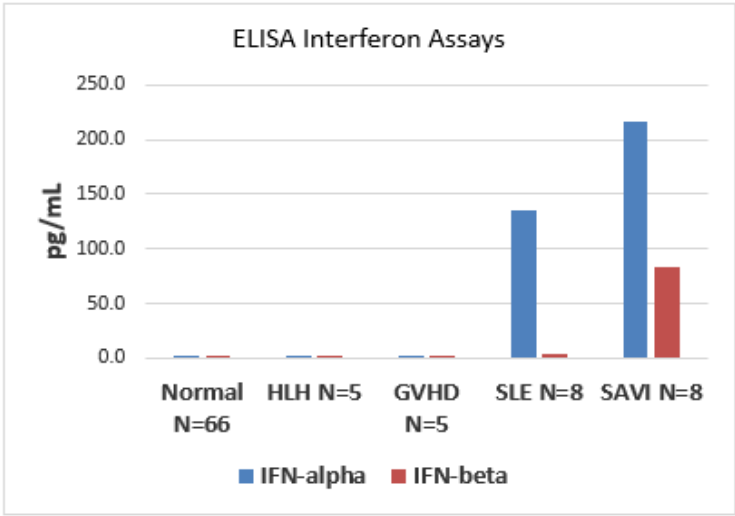

Figure 1. IFN-alpha and IFN-beta were measured using plasma or serum from normal adults and pediatric samples of patients whose diagnoses were scrutinized prior to use.

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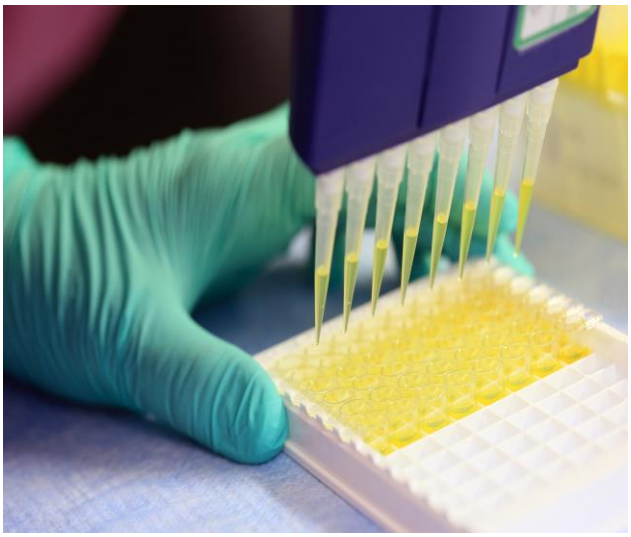
The IFN-alpha and IFN-beta clinical tests utilize the quantitative sandwich enzyme-linked immunosorbent assay (ELISA) method. Results for both tests are expressed in pg/mL. Testing is performed every 2 weeks.

Specimen requirements for either test are 3mL of whole blood collected in a lavender tube (EDTA), gold top tube (SST), or red top tube. Whole blood tubes must be shipped at room temperature and received by our laboratory within 24 hours of collection. Alternatively, whole blood tubes may be spun and preferably two 0.3 mL aliquots of serum or plasma placed at -20^{0}C or lower. When ready to ship, aliquots should be packaged on dry ice in a Styrofoam packing container for delivery the next day. Please refer to our updated requisition or Clinical Laboratory Index for complete sample collection and shipping instructions.

REFERENCES:

1. De Andrea, M. et al., (2002) Eur J Paediatr Neurol 6(Suppl A):A41-6;A55-8.
2. Crow, M.K., (2010) Arthritis Research & Therapy 12(Suppl 1):S5.
3. Axtell, R. et al., (2011) Trends Immunol. 32:6.
4. Volpi, S. et al., (2016) Pediatric Rheumatology 14:35.



IL-6 Stand Alone Testing

Due to high demand, Interleukin-6 (IL-6) is now available as a stand-alone clinical test. This test is listed as IL-6, CIA. The IL-6 method utilized is a Chemiluminescent Immunometric Assay (CIA) and is performed Monday through Friday. IL-6 will still also be available as part of the current multiplex cytokine panel.
NOTE: Values of IL-6 measured with the chemiluminescent method generally run 2 fold lower than when measured with the multiplex method.

Specimen requirements are 3mL of whole blood collected in a lavender tube (EDTA). Whole blood tubes must be shipped at room temperature and received by our laboratory within 24 hours of collection. Alternatively, whole blood tubes may be spun and preferably two 0.3 mL aliquots of plasma placed at -20^{0}C or lower. When ready to ship, aliquots should be packaged on dry ice in a Styrofoam packing container for delivery the next day. Please refer to our updated requisition or Clinical Laboratory Index for complete sample collection and shipping instructions.

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TESTING UPDATES

New CXCL9 method and reference range

A new CXCL9 method is in effect as of 5/03/2021. CXCL9 is now measured by an automated microfluidics immunoassay method. Values of CXCL9 measured with this method generally run 5 to 10 fold higher than when measured with the previous ELISA method (see Figure 2). Reference range for the new method is ≤ 647pg/mL.

Collection and specimen handling remain the same. Briefly, whole blood should be collected using EDTA as an anticoagulant (lavender top tube). Process and freeze separated plasma within 8 hours of collection. Ship on dry ice.

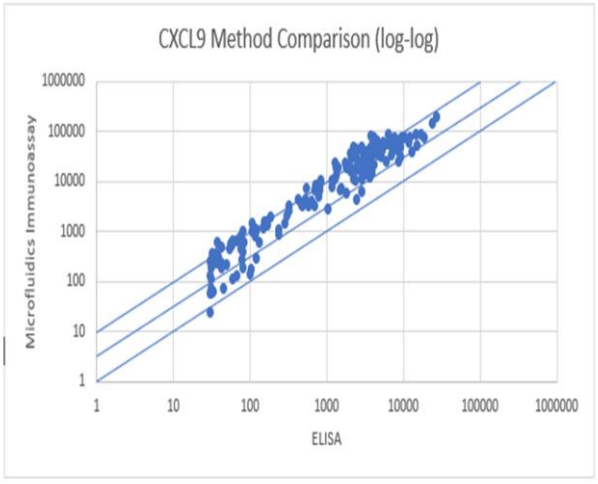


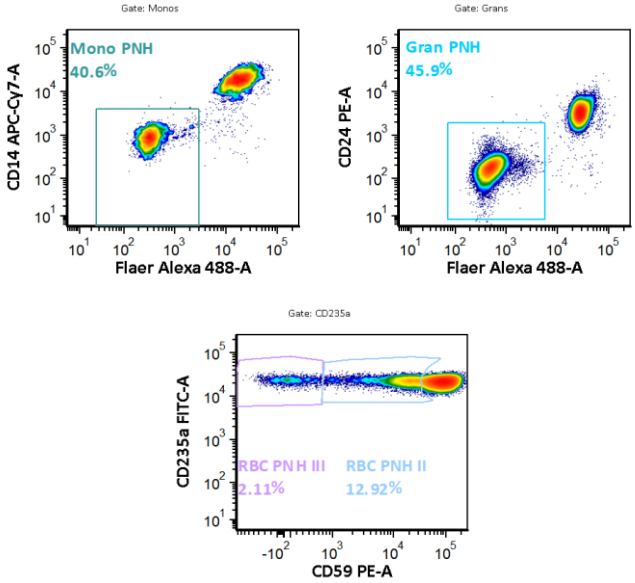
Figure 2. Graph showing the correlation of CXCL9 sample values obtained using the ELISA method versus the new automated microfluidics immunoassay method.

High-resolution PNH testing

High resolution testing for Paroxysmal nocturnal hemoglobinuria (PNH) is available through the Immunopathology Laboratory. PNH is a rare disease in which there is a complete (Type III) or partial absence (Type II) of GPI-anchor proteins. The disease is characterized by destruction of red blood cells, blood clots, and impaired bone marrow function.

The assay includes an assessment of the expression of GPI-anchored proteins as well as the GPI anchors themselves. A positive PNH test is indicated by loss of Flaer and CD14/CD24 on monocytes and granulocytes respectively, as well as a loss of CD59 on RBCs. The limits of sensitivity for this assay are 0.1% for the WBCs and 0.01% for the RBCs.

Whole blood should be collected using EDTA (lavender top tube) and shipped ambient. The specimen must be received so that testing can occur within 24 hours of collection.



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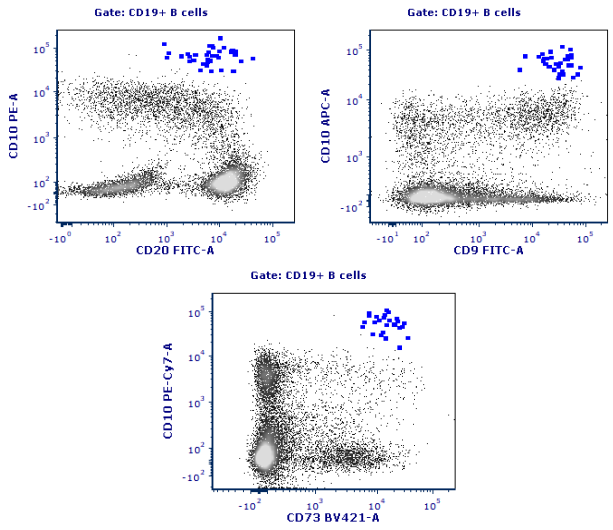
COG-approved Minimal Residual Disease (MRD) Testing for B-ALL

COG-approved Minimal Residual Disease (MRD) testing for B-ALL is available through the Immunopathology Laboratory. The presence of MRD at day 29 following induction therapy is one the most significant prognostic factors in determining outcomes for B-ALL patients. MRD testing can be a useful monitoring tool at any time point during a patient's therapy.

Whole blood or bone marrow aspirate should be collected using Sodium Heparin (dark green top tube) and shipped ambient. Testing is ideally performed with 48 hours following collection. Please send diagnostic flow cytometry dot plots to assist with the interpretation of the MRD flow. The limit of sensitivity is 0.01% or 1 in 10,000 cells.

NOTE: The current MRD assay uses CD19 for gating purposes and should **NOT** be ordered for patients having received any type of anti-B cell therapy recently, including CD19 CAR-T cells or blinatumomab. Please call the Immunopathology Lab at 513-803-2567 with any questions regarding anti-B cell therapy prior to shipping the specimen.



Acknowledgments

*The authors would like to thank **Kristi Smiley and Holly Bonar,** CBDI Labs, CCHMC for their contributions to this newsletter.*

BULLETIN BOARD

Kristi Smiley receives the first Alexandra (Lisa) Hult Filipovich Award

Our Diagnostic Immunology Laboratory established this award in recognition and memory of Dr. Lisa Filipovich who formed the laboratory in 1996 and served as Director until her retirement. This award recognizes individuals who have made a significant contribution to advancing immunologic testing for patients with diseases that involve the immune system.

Kristi Smiley started her career within the Diagnostic Immunology Laboratory under Dr. Filipovich's mentorship in 2006. Kristi has worked to bring over 10 new clinical tests to the laboratory's test menu. These tests help doctors diagnose, monitor, and treat patients with diseases that involve the immune system. Some of these tests are not available anywhere else in the United States.

Kristi Smiley (right) receiving her award from Rebecca Marsh, MD, co- director, Diagnostic Immunology Laboratory.

Content

*The CBDI Labs newsletter content design/writer is **Sabina Sylvest**. The newsletter is edited by **Rebecca Marsh**, MD*

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