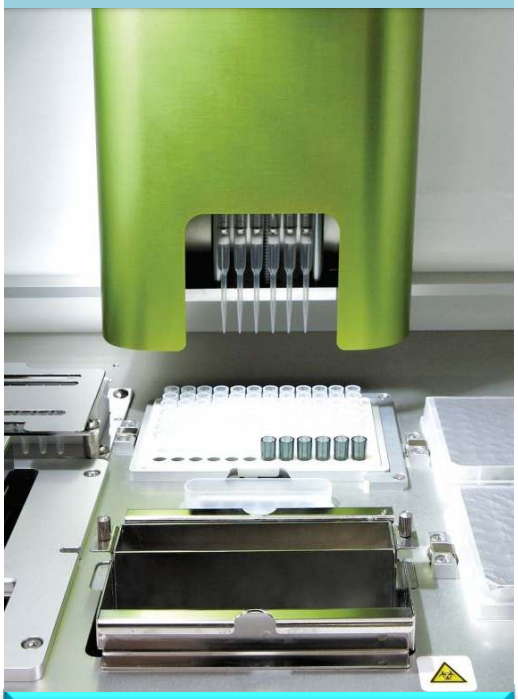
From the Clinical Laboratories of the
Cancer & Blood Diseases Institute

ISSUE 19 | DECEMBER 2021



IN THIS ISSUE:

Cincinnati Children's Hospital Medical Center
Cancer & Blood Diseases Institute
3333 Burnet Avenue
Cincinnati, Ohio 45229
513-636-4685
www.cchmc.org/CBDILabs

INTRODUCTION TO THE LABORATORY OF PRECISION DIAGNOSTICS

The Diagnostic Immunology Laboratories (DIL), part of the CCHMC – Cancer and Blood Diseases Institutes, is excited to announce the creation of the **Laboratory of Precision Diagnostics**.

Before describing the Laboratory in more detail, it might be helpful to touch upon the Iceberg issue in the context of contemporary diagnostics for Inborn Errors of Immunity.

Inborn Errors of Immunity (IEoI) comprise a heterogeneous group of – often complex – disorders that include primary immunodeficiency disorders, autoimmune/lymphoproliferative disorders, as well as hemophagocytic lymphohistiocytic (HLH) disorders, amongst others. We are in the era of genomics, and even though many of these disorders now are linked directly to specific defects in genes, for every patient with a solved genetic puzzle (above water), there are many more patients who lack a (convincing) genetic diagnosis (below water - hence the Iceberg). This *Iceberg* is experienced by patients and their families as the unfulfilled promise of genetic testing (and this essentially applies to all medical disciplines).

Solving diagnostic puzzles is important, not only to provide an accurate diagnosis and enable important genetic counseling, but also to link a definitive diagnosis to treatment options (particularly if "precision therapeutics" are available). Although it is not uncommon to find multiple genetic variants by contemporary genetic testing in genes that seem relevant for the disease in question, but if those genetic alterations do not lead to clear disruption of its gene products, it can be challenging to assign relevance and significance to these (often missense) variants.

Thus, short of "bioinformatics knowledge" derived from a variety of sources (e.g., in silico modeling, frequency of variants in the proper control population, published/submitted cases, etc.), current routine diagnostic testing in Clinical (Immunology) Laboratories is not able to solve cases. To improve this, we need to overcome a significant technology gap: a gap between what is technically possible and what is used in Clinical (Immunology) Laboratories*****.

Cincinnati Children's
changing the outcome together

With this in mind, the **Laboratory of Precision Diagnostics** will focus on Precision Diagnostics as the necessary counterpart to Precision therapeutics. Its goals include screening and diagnosis (i.e., puzzle solving), but also disease classification (for example based on "endotypes"), stratification and therapy selection, and determination of therapy response (e.g., biomarkers), and thus prognosis.

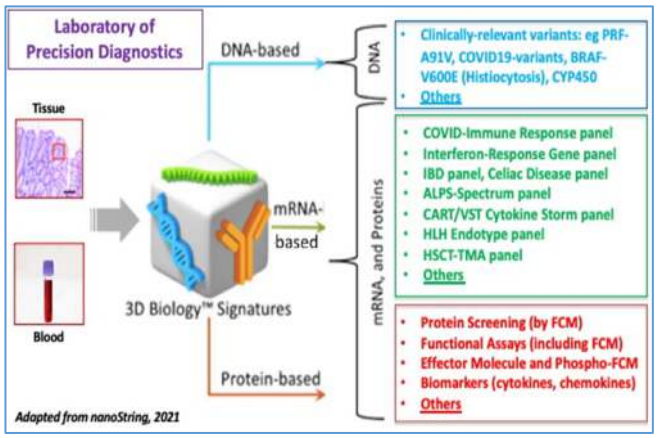
As part of the DIL/CBDI, this entails an integrated approach to protein/protein function detection – as part of the DIL's existing Flow Cytometry, Biomarker and Functional Immune-Assessment Laboratory – transcriptomics (RNA detection) and select mutation/variant detection. The transcriptomics and – future – genomics will be the focus of the **Laboratory of Precision Diagnostics** and will be executed with the nanoString nCounter platform.

The nCounter platform has several clear advantages for the development of clinical-grade transcriptomics, including pre-formatted panels for human diseases, no need for amplification or a reverse transcriptase step, meaning there is less Technician (hands-on) time and better QC performance (less error), a fast result output and total turn-around-time (relevant in biomarker diagnostics) and, important in clinical diagnostics, projected assay costs that are on par with existing clinical laboratory assays.

The inaugural assay will be the **Interferon-Response Gene** panel, a clinical transcriptomics assay, centered on [auto/hyper] inflammatory conditions. This 58-gene panel reflects activity of type I interferons, type II interferons (gamma-interferon), as well as NF-κB–regulated genes. Applications include **Inborn Immunity Errors** of type I interferons (Interferonopathies), inflammasome-associated autoinflammation, hyper-inflammation (cytokine storm) as seen in HLH, MAS, following CART therapy, as well as COVID19 and COVID19-associated MIS-C.

** Consider that translational research flow cytometry laboratories now routinely interrogate 20+ parameters with novel technical platforms and have moved on from Boolean to higher-dimension analytics, while clinical flow labs are in essence "stuck" at ~ 8-10 parameters with Boolean gating/analysis (the 2-parameter dotplots).*

A taste of the future of the DIL/Laboratory of Precision Diagnostics is shown in the figure below. See future Newsletters for updates.



NEW ASSAY: SERUM ANGIOPOIETIN-2

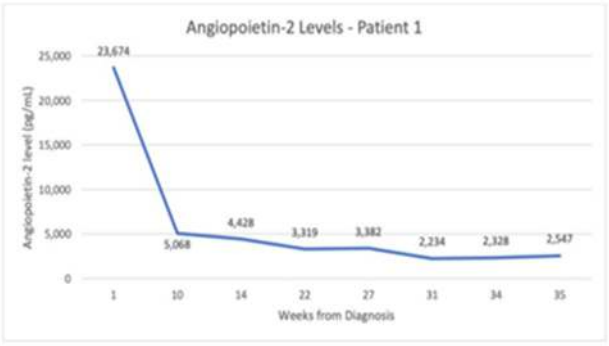
The CBDI Hemostasis and Thrombosis Laboratory is pleased to announce that they are now offering serum Angiopoietin-2 testing. The biomarker angiopoietin-2 (ang-2), a member of a family of growth factors that regulate blood vessel growth and development, has previously been reported to be elevated in certain vascular abnormalities (VA) associated with coagulopathy, including Kaposiform hemangioendotheliomas (KHE) and Kaposiform lymphangiomatosis (KLA).

Ang-2 levels have been reported to decrease in some patients in response to treatment with sirolimus (1, 2). Based upon the published methodology, a clinical serum ang-2 assay was developed that employs a quantitative sandwich enzyme immunoassay technique using a monoclonal antibody specific for human ang-2. The case below demonstrates the potential utility of serum ang-2 as a diagnostic tool and biomarker to monitor response to treatment in selected patients with VA. This patient presented at birth with a tumor suggestive of KHE, confirmed by biopsy.

Cincinnati Children's
changing the outcome together

The patient was started on sirolimus and the Ang-2 level that was elevated at diagnosis showed reductions over time (graph 1). Next steps are to evaluate ang-2 levels in an expanded group of VA to understand which diagnoses can be distinguished by elevated levels at baseline and how treatment impacts ang-2 over time (3).

The sample requirements are a minimum of 0.5 mL frozen serum. Please refer to our updated requisition or Clinical Laboratory Index (Cincinnati Children's | Home (testmenu.com))for complete sample collection and shipping instructions.



Graph 1: Angiopoietin-2 Levels – Patient 1

References

1. Le Cras TD, Mobberley-Schuman PS, Broering M, Fei L, Trenor CC 3rd, Adams DM. Angiopoietins as serum biomarkers for lymphatic anomalies. Angiogenesis. 2017 Feb;20(1):163-173. doi: 10.1007/s10456-016-9537-2. Epub 2016 Dec 18. PMID: 27990590.
2. Crane J, Manfredo J, etal, Pediatr Blood Cancer. 2020 Sep;67(9): e28529. doi: 10.1002/pbc.28529. Epub 2020 Jul 7. PMID: 32634277; PMCID: PMC8554683.
3. Elissa Engel, MD, Kiersten Ricci, MD, Adrienne M Hammill, MD, PhD, Karen Mittermeier, MT, Timothy D. LeCras, PhD, and Lori Luchtman-Jones, MD. Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH; Division of Hematology, Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH

Acknowledgments

The authors for this edition are Jack Bleesing, MD PhD, and Karen Mittermeier.

The newsletter editors are Karen Mittermeier, Cristi Cobb and Mary Reynaud

Transport of Specimens during Winter

Specimen packages shipped overnight by commercial carriers can be subject to extreme seasonal temperatures. Most of our packages route through Memphis, where temperatures during the winter months often dip to 0°C. In order to protect the integrity of **ambient** specimens, extra packing material may be helpful. A study done at the University of Virginia [(1)] found that insulating bagged specimens in paper towels and placing a "warm pack" (heated to 37°C.) on top can extend the amount of time specimens remain protected from extreme cold (see Figure 1). Packing for the weather can mean the difference between moving ahead with testing or asking for a recollection.

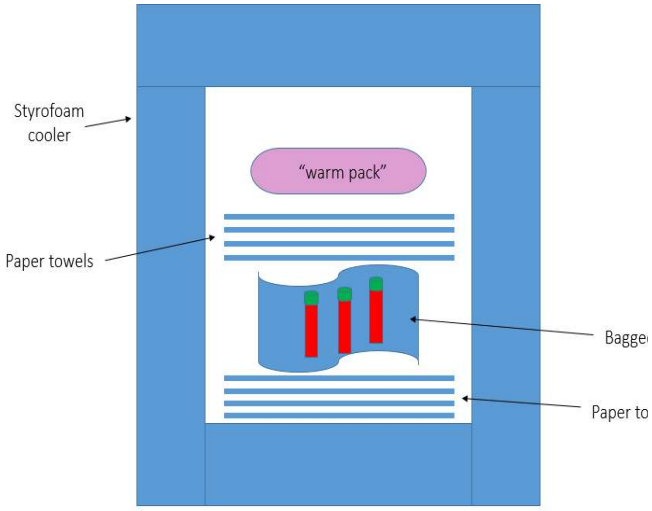


Figure 1. While we do **not** recommend heating patient specimens to 37°C. A gel pack pre-warmed at 37°C and placed inside the shipping container helps to maintain an ambient evironment during winter transport.

References:

1. Olsen Walter C, et al. Shipping blood to a central laboratory in multicenter clinical trials: effect of ambient temperature on specimen temperature, and effects of temperature on mononuclear cell yield, viability, and immunologic function. *Journal of Translational Medicine.* doi: 10.1186/1479-5876-9-26. *Epub* 2011 Mar 8.

Cincinnati Children's
changing the outcome together