Nationwide Children's
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Laboratory Services
Important Test Announcement

GRANULOCYTE OXIDATIVE BURST PANEL

New Test Code: GOBP **Live Date: 8/20/2024**

Effective August 20, 2024, the Granulocyte Oxidative Burst Panel (GOBP) will replace the current Oxidative Burst Assay (DHRB). The new assay, GOBP, will include data from phorbol myristate acetate (PMA) stimulation along with an additional stimulant, N-formyl-methionyl-leucyl-phenylalanine (fMLP), for diagnosis of hypomorphic Chronic Granulomatous Disease (CGD) as well as autosomal recessive RAC2 deficiency. Both frequency (%) data, for post-transplant assessment, and identification of skewed lyonization in female carriers of X-linked CGD, and stimulation index (SI) will be reported for this assay for each stimulant. The assay will also include the absolute neutrophil count (ANC) from the sample. Another new feature of this assay is the analysis of oxidative burst only in viable granulocytes, eliminating potential confounders in interpretation from poor-quality samples.

Methodology: Flow cytometry
Performed: Monday-Friday (samples must be received by 4 p.m.)
Turnaround Time: 72 hours
Specimen Required:

- **Collect :** 3 mL Sodium Heparin tube (dark green)
- **Specimen Volume:** 2 mL
- **Specimen Preparation:** Recommend at least a 48-hour interval without acetaminophen for this assay
- **Storage/Transport/Temperature:** Room temperature
- **Unacceptable Conditions:** Refrigerated/frozen samples
- **Stability:** 48 hours
- **Comments:**
  - The use of acetaminophen as well as acute and critical illness can potentially affect interpretation of results. Recommend at least a 48-hour interval without acetaminophen for this assay. An interpretive report will be provided in this assay.
- **Clinical Utility:**
  - Chronic Granulomatous Disease (CGD) is primarily an inborn error of immunity, associated with defects in neutrophil function, caused by genetic defects in the components of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme complex. These defects result in an inability to produce superoxide anions required for killing of bacterial and fungal organisms. Other clinical features include a predisposition to systemic granulomatous complications and autoimmunity. There are 5 known genetic defects associated with the clinical phenotype of CGD. The gene defects include pathogenic variants in the *CYBB* gene, encoding the gp91phox protein, which is X-linked and accounts for approximately 70% of CGD cases. Other gene defects are autosomal recessive: *NCF1* (p47phox), *NCF2* (p67phox), *CYBA* (p22phox), *NCF4* (p40phox), and Eros (*CYBC1)*. Typically, patients with X-linked CGD have the most severe disease, while patients with autosomal recessive NADPH oxidase defects tend to have the best outcomes. Variants in *NCF4* encoding the p40phox protein are mainly associated with inflammatory features and fewer infections.

There is significant clinical variability even among individuals with similar variants, in terms of NADPH oxidase function, indicating that there can be several modulating factors including genetic defect, infection history, and granulomatous and autoimmune complications. There appears to be a correlation between very low NADPH superoxide production and poor clinical outcomes. CGD can be managed with antimicrobials and in certain cases, recombinant interferon-gamma, and curative therapy includes hematopoietic cell transplantation (HCT) and potentially gene therapy, which can be effective for the inflammatory and autoimmune manifestations.

- Measurement of NADPH oxidase activity through the dihydrorhodamine 123 (DHR) flow cytometry assay contributes to the assessment of reactive oxygen intermediates (ROI). The diagnostic laboratory assessment for CGD includes evaluation of NADPH oxidase function in neutrophils and the flow cytometry-based DHR test. Activation of neutrophils with phorbol myristate acetate (PMA) results in oxidation of DHR to a fluorescent compound, rhodamine 123, which can be measured by flow cytometry. Flow cytometry can distinguish between the different genetic forms of CGD. Complete myeloperoxidase (MPO) deficiency can cause a false-positive result for CGD in the DHR flow cytometric assay; however, this can be identified by the pattern of the DHR flow assay, and confirmed by flow cytometry for myeloperoxidase in neutrophils or by measuring NADPH oxidase function in eosinophils.
- The DHR assay can be used for both diagnosis and monitoring response to treatment, especially post-HCT.
- In addition to PMA stimulation, this assay includes fMLP (N-formyl-methionyl-leucyl-phenylalanine), a physiological activator of neutrophils. Therefore, this assay can also be used for the diagnosis of loss-of-function autosomal recessive defects in *RAC2,* which encodes a Rho-family GTPase, essential for neutrophil activation and NADPH oxidase function.
- Female carriers of X-linked CGD can become symptomatic for CGD due to skewed lionization (X chromosome inactivation). Age-related skewing of lyonization can also cause increased susceptibility to infections in carriers of X-linked CGD. While germline variants are more common in CGD, there have been reports of de novo, sporadic variants in the *CYBB* gene, causing X-linked CGD in males.

If you have any additional questions about **GOBP**, please refer to the Laboratory Test Directory or call Client Services at 614-722-5477.