Laboratory Testing for Chronic Granulomatous Disease: Challenges and Recommendations

Take-Home Points
The take-home points to consider for diagnostic testing for CGD: CGD is a complex and heterogeneous disease due to mutations in 1 of the components of the NADPH oxidase pathway. Functional assessment of NADPH oxidase activity is a sensitive and specific diagnostic tool for CGD. The DHR / Dihydrorhodamine (DHR) Flow Cytometric Test, Blood test established more than 20 years ago is considered superior to the NBT assay and is most commonly used these days for the diagnosis of CGD. The assay involves stimulation of neutrophils with a stimulant such as PMA (phorbol myristate acetate) along with the use of dihydrorhodamine 123 (DHR123) as the substrate to measure neutrophil oxidative burst. Nonfluorescent DHR is oxidized by H₂O₂ produced by activated neutrophils and peroxidase (produced by neutrophils-myeloperoxidase) or eosinophils (eosinophil peroxidase) to fluorescent rhodamine 123. This is a quick and robust method for assessing neutrophil oxidative function in whole blood and can also be used to identify the underlying genetic defect in most cases. Genetic diagnosis by gene sequencing methods is most useful to identify specific genetic defect and mutation (for counseling purposes) and perform genotype-phenotype correlations. Genetic testing for X-linked and the more common autosomal recessive forms are available at several molecular reference labs (see Gene Tests for more information on labs).
However, there are some points to note about the DHR flow assay that can affect interpretation of results. The sample quality is very important. Optimal results are obtained in samples transported within 24 hours of blood collection under strict ambient conditions. Mayo Medical Labs provides critical ambient shipping boxes free of cost for appropriate transport of samples. Assessment of sample viability and neutrophil count (ANC) on receipt of sample is essential to determining quality of the sample and cell loss during transportation. Further gating on viable neutrophils removes the possibility of incorporating artifact in the results. It is essential to include both percent-positive neutrophils and mean fluorescence intensity (MFI) in the analysis and interpretation of results. Complete myeloperoxidase deficiency (CMPO) can provide a false-positive result for the DHR assay and this can also overlap with the pattern seen in atypical X-linked CGD. Patients with p40phox deficiency can also demonstrate a similar pattern of oxidative burst.
Though DHR flow is a sensitive test, it can miss patients with atypical autosomal recessive CGD and, therefore, the result has to be interpreted in context of clinical history and additional testing, including evaluation of specific protein should be performed if clinical phenotype is suggestive.

- To rule-out cMPO deficiency as a cause for a false-positive result, genetic testing can be performed for sequencing of the MPO gene.
- Alternatively, neutrophils can be stained for MPO using a peripheral smear.
- The DHR assay can be performed with gating on eosinophils since eosinophil peroxidase will provide a normal DHR result in the absence of myeloperoxidase.
- Most patients with cMPO deficiency are asymptomatic but a small subset (~5%) may have serious complications, including candida infection.
- The measurement of MFI can be used as a surrogate for NADPH oxidase activity.
- The DHR flow assay can be used to identify carrier females of X-linked CGD as well as donor chimerism in CGD patients postallogeneic HCT.
- Survival in CGD is strongly dictated by the specific mutation and amount of ROS (reactive oxygen species) produced.

The measurement of mean fluorescence intensity (or MFI) can be used as a surrogate for NADPH oxidase activity. The DHR flow assay can be used to identify carrier females of X-linked CGD as well as measure donor chimerism in CGD patients postallogeneic hematopoietic cell transplant. Survival in CGD is strongly dictated by the specific mutation and the amount of reactive oxygen species produced on neutrophil activation.
Female carriers can show significantly skewed lyonization and clinically symptomatic disease. Also there is age-related skewing of lyonization. To facilitate proper interpretation of results on shipped samples, it is important to include a healthy control with patient sample, as a shipping control, to account for transportation conditions.

Stimulation with PMA is the most commonly used version of the DHR flow test for neutrophil oxidative burst. Stimulation with fMLP (N-formylmethionine-leucyl-phenylalanine) is a chemoattractant peptide for neutrophils and is most commonly used for the diagnosis of Rac2 deficiency, a different neutrophil disorder; but it may also be used as an adjunct to PMA for CGD diagnosis. If assessing specifically for CGD, it is recommended to use the DHRP test, which involves just PMA stimulation. A panel with both stimulants, that is PMA and fMLP, is available if considering both diagnoses, that is CGD and Rac2 deficiency, or it may be useful in the detailed evaluation of CGD patients, especially those with atypical presentation.

All interpretive reports provided include: patient sample, absolute neutrophil count, the percent positive, and MFI results for PMA for patient and shipping control (if provided). If the DHR panel is ordered the same data is provided for fMLP as well. Patient and control viability are included in the interpretation section of the report along with an interpretation of the result.

Optimal neutrophil viability is ≥80%.
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