Overview

Useful For
Diagnosis of chronic granulomatous disease (CGD), X-linked and autosomal recessive forms, Rac2 deficiency, complete myeloperoxidase (MPO) deficiency; monitoring chimerism and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase function posthematopoietic cell transplantation

Assessing residual NADPH oxidase activity pretransplant

Identification of carrier females for X-linked CGD; assessment of changes in lyonization with age in carrier females

Method Name
Flow Cytometry

NY State Available
Yes

Specimen

Specimen Type
WB Sodium Heparin

Shipping Instructions
Specimens are required to be received in the laboratory weekdays and by 4 p.m. on Friday. Draw and package specimen as close to shipping time as possible. Ship specimen overnight in an Ambient Shipping Box-Critical Specimens Only (T668) following the instructions in the box.

It is recommended that specimens arrive within 24 hours of draw.

Samples arriving on the weekend and observed holidays may be canceled.

Necessary Information
Ordering physician name and phone number are required.

Specimen Required
Both a whole blood sodium heparin specimen and a whole blood sodium heparin control specimen from an unrelated, healthy donor are required.

Supplies: Ambient Shipping Box-Critical Specimens Only (T668)

Patient:

Container/Tube: Green top (sodium heparin)

Specimen Volume: 5 mL

Collection Instructions: Send specimen in original tube. Do not aliquot.

Normal Control:
Container/Tube: Green top (sodium heparin)

Specimen Volume: 5 mL

Collection Instructions:

1. Draw a control specimen from a normal (healthy), unrelated person within an hour of the patient.

2. Label clearly on outermost label normal control.

3. Send specimen in original tube. Do not aliquot.

Specimen Minimum Volume

1 mL

Reject Due To

<table>
<thead>
<tr>
<th>Gross hemolysis</th>
<th>Reject</th>
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<tbody>
<tr>
<td>Gross lipemia</td>
<td>Reject</td>
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Specimen Stability Information

<table>
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<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
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<tbody>
<tr>
<td>WB Sodium Heparin</td>
<td>Ambient</td>
<td>48 hours</td>
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Clinical and Interpretive

Clinical Information

Chronic granulomatous disease (CGD) is caused by genetic defects in the gene components that encode the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme complex. These defects result in an inability to produce superoxide anions required for killing bacterial and fungal organisms. Other clinical features include a predisposition to systemic granulomatous complications and autoimmunity. (1) There are 5 known genetic defects associated with the clinical phenotype of CGD. (2) The gene defects include mutations 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), and NCF4 (p40phox). Typically, patients with X-linked CGD have the most severe disease, while patients with p47phox defects tend to have the best outcomes. Mutations in NCF4 encoding the p40phox protein have been the most recently described (3) and appears to be associated with more gastrointestinal disease with fewer infections. There is significant clinical variability even among individuals with similar mutations, in terms of NADPH oxidase function, indicating that there can be several modulating factors including the genetic defect, infection history, and granulomatous and autoimmune complications. There appears to be a correlation between very low NADPH superoxide production and worse outcomes. CGD can be treated with hematopoietic cell transplantation (HCT), which can be effective for the inflammatory and autoimmune manifestations.

It has been shown that survival of patients with CGD was strongly associated with residual reactive oxygen intermediate (ROI) production, independent of the specific gene defect. (4) Measurement of NADPH oxidase activity through the dihydrorhodamine (DHR) flow cytometry assay contributed to the assessment of ROI. The diagnostic laboratory assessment for CGD includes evaluation of NADPH oxidase function in neutrophils, using either the
Test Definition: DHR
DHR Flow, B

nitroblue tetrazolium test (NBT) or the more analytically sensitive DHR test, as described here. 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.\(^{(5, 6)}\) Complete myeloperoxidase (MPO) deficiency can cause a false-positive result for CGD in the DHR flow cytometric assay \(^{(7)}\); however, there is a difference between the percent DHR+ neutrophils and the mean fluorescence intensity (MFI) after PMA stimulation that allows discrimination between true X-linked CGD and complete MPO deficiency. Further, the addition of recombinant human MPO enhances the DHR signal in MPO-deficient neutrophils but not in CGD neutrophils.\(^{(7)}\)

It is important to have quantitative measures in the DHR flow cytometry assay to effectively use the test for diagnosis of the different forms of CGD as well as for monitoring chimerism and NADPH oxidase activity post-HCT. These quantitative measures include assessment of the relative proportion (\%) of neutrophils that are positive for DHR fluorescence after PMA stimulation and the relative fluorescence intensity of DHR (MFI) on neutrophils after activation. This assay can also be used for the diagnostic evaluation of Rac2 deficiency, which is a neutrophil defect that causes profound neutrophil dysfunction with decreased chemotaxis, polarization, superoxide anion production, azurophilic granule secretion. This disease is caused by inhibitory mutations in the \textit{RAC2} gene, which encodes a Rho family GTPase essential to neutrophil activation and NADPH oxidase function.\(^{(8)}\) Patients with Rac2 deficiency have been shown to have normal neutrophil oxidative burst when stimulated with PMA, indicating normal NADPH oxidase activity, but abnormal neutrophil responses to N-formyl-methionyl-leucyl-phenylalanine (fMLP), which is a physiological activator of neutrophils. The defective oxidative burst to fMLP, but not to PMA, indicates a signaling defect in Rac2 deficiency.\(^{(9)}\)

Female carriers of X-linked CGD can become symptomatic for CGD due to skewed lyonization (X chromosome inactivation).\(^{(10)}\) Age-related acquired skewing of lyonization can also cause increased susceptibility to infections in carriers of X-linked CGD.\(^{(11)}\) While germline mutations are more common in CGD, there have been reports of de novo, sporadic mutations in the \textit{CYBB} gene, causing X-linked CGD in male patients whose mothers are not carriers for the affected allele. Additionally, somatic mosaicism has been reported in patients with X-linked CGD who have small populations of normal cells.\(^{(12)}\) There are also reports of triple somatic mosaicism in female carriers (13,14) as well as late-onset disease in an adult female who was a somatic mosaic for a novel mutation in the \textit{CYBB} gene.\(^{(15)}\)

Therefore, the clinical, genetic, and age spectrum of CGD is varied and laboratory assessment of NADPH oxidase activity after neutrophil stimulation, coupled with appropriate interpretation, is critical to achieving an accurate diagnosis or for monitoring patients posttransplant.

**Reference Values**

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<th>Result Name</th>
<th>Unit</th>
<th>Cutoff for Defining Normal</th>
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<td>% PMA ox-DHR+</td>
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<td>%</td>
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The appropriate age-related reference values for Absolute Neutrophil Count will be provided on the report.
**Interpretation**

An interpretive report will be provided, in addition to the quantitative values.

Interpretation of the results of the quantitative dihydrorhodamine (DHR) flow cytometric assay has to include both the proportion of positive neutrophils for DHR after phorbol myristate acetate (PMA) and/or N-formyl-methionyl-leucyl-phenylalanine (fMLP) stimulation, and the mean fluorescence intensity (MFI). Additionally, visual assessment of the pattern of DHR fluorescence is helpful in discriminating between the various genetic defects associated with chronic granulomatous disease (CGD) and complete myeloperoxidase (MPO) deficiency.

**Cautions**

Specimens are optimally tested within 24 hours of blood draw, though the stability of the assay is within 48 hours of collection. Specimens should be collected in sodium heparin and transported under strict ambient conditions. Use of the Ambient Mailer-Critical Specimens Only box (T668) is encouraged to ensure appropriate transportation of the specimen.

Hemolyzed specimens may give high background. Specimens with an absolute neutrophil count (ANC) below 200 will not be accepted for this assay. Complete myeloperoxidase (MPO) deficiency can yield a false-positive result.

**Supportive Data**

Dihydrorhodamine (DHR) analysis was performed to assess neutrophil oxidative burst in 157 healthy donors, 74 children and 83 adults.

**Clinical Reference**


Performance

Method Description

A sodium heparin whole blood specimen is incubated at 37 degrees C in the presence of DHR123. Phorbol myristate acetate (PMA) or formyl-methionyl-leucyl-phenylalanine (fMLP) stimulant is added and mixed with the whole blood specimen for additional incubation at 37 degrees C. The specimen is then centrifuged and the cell pellet is subsequently lysed with ammonium chloride at ambient temperature. Lysed specimens are then washed with azide-free phosphate buffered saline (PBS) prior to staining with LIVE/DEAD viability marker and CD15 at ambient temperature. Finally, cells are washed, centrifuged, and resuspended in 1% para-formaldehyde prior to analysis. Viable neutrophils are identified by the use of the viability dye and further confirmed by the presence of CD15. Approximately 20,000 viable neutrophil events in the unstimulated specimen are used to set the limits for number of events collected for flow cytometry. The results are derived as delta % DHR-positive neutrophils after PMA or fMLP stimulation and mean fluorescence intensity (MFI) for each stimulant for DHR flow cytometry. (O’Gorman MR, Corrochano V: Rapid whole-blood flow cytometry assay for diagnosis of chronic granulomatous disease. Clin Diagn Lab Immunol 1995;2[2]:227-232)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Friday

Specimen must be received by 4 p.m. on Friday.

Analytic Time

3 days

Maximum Laboratory Time

4 days

Specimen Retention Time

4 days

Performing Laboratory Location

Rochester
Fees and Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification

This test was developed using an analyte specific reagent. Its performance characteristics were determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

86352 x2

LOINC® Information

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