

Overview

Useful For

- Evaluating chronic granulomatous disease (CGD), X-linked and autosomal recessive forms, complete myeloperoxidase deficiency
- Monitoring chimerism and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase function post-hematopoietic cell transplantation
- Assessing residual NADPH oxidase activity pretransplant
- Identifying of female carriers for X-linked CGD
- Assessing changes in lyonization with age in female carriers

Genetics Test Information

Approximately 70% of chronic granulomatous disease cases are X-linked and are due to disease-causing variants in the *CYBB* gene, encoding the gp91phox protein. The following genes may have genetic variants inherited in an autosomal recessive pattern: *NCF1* (p47phox), *NCF2* (p67phox), *CYBA* (p22phox), and *NCF4* (p40phox). Disease-causing variants in *NCF1* account for 25% of cases, while variants in *NCF2* and *CYBA* account for 5% of cases each. Disease-causing variants in the *NCF4* gene have been described but are rare.

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. Collect 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 collection.

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 whole blood specimen in original tube. Do not aliquot.

Normal Control:

Container/Tube: Green top (sodium heparin)

Specimen Volume: 5 mL

Collection Instructions:

1. Collect a control specimen from a normal (healthy), unrelated person within an hour of the patient's specimen collection.
2. Label clearly on outermost label **normal control**.
3. Send whole blood specimen in original tube. Do not aliquot.

Specimen Minimum Volume

1 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
WB Sodium Heparin	Ambient	48 hours	GREEN TOP/HEP

Clinical & Interpretive

Clinical Information

Chronic granulomatous disease (CGD) is caused by genetic alterations in the gene components that encode the

nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme complex. These alterations 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.(1) There are 6 known genes associated with the clinical phenotype of CGD.(2) The gene defects include disease-causing variants in the *CYBB* gene, encoding the gp91phox protein, which is X-linked and accounts for approximately 70% of CGD cases. Other genetic causes are autosomal recessive in inheritance and occur in one of the following genes: *NCF1* (p47phox), *NCF2* (p67phox), *CYBA* (p22phox), *NCF4* (p40phox) and *CYBC1*(3). Typically, patients with X-linked CGD have the most severe disease, while patients with p47phox defects tend to have the best outcomes. Disease-causing variants in *NCF4* and *CYBC1* have been the most recently described rare causes of disease.(3,4) 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 the genetic alteration, 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, 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 alteration.(5) 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 historically the nitroblue tetrazolium test or currently 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.(6,7) Complete myeloperoxidase (MPO) deficiency can cause a false-positive result for CGD in the DHR flow cytometric assay (8); however, there is a difference between the percent DHR+ neutrophils and the mean fluorescence intensity 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.(8)

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-hematopoietic cell transplantation. 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 on neutrophils after activation.

Female carriers of X-linked CGD can become symptomatic for CGD due to skewed lyonization (X chromosome inactivation).(9) Age-related acquired skewing of lyonization can also cause increased susceptibility to infections in carriers of X-linked CGD.(10) While inherited disease-causing variants are more common in CGD, there have been reports of *de novo*, variants in the *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.(11) There are also reports of triple somatic mosaicism in female carriers (12,13) as well as late-onset disease in an adult female who was a somatic mosaic for a novel variant in the *CYBB* gene.(14)

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

Result Name	Unit	Cutoff for defining normal
% PMA ox-DHR+	%	> or =95%
MFI PMA ox-DHR+	MFI	> or =60
Control % PMA ox-DHR+	%	> or =95%
Control MFI PMA ox-DHR+	MFI	> or =60

MFI = mean fluorescence intensity
PMA = phorbol myristate acetate
DHR = dihydrorhodamine

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 described in Clinical Information.

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 stimulation, and the mean fluorescence intensity. Additionally, visual assessment of the pattern of DHR fluorescence is helpful in discriminating between the various genetic defects associated with chronic granulomatous disease and complete myeloperoxidase 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 Shipping Box-Critical Specimens Only (T668) is encouraged to ensure appropriate transportation of the specimen.

Some disease-causing variants in *NCF4* cause only a mild atypical form of CGD and may not be detected by this assay.

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

Supportive Data

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

Clinical Reference

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12. de Boer M, Bakker E, Van Lierde S, Roos D: Somatic triple mosaicism in a carrier of X-linked chronic granulomatous disease. *Blood*. 1998 Jan;91(1):252-257
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15. Kuhns DB: Diagnostic testing for chronic granulomatous disease. *Methods Mol Biol*. 2019;1982:543-571
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17. Knight V, Heimall JR, Chong H, et al: A toolkit and framework for optimal laboratory evaluation of individuals with suspected primary immunodeficiency. *J Allergy Clin Immunol Pract*. 2021 Sep;9(9):3293-3307.e6

Performance

Method Description

A sodium heparin whole blood specimen is incubated at 37 degrees C in the presence of DHR123. Phorbol myristate acetate (PMA) stimulant is added and mixed with the whole blood specimen for additional incubation at 37 degrees C. The sample is then centrifuged, and the cell pellet is subsequently lysed with ammonium chloride at room temperature. Lysed samples are then washed with azide-free phosphate buffered saline prior to staining with LIVE/DEAD viability marker and CD15 at room temperature. Finally, cells are washed, centrifuged, and resuspended in 1% paraformaldehyde 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 sample are used to set the limits for number of events collected for flow cytometry. The results are derived as delta % DHR+ neutrophils after PMA stimulation and mean fluorescence intensity.(O'Gorman MR, Corrochano V: Rapid whole-blood flow cytometry assay for

diagnosis of chronic granulomatous disease. Clin Diagn Lab Immunol. 1995 Mar;2[2]:227-232; Kuhns DB: Diagnostic testing for chronic granulomatous disease. Methods Mol Biol. 2019;1982:543-571)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

3 to 4 days

Specimen Retention Time

4 days

Performing Laboratory Location

Rochester

Fees & 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 account representative. 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 US Food and Drug Administration.

CPT Code Information

86352

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
DHRP	DHR Flow PMA, B	98124-1

Result ID	Test Result Name	Result LOINC® Value
ANC	Absolute Neutrophil Count	751-8
PMAP	% PMA ox-DHR+	85376-2
PMAM	MFI PMA ox-DHR+	85374-7
ANCC	Control Absolute Neutrophil Count	85369-7

Test Definition: DHRP

Dihydrorhodamine Flow Cytometric Phorbol
Myristate Acetate Test, Blood

PMAPC	Control % PMA ox-DHR+	85377-0
PMAMC	Control MFI PMA ox-DHR+	85375-4
DHRPI	Interpretation	69052-9