Test Definition: DHR

DHR Flow, B

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.**

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject

Specimen Minimum Volume

1 mL

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container

WB Sodium Heparin	Ambient (preferred)		GREEN TOP/HEP

Clinical & 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 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 *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 *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 *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

Result Name	Unit	Cutoff for Defining Normal
% PMA ox-DHR+	%	> or =95%
MFI PMA ox-DHR+	MFI	> or =60
% fMLP ox-DHR+	%	> or =10%
MFI fMLP ox-DHR+	MFI	> or =2
Control % PMA ox-DHR+	%	> or =95%
Control MFI PMA ox-DHR+	MFI	> or =60
Control % fMLP ox-DHR+	%	> or =10%
Control MFI fMLP ox-DHR+	MFI	> or =2

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

1. Kang EM, Marciano BE, DeRavin SS, et al: Chronic Granulomatous Disease: Overview and hematopoietic stem cell transplantation. J Allergy Clin Immunol 2011;127:1319-1326

2. Segal BH, DeCarlo ES, Kwon-Chung KJ, et al: Aspergillus nidulans infection in chronic granulomatous disease. Medicine 1998;77:345-354

3. Matute JD, Arias AA, Wright NA, et al: A new genetic subgroup of CGD with autosomal recessive mutations in p40phox and selective defects in neutrophil NADPH oxidase activity. Blood 2009;114:3309-3315

4. Kuhns DB, Alvord WG, Heller T, et al: Residual NADPH oxidase and survival in Chronic Granulomatous Disease. N Engl J Med 2010;363:2600-2610

5. Vowells SJ, Fleisher TA, Sekhsaria S, et al: Genotype-dependent variability in flow cytometric evaluation of reduced NADPH oxidase function in patients with CGD. J Pediatr 1996;128:104-107

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7. Mauch L, Lun A, Oâ€™Gorman MRG, et al: CGD and complete MPO deficiency both yield strongly reduced DHR 123 test signals but can be easily discerned in routine testing for CGD. Clin Chem 2007;53:890-896

8. Ambruso DR, Knall C, Abell AN, et al: Human neutrophil immunodeficiency syndrome is associated with an inhibitory Rac2 mutation. Proc Natl Acad Sci U S A 2000;97:4654-4659

9. Accetta D, Syverson G, Bonacci B, et al: Human phagocyte defect caused by a RAC2 mutation detected by means of neonatal screening for T cell lymphopenia. J Allergy Clin Immunol 2011;127:535-538

10. Roesler J: Carriers of X-linked CGD at risk. Clin Immunol 2009;130:233

11. Rosen-Wolff A, Soldan W, Heyne K, et al: Increased susceptibility of a carrier of X-linked CGD to Aspergillus fumigatus infection associated with age-related skewing of lyonization. Ann Hematol 2001:80:113-115

12. Yamada M, Okura Y, Suzuki Y, et al: Somatic mosaicism in two unrelated patients with X-linked CGD characterized by the presence of a small population of normal cells. Gene 2012:497:110-115

13. de Boer M, Bakker E, Van Lierde S, et al: Somatic triple mosaicism in a carrier of X-linked CGD. Blood 1998;91:252-257

14. Noack D, Heyworth PG, Kyono W, et al: A second case of somatic triple mosaicism in the *CYBB* gene causing CGD. Hum Genet 2001;109:234-238

15. Wolach B, Scharf Y, Gavrieli R, et al: Unusual late presentation of X-linked CGD in an adult female with a somatic mosaic for a novel mutation in *CYBB*. Blood 2005;105:61-66

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

Specimen Retention Time

4 days

Performing Laboratory Location

Rochester

Fees & Codes

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 x2

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
DHR	DHR Flow, B	98122-5

Result ID	Reporting Name	LOINC®
ANC	Absolute Neutrophil Count	751-8
PMAP	% PMA ox-DHR+	85376-2
PMAM	MFI PMA ox-DHR+	85374-7
FMPPP	% FMLP ox-DHR+	85373-9
FMPM	MFI fMLP ox-DHR+	85370-5
ANCC	Control Absolute Neutrophil Count	85369-7
PMAPC	Control % PMA ox-DHR+	85377-0
PMAMC	Control MFI PMA ox-DHR+	85375-4
FMPPC	Control % fMLP ox-DHR+	85372-1
FMPMC	Control MFI fMLP ox-DHR+	85371-3
DHRI	Interpretation	69052-9