

Overview

Useful For

Evaluation of RAC2 deficiency and RAC2 gain of function

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 mailer.

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 time.
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

This assay can 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 variants in the *RAC2* gene, which encodes a Rho family GTPase essential to neutrophil activation and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase function.(1) Patients with RAC2 deficiency have been shown to have normal neutrophil oxidative burst when stimulated with phorbol myristate acetate (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, is consistent with RAC2 deficiency.(2) By contrast, gain of function variants in RAC2 would lead to a an exaggerated response to fMLP.(3)

Reference Values

Result name	Unit	Cutoff for defining normal
% fMLP ox-DHR+	%	> or =10%
MFI fMLP ox-DHR+	MFI	> or =2
Control % fMLP ox-DHR+	%	> or =10%

Control MFI fMLP ox-DHR+	MFI	> or =2
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fMLP = N-formyl-methionyl-leucyl-phenylalanine
DHR = dihydrorhodamine
MFI = mean fluorescence intensity

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 N-formyl-methionyl-leucyl-phenylalanine stimulation, and the mean fluorescence intensity.

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 below 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

1. 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 Apr 25;97(9):4654-4659

2. 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 Feb;127(2):535-538

3. Hsu AP, Donko A, Arrington ME, et al: Dominant activating RAC2 mutation with lymphopenia, immunodeficiency, and cytoskeletal defects. Blood. 2019 May 2;133(18):1977-1988

Performance

Method Description

A sodium heparin whole blood specimen is incubated at 37 degrees C in the presence of DHR123. N-formyl-methionyl-leucyl-phenylalanine (fMLP) stimulant is added and mixed with the whole blood specimen for additional incubation at 37 degrees C. The sample is then centrifuged, and 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 ambient 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 fMLP 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;2[2]:227-232; Kuhns DB: Diagnostic testing for chronic granulomatous disease. Methods Mol Biol. 2019;1982:543-571)

PDF Report

No

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
DHRF	DHR Flow fMLP, B	98123-3

Test Definition: DHRF

Dihydrorhodamine Flow Cytometric
N-Formyl-Methionyl-Leucyl-Phenylalanine
Test, Blood

Result ID	Test Result Name	Result LOINC® Value
ANC	Absolute Neutrophil Count	751-8
FMPPP	% FMLP ox-DHR+	85373-9
FMPM	MFI fMLP ox-DHR+	85370-5
ANCC	Control Absolute Neutrophil Count	85369-7
FMPPC	Control % fMLP ox-DHR+	85372-1
FMPMC	Control MFI fMLP ox-DHR+	85371-3
DHRFI	Interpretation	69052-9