Overview

Useful For
Screening for X-linked hyper-IgM (XL-HIGM) or CD40L deficiency, primarily in male patients younger than 10 years of age

Ascertaining XL-HIGM carrier status in females of child-bearing age younger than 45 years of age

Method Name
FlowCytometry

NY State Available
No

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.

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's name and phone number are required.

Specimen Required
For serial monitoring, we recommend that specimen draws be performed at the same time of day.

Container/Tube: Green top (sodium heparin)

Specimen Volume: 4 mL

Collection Instructions:
1. Send specimen in original tube. Do not aliquot.
2. Specimens received more than 72 hours after collection will be rejected and the assay will not be performed.

Specimen Minimum Volume
1.2 mL

Reject Due To

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<th>Gross hemolysis</th>
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Document generated February 6, 2020 at 3:39am CST
Test Definition: XHIM
X-Linked Hyper IgM Syndrome, B

Specimen Stability Information

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Clinical and Interpretive

Clinical Information

CD154 (CD40 ligand: CD40L) is required for the interaction of T cells and B cells as part of the normal adaptive immune response. Activation of T cells leads to the expression of the CD40L molecule on the cell surface. CD40L binds the CD40 receptor that is always present on B cells, monocytes, and macrophages (regardless of environmental conditions). This interaction of CD40L with CD40 is important in B-cell proliferation, differentiation, and class-switch recombination (isotype class-switching).

Patients with X-linked hyper-IgM (XL-HIGM) syndrome have defective CD40L expression on their activated helper CD4 T cells.(1,2) This leads to defective B-cell responses and the absence of immunoglobulin class-switching. These features are typified in these patients by a profound reduction or absence of isotype class-switched memory B cells (CD19+CD27+IgM-IgD-) with low or absent secreted IgG and IgA, and normal or elevated serum IgM levels.(1,2) Due to the impairment of T-cell function and macrophage activation, XL-HIGM patients are particularly prone to opportunistic infections with Pneumocystis jiroveci, Cryptosporidium, and Toxoplasma gondii.(1)

To date, more than 100 unique mutations of CD40LG, the gene that encodes CD40L, have been described, affecting the intracellular, transmembrane and, more commonly, extracellular domain containing the CD40-binding region.

A defect in surface expression of CD40L on activated CD4 T cells can be demonstrated using an anti-CD40L antibody and flow cytometry.(3,4) Since certain CD40LG mutations can maintain surface protein expression, albeit with loss of function, it is important to also evaluate CD40L-binding capacity to eliminate the possibility of false-negative results. A soluble recombinant, chimeric receptor protein, CD40-ulig, is incorporated into the assay, which assesses CD40L function by determining receptor-binding activity. Approximately 20% of XL-HIGM patients have activated CD4 T cells with normal surface expression of CD40L, but aberrant function.(4)

XL-HIGM is a severe type of primary immunodeficiency that affects males, and most patients are diagnosed within a few months to the first year of life. Females are typically carriers and asymptomatic. Consequently, this test is only indicated in young males (<10 years of age) or, to identify carriers, in females of child-bearing age (<45 years).

Reference Values

Present

Interpretation

This is a qualitative assay; CD40L-protein expression and function is reported as present or absent. Absence of CD40L-protein expression and function is consistent with X-linked hyper-IgM (XL-HIGM). In females, the presence of 2 populations-normal and abnormal-is consistent with carrier status.

Most patients (80%-90%) with XL-HIGM have absent or significantly reduced CD40L expression on their activated CD4 T cells. Patients with normal CD40L expression, but abnormal function, show an absence of binding with
soluble chimeric CD40-ulg antibody, substantiating a diagnosis of XL-HIGM. Females who are carriers for this disease will show a typical bimodal pattern of CD40L expression, with 50% of the T cells lacking any CD40L expression. In the case of aberrant protein function, a similar profile will be obtained with the CD40-ulg antibody.

CD69 is a marker for T-cell activation and serves as a positive control; in the absence of induced CD69 expression on T cells, the presence of XL-HIGM cannot be assessed.

**Cautions**

This test is typically not indicated in males over 10 years of age or females beyond child-bearing age (>45 years). For questions about appropriate test selection, call 800-533-1710.

The test must be performed on fresh, heparinized whole blood cells for appropriate CD40L expression on activated CD4 T cells; specimen handling instructions must be followed. T-cell activation is variable on specimens tested between 48 and 72 hours after blood collection. These specimens will be analyzed and results will be reported after the laboratory director's review. Specimens received more than 72 hours after collection will be rejected and the assay will not be performed.

Patients with normal CD40L expression and normal receptor binding with the CD40-ulg antibody, yet presenting with the clinical phenotype of hyper-IgM (HIGM) syndrome, should be evaluated for autosomal recessive forms of this syndrome including mutations in CD40, AICDA (AID), and UNG.(1,2) A combination of clinical features and laboratory analyses should permit identification of an underlying HIGM defect, if present.

The other form of X-linked hyper-IgM (XL-HIGM) involving mutations in the NEMO (NF-kappa B essential modulator) gene (official symbol IKBKG) can be easily discriminated from the CD40LG deficiency due to the unusual and characteristic clinical findings including abnormal development of ectoderm-derived skin structures and immunodeficiency with increased susceptibility to mycobacterial infections.(1,2)

Previous studies have reported mutations involving splice sites that result in the generation of small amounts of wild-type CD40L, associated with a milder clinical phenotype.(4) In these cases, the CD40-ulg fusion protein may show some binding, albeit at lower intensity and, therefore, the final molecular diagnosis depends on sequencing of the CD40LG gene.

This is not a confirmatory test for CD40L deficiency, and genetic testing must be performed to determine the specific mutation involved. Information about genetic testing for CD40L deficiency is available by calling 800-533-1710.

**Clinical Reference**


**Performance**

**Method Description**

The assay measures the expression of CD40L on activated CD4 T cells. Heparinized whole blood is incubated with phorbol myristate acetate (PMA) and ionomycin (calcium ionophore) for lymphocyte activation. The red blood cells
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X-Linked Hyper IgM Syndrome, B

are lysed and the remaining white blood cells are stained with a 4-color panel of antibodies on a single platform. The assay involves 4 tubes, which include an unstimulated control for both the CD40L and CD40-ulg antibodies. CD69 expression is measured as a positive control for appropriate T-cell activation. A combination of CD3, CD8, CD154 (CD40L), and CD40-ulg antibodies enables assessment of CD40L expression and binding (with CD40-ulg) on total T cells (CD3+), suppressor T cells (CD3+CD8+), and helper T cells (CD3+CD8-). A normal, healthy control will be included with each experiment to ensure the optimal performance of the assay. (O’Gorman MR, Zaas D, Paniagua M, et al: Development of a rapid whole blood flow cytometry procedure for the diagnosis of X-linked hyper-IgM syndrome patients and carriers. Clin Immunol Immunopathol 1997 November;85[2]:179-181; unpublished Mayo method)

PDF Report
No

Day(s) and Time(s) Test Performed
Monday through Friday

Specimens are required to be received in the laboratory on weekdays and by 4 p.m. on Friday. No weekend processing

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
88184-Flow cytometry, cell surface, cytoplasmic

88185 x 6-Each additional marker

LOINC® Information
### Test Definition: XHIM
X-Linked Hyper IgM Syndrome, B

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