Test Definition: XHIM

X-Linked Hyper IgM Syndrome, Blood

Overview

Useful For

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

Ascertaining XL-HIGM carrier status in women of child-bearing age (younger than 45 years)

Genetics Test Information

*CD40L*G is located on the long arm of the X-chromosome (Xq 21.3-22) and encodes the surface protein CD40 ligand (CD154). It is critical for the formation of germinal centers and, therefore, class switch recombination and somatic hypermutation. More than 100 unique genetic variants of *CD40LG* have been described. The observed variants are scattered throughout the gene but are more prevalent in exon 5.

Method Name

Flow Cytometry

NY State Available

No

Specimen

Specimen Type

WB Sodium Heparin

Shipping Instructions

Testing is not performed on Saturday, Sunday, or observed holidays. Only collect and ship specimens for arrival on days when testing is performed.

Specimens received on days when testing is not performed or after 5 p.m. Central on Friday will be canceled if specimen is outside of stability when testing is next performed.

Collect and package specimen as close to shipping time as possible. It is recommended that specimens arrive within 24 hours of collection.

Necessary Information

The ordering healthcare professional's name and phone number are required.

Specimen Required

Container/Tube: Green top (sodium heparin)
Specimen Volume: 4 mL
Collection Instructions: Send whole blood specimen in original tube. **Do not aliquot.**
Additional Information: For serial monitoring, it is recommended that specimen collection be performed at the same

time of day.

Specimen Minimum Volume

1.2 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
WB Sodium Heparin	Ambient	72 hours	GREEN TOP/HEP

Clinical & 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 constitutively expressed on B cells, monocytes, and macrophages. 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) It is the most common class switch recombination defect and accounts for approximately 50% of the patients in this category. It leads to defective B-cell responses and the absence of immunoglobulin class-switching, which are typified 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, patients with XL-HIGM are particularly prone to opportunistic infections with *Pneumocystis jiroveci*, *Cryptosporidium*, and *Toxoplasma gondii*.(1)

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* variants 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-uIg, is incorporated into the assay, which assesses CD40L function by determining receptor-binding activity. Approximately 20% of patients with XL-HIGM have activated CD4 T cells with normal surface expression of CD40L but aberrant function.(4)

X-linked hyper-IgM is a severe primary immunodeficiency that affects male patients, and most patients are diagnosed within a few months to the first year of life. Female patients are typically carriers and asymptomatic. Consequently, this test is only indicated for boys (<10 years) or to identify carriers, women of child-bearing age (<45 years).

Reference Values

Present

Interpretation

This is a qualitative assay; CD40L-protein expression and function are reported as present or absent. Absence of CD40L-protein expression and function is consistent with X-linked hyper-IgM (XL-HIGM). In female patients, 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-uIg antibody, substantiating a diagnosis of XL-HIGM. Female patients 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-uIg 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 male patients older than 10 years or women older than 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.

Patients with normal CD40L expression and normal receptor binding with the CD40-uIg antibody yet presenting with the clinical phenotype of hyper-IgM (HIGM) syndrome should be evaluated for autosomal recessive forms of this syndrome including genetic variants 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 X-linked form of hyper-IgM can rarely be caused by disease-causing variants in the *NEMO* (NF-kappa B essential modulator) gene (official symbol *IKBKG*), which can be 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 variants 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-uIg fusion protein may show some binding, albeit at lower intensity and, therefore, the final molecular diagnosis depends on sequencing of the *CD40LG* gene (see BCELL / B-Cell and Antibody Deficiency Gene Panel, Varies).

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

Clinical Reference

1. Etzioni A, Ochs HD. The hyper IgM syndrome-an evolving story. Pediatr Res. 2004:56(4):519-525
2. Durandy A, Peron S, Fischer A. Hyper-IgM syndromes. Curr Opin Rheumatol. 2006;18(4):369-376

3. Lee WI, Torgerson TR, Schumacher MJ, Yel L, Zhu Q, Ochs HD. Molecular analysis of a large cohort of patients with the hyper immunoglobulin M (IgM) syndrome. Blood. 2005;105(5):1881-1890
4. Seyama K, Nonoyama S, Gangsaas I, et al. Mutations of the CD40 ligand gene and its effect on CD40 ligand expression in patients with X-linked hyper IgM syndrome. Blood. 1998;92(7):2421-2434
5. Vargas-Hernandez A, Berron-Ruiz L, Staines-Boone T, et al. Clinical and genetic analysis of patients with X-linked hyper-IgM syndrome. Clin Genet. 2013;83(6):585-587
6. Vavassori V, Mercuri E, Marcovecchio GE, et al. Modeling, optimization, and comparable efficacy of T cell and hematopoietic stem cell gene editing for treating hyper-IgM syndrome. EMBO Mol Med. 2021;13(3):e13545

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 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-uIg antibodies. CD69 expression is measured as a positive control for appropriate T-cell activation. A combination of CD3, CD8, CD154 (CD40L), and CD40-uIg antibodies enables assessment of CD40L expression and binding (with CD40-uIg) 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;85[2]:179-181; Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

3 to 4 days

Specimen Retention Time

4 days

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Superior Drive

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

88184-Flow cytometry, cell surface, cytoplasmic
88185 x 6-Each additional marker

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
XHIM	X-Linked Hyper IgM Syndrome, B	98239-7

Result ID	Test Result Name	Result LOINC® Value
29040	CD40muIg (Function)	98241-3
82964	CD40 Ligand Expression	98240-5
23901	Interpretation	69052-9