Overview

**Useful For**
Screening for common variable immunodeficiency (CVID) and hyper-IgM syndromes

Assessing B-cell subset reconstitution after stem cell or bone marrow transplant

Assessing response to B-cell-depleting immunotherapy

Identifying defects in transmembrane activator and calcium modulator and cyclophilin ligand (CAML) interactor (TACI) and B-cell-activating factor receptor (BAFF-R) in patients presenting with clinical symptoms and other laboratory features consistent with CVID

**Profile Information**

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<tr>
<td>IABC</td>
<td>Immune Assessment B Cell Subsets, B</td>
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**Reflex Tests**

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<tr>
<td>CVID</td>
<td>CVID Confirmation Flow Panel</td>
<td>Yes</td>
<td>No</td>
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**Testing Algorithm**
If immune assessment B-cell subsets test is abnormal, then confirmation will be performed at an additional charge.

When multiple specimen types are required to perform a panel of tests, the laboratory will perform the tests for which the appropriate specimen type was received and the laboratory will cancel those for which the appropriate specimen was not received. Please be advised that this may change the degree of interpretation received with the report. If only the refrigerate EDTA sample is received, this test will be canceled and converted to RBCS / Relative B-Cell Subset Analysis Percentage which provides the relative B-cell subset values without quantitation.

**Method Name**
TBBS: Flow Cytometry

IABC: Fluorescent Flow Cytometry

**NY State Available**
Yes
Specimen

Specimen Type
Whole Blood EDTA

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
1. Date of draw is required.

2. Ordering physician's name and phone number are required.

Specimen Required
Two separate EDTA specimens are required: 1 refrigerated and 1 at ambient transport temperature.

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

Specimen Type: Whole blood for TBBS / Quantitative Lymphocyte Subsets: T, B, and NK

Container/Tube: 4 mL Lavender top (EDTA)

Specimen Volume: 3 mL

Collection Instructions:
1. Send specimen in original tube. Do not aliquot.

2. Label specimen as blood for TBBS / Quantitative Lymphocyte Subsets: T, B, and NK.

Specimen Stability Information: Ambient <52 hours

Specimen Type: Whole blood for IABC / B-Cell Phenotyping Screen for Immunodeficiency and Immune Competence Assessment, Blood

Container/Tube: Lavender top (EDTA)

Specimen Volume:
< or =14 years: 4 mL
>14 years: 10 mL

Collection Instructions:
1. Send specimen in original tube. **Do not aliquot.**

2. Label specimen as blood for IABC / B-Cell Phenotyping Screen for Immunodeficiency and Immune Competence Assessment, Blood.

**Specimen Stability Information:** Refrigerated <48 hours

**Specimen Minimum Volume**

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**Reject Due To**

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**Specimen Stability Information**

**Clinical and Interpretive**

**Clinical Information**

Quantitative Lymphocyte Subsets: T, B, and NK:

Normal immunity requires a balance between the activities of various lymphocyte subpopulations with different effector and regulatory functions.

Different immune cells can be characterized by unique surface membrane antigens described by a cluster of differentiation nomenclature (eg, CD3 is an antigen found on the surface of T lymphocytes). Abnormalities in the number and percent of T (CD3, CD4, CD8), B (CD19), and natural killer (CD16+CD56) lymphocytes have been described in a number of different diseases. In patients who are infected with HIV, the CD4 count is measured for AIDS diagnosis and for initiation of antiviral therapy. The progressive loss of CD4 T lymphocytes in patients infected with HIV is associated with increased infections and complications.

The US Public Health Service has recommended that all HIV-positive patients be tested every 3 to 6 months for the level of CD4 T lymphocytes.

The absolute counts of lymphocyte subsets are known to be influenced by a variety of biological factors, including hormones, the environment, and temperature. The studies on diurnal (circadian) variation in lymphocyte counts have demonstrated progressive increase in CD4 T-cell count throughout the day, while CD8 T cells and CD19+ B cells increase between 8:30 a.m. and noon, with no change between noon and afternoon. Natural killer (NK) cell counts, on the other hand, are constant throughout the day. (1) Circadian variations in circulating T-cell counts have been shown to be negatively correlated with plasma cortisol concentration. (2-4) In fact, cortisol and catecholamine
concentrations control distribution and, therefore, numbers of naive versus effector CD4 and CD8 T cells.(2) It is generally accepted that lower CD4 T-cell counts are seen in the morning compared with the evening,(5) and during summer compared to winter.(6) These data, therefore, indicate that timing and consistency in timing of blood collection is critical when serially monitoring patients for lymphocyte subsets.

Immune Assessment B Cell Subsets, Blood:

The adaptive immune response includes both cell-mediated (mediated by T cells and natural killer: NK cells) and humoral immunity (mediated by B cells). After antigen recognition and maturation in secondary lymphoid organs, some antigen-specific B cells terminally differentiate into antibody-secreting plasma cells or become memory B cells. Memory B cells are 3 subsets: marginal zone B cells (MZ or nonswitched memory), class-switched memory B cells, and IgM-only memory B cells. Decreased B-cell numbers, B-cell function, or both, result in immune deficiency states and increased susceptibility to infections. These decreases may be either primary (genetic) or secondary. Secondary causes include medications, malignancies, infections, and autoimmune disorders.

Common variable immunodeficiency (CVID), a disorder of B-cell function, is the most prevalent primary immunodeficiency with a prevalence of 1 to 25,000 to 1 to 50,000.(1) CVID has a bimodal presentation with a subset of patients presenting in early childhood and a second set presenting between 15 and 40 years of age, or occasionally even later. Four different genetic defects have been associated with CVID including mutations in the ICOS, CD19, BAFF-R, and TACI genes. The first 3 genetic defects account for approximately 1% to 2%, and TACI mutations account for 8% to 15% of CVID cases.

CVID is characterized by hypogammaglobulinemia usually involving most or all of the Ig classes (IgG, IgA, IgM, and IgE), impaired functional antibody responses, and recurrent sinopulmonary infections.(1,2) B-cell numbers may be normal or decreased. A minority of CVID patients (5%-10%) have very low B-cell counts (<1% of peripheral blood leukocytes), while another subset (5%-10%) exhibit noncaseating, sarcoid-like granulomas in different organs and also tend to develop a progressive T-cell deficiency.(1) Of all patients with CVID, 25% to 30% have increased numbers of CD8 T cells and a reduced CD4 to CD8 ratio (<1). Studies have shown the clinical relevance of classifying CVID patients by assessing B-cell subsets, since changes in different B-cell subsets are associated with particular clinical phenotypes or presentations.(3,4)

The B-cell phenotyping assay can be used in the diagnosis of hyper-IgM syndromes, which are characterized by increased or normal levels of IgM with low IgG and/or IgA.(5) Patients with hyper-IgM syndromes can have 1 of 5 known genetic defects—mutations in the CD40L, CD40, AID (activation-induced cytidine deaminase), UNG (uracil DNA glycosylase), and NEMO (NF-kappa B essential modulator) genes. Mutations in CD40L and NEMO are inherited in an X-linked fashion, while mutations in the other 3 genes are inherited in an autosomal recessive fashion. Patients with hyper-IgM syndromes have a defect in isotype class-switching, which leads to a decrease in class-switched memory B cells, with or without an increased in nonswitched memory B cells and IgM-only memory B cells.

In addition to its utility in the diagnosis of the above-described primary immunodeficiencies, B-cell phenotyping may be used to assess reconstitution of B-cell subsets after hematopoietic stem cell or bone marrow transplant. This test is also used to monitor B-cell-depicting therapies, such as Rituxan (Rituximab) and Zevalin (Ibritumomab tiuxetan).

CVID Confirmation Flow Panel:

The etiology of CVID is heterogeneous, but recently 4 genetic defects were described that are associated with the CVID phenotype. Specific mutations, all of which are expressed on B cells, have been implicated in the pathogenesis of CVID.

These mutations encode for:

-ICOS-inducible costimulator expressed on activated T cells(1)
-TACI-transmembrane activator and CAML (calcium modulator and cyclophilin ligand) interactor(2)
-CD19(3)
-BAFF-R-B cell activating factor belonging to the tumor necrosis factor (TNF) receptor family(4)

Of these, the TACI mutations probably account for about 10% of all CVID cases.(2) Patients with mutations in the TACI gene are particularly prone to developing autoimmune disease, including cytopenias as well as lymphoproliferative disease. The other mutations each have been reported in only a handful of patients. The etiopathogenesis is still undefined in more than 50% of CVID patients.

A BAFF-R defect should be suspected in patients with low to very low class switched and nonswitched memory B cells and very high numbers of transitional B cells (see IABC/87994 B-Cell Phenotyping Screen for Immunodeficiency and Immune Competence Assessment, Blood). Class switching is the process that allows B cells, which possess IgD and IgM on their cell surface as a part of the antigen-binding complex, to produce IgA, IgE, or IgG antibodies. A TACI defect is suspected in patients with low IgM with normal to low switched B cells, with autoimmune and/or lymphoproliferative manifestations, and normal B cell responses to mitogens.

The absolute counts of lymphocyte subsets are known to be influenced by a variety of biological factors, including hormones, the environment, and temperature. The studies on diurnal (circadian) variation in lymphocyte counts have demonstrated progressive increase in CD4 T-cell count throughout the day, while CD8 T cells and CD19+ B cells increase between 8:30 a.m. and noon, with no change between noon and afternoon. Natural killer (NK) cell counts, on the other hand, are constant throughout the day.(5) Circadian variations in circulating T-cell counts have been shown to be negatively correlated with plasma cortisol concentration.(6-8) In fact, cortisol and catecholamine concentrations control distribution and, therefore, numbers of naive versus effector CD4 and CD8 T cells.(6) It is generally accepted that lower CD4 T-cell counts are seen in the morning compared with the evening,(9) and during summer compared to winter.(10) These data, therefore, indicate that timing and consistency in timing of blood collection is critical when serially monitoring patients for lymphocyte subsets.

Reference Values
The appropriate age-related reference values will be provided on the report.

Interpretation
Quantitative Lymphocyte Subsets: T, B, and NK:

When the CD4 count falls below 500 cells/mcL, HIV-positive patients can be diagnosed with AIDS and can receive antiretroviral therapy.

When the CD4 count falls below 200 cells/mcL, prophylaxis against Pneumocystis jiroveci pneumonia is recommended.

Immune Assessment B Cell Subsets, Blood:

The assay provides quantitative information on the various B-cell subsets (percentage and absolute counts in cells/microliter). Each specimen is evaluated for B-cell subsets with respect to the total number of CD19+ B cells present in the peripheral blood mononuclear cell population, compared to the reference range. In order to verify that there are no CD19-related defects, CD20 is used as an additional pan-B-cell marker (expressed as percentage of CD45+ lymphocytes).

The B-cell panel assesses the following B-cell subsets:
-CD19+=B cells expressing CD19 as a percent of total lymphocytes
Test Definition: IABCS

Immune Assessment B Cell Subsets, B

-CD19+ CD27+ = total memory B cells
-CD19+ CD27+ IgD+ IgM+ = marginal zone or nonswitched memory B cells
-CD19+ CD27+ IgD- IgM+ = IgM-only memory B cells
-CD19+ CD27+ IgD- IgM- = class-switched memory B cells
-CD19+ IgM+ = IgM B cells
-CD19+ CD38+ IgM+ = transitional B cells
-CD19+ CD38+ IgM- = plasmablasts
-CD19+ CD21-=CD21 low ("immature") B cells
-CD19+ CD21+= mature B cells
-CD19+ CD20+= B cells co-expressing both CD19 and CD20 as a percent of total lymphocytes

For isotype class-switching and memory B-cell analyses, the data will be reported as being consistent or not consistent with a defect in memory and/or class switching. If a defect is present in any of these B-cell subpopulations, further correlation with clinical presentation and additional functional, immunological, and genetic laboratory studies will be suggested.

Since each of the 11 B-cell subsets listed above contributes to the diagnosis of common variable immunodeficiency (CVID) and hyper-IgM syndromes and provides further information on the likely specific genetic defect, all the B-cell subsets are carefully evaluated to determine if further testing is needed for confirmation, including functional assays and genotyping, which is then suggested as follow-up testing in the interpretive report as detailed below.

If abnormalities are found in the B-cell phenotyping panel, the specimen will be reflexed to the CVID confirmation panel for assessment of defects in surface expression of B-cell-activating factor receptor (BAFF-R) and transmembrane activator and calcium modulator and cyclophilin ligand (CAML) interactor (TACI) (2 genes/proteins associated with CVID). To conclusively determine if TACI mutations are present, the TACI mutation analysis test by gene sequencing can be ordered (TACIF / Transmembrane Activator and CAML Interactor [TACI] Gene, Full Gene Analysis).

CVID Confirmation Flow Panel:

BAFF-R is normally expressed on over 95% of B cells, while TACI is expressed on a smaller subset of B cells and a proportion of activated T cells.

The lack of TACI or BAFF-R surface expression on the appropriate B-cell population is consistent with a CVID defect.

Results will be interpreted in the context of the B-cell phenotyping results and correlation to clinical presentation will be recommended.

Cautions

This assay and the reference range reported are based on analysis of B cells derived from the mononuclear cell fraction of peripheral whole blood and, therefore, results may not be identical to those performed on whole blood (eg, TBBS / Quantitative Lymphocyte Subsets: T, B, and NK).
This test is a screening test and further analyses will be required to complete a diagnostic workup for common variable immunodeficiency (CVID) (eg, CVID / Common Variable Immunodeficiency Confirmation Flow Panel; TACIF / Transmembrane Activator and CAML Interactor [TACI] Gene, Full Gene Analysis) and hyper-IgM (XHIM / X-Linked Hyper IgM Syndrome, Blood and CD40 / B-Cell CD40 Expression by Flow Cytometry, Blood for CD40 ligand and CD40 expression, respectively).

This test is not indicated for the evaluation of lymphoproliferative disorders (eg, leukemia, lymphoma, multiple myeloma).

Timing and consistency in timing of blood collection is critical when serially monitoring patients for lymphocyte subsets. See data under Clinical Information.

**Clinical Reference**

Quantitative Lymphocyte Subsets: T, B, and NK:


Immune Assessment B Cell Subsets, Blood:


CVID Confirmation Flow Panel:


Performance

Method Description

Quantitative Lymphocyte Subsets: T, B, and NK:
The T, B, and natural killer (NK)-cell surface marker assay uses monoclonal antibodies to identify the various membrane antigens, and flow cytometry to enumerate the number of cells expressing these differentiation antigens. CD14 is used to exclude monocytes, thereby improving accuracy and enhancing the purity of the lymphocyte population. The results are reported as the percent of lymphocytes that are total T cells (CD3+), CD3+CD4+ T cells, CD3+CD8+ T cells, natural killer (CD16+56+, CD3-), and B-lymphocytes (CD19+), and the absolute number of each cell type per mL of blood. The assay is a 7-color no-wash procedure and the absolute counts are calculated from internal bead standards. In addition, the total lymphocyte count and the CD4:CD8 ratio are reported. (Hoffman RA, Kung PC, Hansen WP, Goedstien G: Simple and rapid measurement of human T lymphocytes and their subclasses in peripheral blood. Proc Natl Acad Sci USA, 1980;77:4914-4917; US Department of Health and Human Services: Guidelines for performance of CD4+ T-cell determinations in persons with human immunodeficiency virus infection. MMWR Morb Mortal Wkly Rep 1997;46 no. RR-2 pp 1-29).

Immune Assessment B Cell Subsets, Blood:

Peripheral blood mononuclear cells (PBMC) are isolated from whole blood using a Ficoll gradient and used in the staining protocol. The assay involves a multicolor 5-tube panel for the following antibodies: CD45, CD19, CD20, CD27, IgD, IgM, CD38, and CD21. After the staining with specific antibody, the cells are washed and fixed with paraformaldehyde and then analyzed by flow cytometry on a Becton Dickinson FACS Canto instrument. The cell-surface expression is denoted as the percent of CD19+ B cells expressing each of the specific markers. CD19+ and CD20+ B cells are expressed as the percent of the total lymphocytes (CD45+). The absolute counts for the B-cell subsets are derived from flow cytometry analysis of whole blood using monoclonal antibodies to identify CD45, CD3, CD4, CD8, CD19, and CD16+CD56+. CD14 is used to exclude monocytes, thereby improving accuracy and enhancing the purity of the lymphocyte population. The assay is a 7-color, lyse-no wash procedure and the absolute counts are calculated from internal bead standards. The absolute lymphocyte count per microliter is used to calculate the absolute counts of the various B-cell subsets in this assay (Unpublished Mayo method).

Common Variable Immunodeficiency Confirmation Flow Panel:

Peripheral blood mononuclear cells are isolated and stained with CD19, transmembrane activator and calcium modulator and cyclophilin ligand (CAML) interactor (TACI), and B-cell-activating factor receptor (BAFF-R), each conjugated to a fluorochrome. After the staining with specific antibody, the cells are washed, fixed with paraformaldehyde, and then analyzed by flow cytometry on a Becton Dickinson FACS Canto instrument. The cell-surface expression is denoted as the percent of CD19+ B cells expressing TACI and BAFF-R. (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
PBMC’s are stored for 7 days at -70 degrees C
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
See Individual Test IDs

CPT Code Information
T- and B-Cell Quantitation by Flow Cytometry

86355-B cells, total count
86357-Natural killer (NK) cells, total count
86359-T cells, total count
86360-Absolute CD4/CD8 count with ratio

B-Cell Phenotyping Screen for Immunodeficiency and Immune Competence Assessment, Blood
86356 x7 - Mononuclear cell antigen, quantitative

Common Variable Immunodeficiency Confirmation Flow Panel
88184-Flow cytometry, first marker (if appropriate)
88185 x 2-Flow cytometry, each additional marker (if appropriate)

LOINC® Information

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