MAYO CLINIC LABORATORIES

Test Definition: IABCS

B-Cell Phenotyping Profile for Immunodeficiency and Immune Competence Assessment, Blood

Overview

Useful For

Screening for common variable immunodeficiency and hyper-IgM syndromes

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

Assessing response to B-cell-depleting immunotherapy

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

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
TBBS	QN Lymphocyte Subsets: T, B, and NK	Yes	Yes
IABC	Immune Assessment B Cell Subsets, B	No	Yes

Testing Algorithm

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. The laboratory will cancel those for which the appropriate specimen was not received. Be advised that this may change the degree of interpretation received with the report.

Method Name

Flow Cytometry

NY State Available

Yes

Specimen

Specimen Type

Whole Blood EDTA

Ordering Guidance

This test requires multiple whole blood specimens to perform all testing. If only one whole blood specimen type is

received, only the testing associated with that specimen type will be performed. 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, Blood, which provides the relative B-cell subset values without quantitation.

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, Blood) and hyper-IgM (XHIM / X-Linked Hyper IgM Syndrome, Blood and CD40 / B-Cell CD40 Expression by Flow Cytometry, Blood).

Shipping Instructions

Specimens must be received in the laboratory weekdays and by 4 p.m. on Friday. Collect and package specimens as close to shipping time as possible.

It is recommended that specimens arrive within 24 hours of collection.

Samples arriving on the weekend and observed holidays may be canceled.

Necessary Information

1. Date of collection is required.
2. Ordering physician's name and phone number are required.

Specimen Required

Two separate EDTA whole blood specimens are required: 1 refrigerated and 1 at ambient transport temperature.

For serial monitoring, it is recommended that specimens are collected at the same time of day.

Specimen Type: Whole blood for TBBS / Quantitative Lymphocyte Subsets: T, B, and Natural Killer (NK) Cells, Blood
Container/Tube: 4 mL Lavender top (EDTA)
Specimen Volume: 3 mL
Collection Instructions:
1. Send whole blood specimen in original tube. **Do not aliquot.**
2. Label specimen as blood for TBBS
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: 10 mL
Pediatric (< or =14 years old) Volume: 4 mL
Collection Instructions:
1. Send whole blood specimen in original tube. **Do not aliquot.**
2. Label specimen as blood for IABC.
Specimen Stability Information: Refrigerated <48 hours

Specimen Minimum Volume

TBBS: 1 mL
IABC: 5 mL; for pediatric patients: 3 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole Blood EDTA	Varies	48 hours	PURPLE OR PINK TOP/EDTA

Clinical & Interpretive

Clinical Information

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 infected with HIV, the CD4 count is measured for AIDS diagnosis and 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 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 non-switched 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. Many different genetic defects have been associated with CVID, including variants in the *ICOS, CD19, BAFF-R*, and *TACI* genes. *TACI* variants 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 patients with CVID (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 patients with CVID 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- in the *CD40L, CD40, AID* (activation-induced cytidine deaminase), *UNG* (uracil DNA glycosylase), and *NEMO* (NF-kappa B essential modulator) genes.(5) Variants in *CD40L* and *NEMO* are inherited in an X-linked fashion, while variants 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 increase in non-switched 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).

The etiology of CVID is heterogeneous. Variants of the gene that encodes TACI, *TNFRSF13B* (tumor necrosis factor receptor superfamily, member 13B), probably account for about 10%to 15% of all CVID cases.(6-8) Patients with variants in the *TACI* gene are particularly prone to developing autoimmune diseases, including cytopenias and lymphoproliferative disease. The other variants each have been reported in only a handful of patients. The etiopathogenesis is still undefined in 65% to 75% of patients with CVID.

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.(9) Circadian variations in circulating T-cell counts have been shown to be negatively correlated with plasma cortisol concentration.(10-12) In fact, cortisol and catecholamine concentrations control distribution and, therefore, numbers of naive versus effector CD4 and CD8 T cells.(10) It is generally accepted that lower CD4 T-cell counts are seen in the morning compared with the evening(13) and during summer compared to winter.(14) 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 natural killer:
When the CD4 count falls below 500 cells/mcL, patients who are HIV-positive 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:
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
-CD19+ CD27+=total memory B cells
-CD19+ CD27+ IgD+ IgM+=marginal zone or non-switched 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 coexpressing 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.

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 Natural Killer [NK] Cells, Blood).

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

Clinical Reference

1. Warnatz K, Denz A, Drager R, et al: Severe deficiency of switched memory B cells (CD27+ IgM- IgD-) in subgroups of

patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. Blood. 2002 Mar 1;99(5):1544-1551
2. Brouet JC, Chedeville A, Fermand JP, Royer B: Study of the B cell memory compartment in common variable immunodeficiency. Eur J Immunol. 2000 Sept;30(9):2516-2520
3. Wehr C, Kivioja T, Schmitt C, et al: The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood. 2008 Jan 1;111(1):77-85
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7. Salzer U, Chapel HM, Webster ADB, et al: Mutations in TNFRSF13B encoding TACI are associated with common variable immunodeficiency in humans. Nat Genet. 2005 Aug;37(8):820-828
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10. Dimitrov S, Benedict C, Heutling D, et al: Cortisol and epinephrine control opposing circadian rhythms in T-cell subsets. Blood. 2009 May 21;113(21);5134-5143
11. Dimitrov S, Lange T, Nohroudi K, Born J: Number and function of circulating antigen presenting cells regulated by sleep. Sleep 2007 Apr;30(4):401-411
12. Kronfol Z, Nair M, Zhang Q, et al: Circadian immune measures in healthy volunteers: relationship to hypothalamic-pituitary-adrenal axis hormones and sympathetic neurotransmitters. Pyschosom Med. 1997 Jan-Feb;59(1):42-50
13. Malone JL, Simms TE, Gray GC, et al: Sources of variability in repeated T-helper lymphocyte counts from HIV 1-infected patients: total lymphocyte count fluctuations and diurnal cycle are important. J AIDS. 1990;3(2):144-151
14. Paglieroni TG, Holland PV: Circannual variation in lymphocyte subsets, revisited. Transfusion. 1994 Jun;34(6):512-516
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16. Thompson MA, Horberg MA, Agwu AL, et al: Primary care guidance for persons with human immunodeficiency virus: 2020 update by the HIV Medicine Association of the Infectious Diseases Society of America. Clin Infect Dis. 2021 Dec 6;73(11):e3572-e3605. Erratum in: Clin Infect Dis. 2021 Dec 08

Performance

Method Description

Quantitative Lymphocyte Subsets: T, B, and Natural Killer:

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, NK (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, Goldstien G: Simple and rapid measurement of human T lymphocytes and their subclasses in peripheral blood. Proc Natl Acad Sci USA. 1980 Aug:77[8]:4914-4917; Mandy FF, Nicholson JK, McDougal JS: Guidelines for performing single-platform absolute CD4+T-cell determinations with CD45 gating for persons infected with human immunodeficiency virus. Center for Disease Control and Prevention. MMWR Morb Mortal Wkly Rep. 2003 Jan 31;52[RR-2]:1-13)

Immune Assessment B Cell Subsets:
Peripheral blood mononuclear cells 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 a 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)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
3 to 4 days

Specimen Retention Time
PBMC:7 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

86355-B cells, total count
86357-Natural killer (NK) cells, total count
86359-T cells, total count
86360-Absolute CD4/CD8 count with ratio
86356 x7 - Mononuclear cell antigen, quantitative

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
IABCS	Immune Assessment B Cell Subsets, B	90416-9

Result ID	Test Result Name	Result LOINC® Value
30300	CD27+ IgM+ IgD+ % of CD19+ B cells	89352-9
30304	CD27+ IgM+ IgD- % of CD19+ B cells	89348-7
30308	CD38+ IgM- % of CD19+ B cells	89344-6
30310	CD38+ IgM+ % of CD19+ B cells	89341-2
30315	CD21-	89354-5
30301	CD27+ IgM+ IgD+	89351-1
30303	CD27+ IgM- IgD-	89349-5
30311	CD38+ IgM+	89357-8
30297	CD19+	8116-6
30313	CD21+	25164-5
30299	CD27+	89353-7
30309	CD38+ IgM-	89343-8
30307	IgM+	89345-3
30296	CD19+ % of total Lymphocytes	8117-4
29095	CD20+	9558-8
29094	CD20+ % of total Lymphocytes	8119-0
30314	CD21- % of CD19+ B cells	89355-2
30312	CD21+ % of CD19+ B cells	89356-0
30305	CD27+ IgM+ IgD-	89347-9

30298	CD27+ % of CD19+ B cells	89358-6
30306	IgM+ % of CD19+ B cells	89346-1
30302	CD27+ IgM- IgD- % of CD19+ B cells	89350-3
30316	Interpretation	80722-2
4054	% CD16+CD56 (NK cells)	8112-5
4055	CD16+CD56 (NK cells)	20402-4
3324	CD19 (B Cells)	8116-6
3322	CD3 (T Cells)	8122-4
3319	% CD4 (T Cells)	8123-2
3325	CD4 (T Cells)	24467-3
3326	CD8 (T Cells)	14135-8
3327	4/8 Ratio	54218-3
3321	CD45 Total Lymph Count	27071-0
3318	% CD19 (B Cells)	8117-4
3316	% CD3 (T Cells)	8124-0
3320	% CD8 (T Cells)	8101-8
6657	Comment	80722-2