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

The IABCS is a panel test. This test requires two separate whole blood EDTA specimens at two different transport

temperatures: one ambient and one refrigerate.

The TBBS / Quantitative Lymphocyte Subsets: T, B, and Natural Killer (NK) Cells, Blood is automatically performed, a separate order is **not** required.

If only an ambient EDTA specimen is received, only the TBBS / Quantitative Lymphocyte Subsets: T, B, and Natural Killer (NK) Cells, Blood will be performed. If only a 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 hyper-IgM (XHIM / X-Linked Hyper IgM Syndrome, Blood and CD40 / B-Cell CD40 Expression by Flow Cytometry, Blood).

Shipping Instructions

Testing is performed Monday through Friday. The test may be canceled if specimens are not received in the laboratory by Friday at 8 p.m. Central time.

Samples arriving on weekends and observed holidays may be canceled.

Collect and package specimens as close to shipping time as possible.

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

Necessary Information

1. Date of collection is required.
2. Ordering healthcare professional name and phone number are required.

Specimen Required

Two separate EDTA whole blood specimens are required: 1 refrigerate and 1 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 TBBS.
3. Ship ambient.
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 whole blood specimen in original tube. **Do not aliquot.**
2. Label specimen IABC.
3. Ship refrigerate.
Specimen Stability Information: Refrigerated <48 hours

Specimen Minimum Volume

TBBS: 1 mL; IABC: > 14 years: 5 mL; < or = 14 years: 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 United States 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 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.

Common variable immunodeficiency is characterized by hypogammaglobulinemia, usually involving most or all of the immunoglobulin 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 specific 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 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

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

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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
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17. Kumanovics A, Sadighi Akha AA. Flow cytometry for B-cell subset analysis in immunodeficiencies. J Immunol Methods. 2022;509:113327. doi:10.1016/j.jim.2022.113327
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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. 1980:77[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;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

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

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
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
624173	CD19 (B Cells) (TBBS)	8116-6
624009	%_Sample Viability	33193-4
624010	%_Lymphocyte Viability	33193-4