MAYO CLINIC LABORATORIES

Test Definition: RBCS

Relative B-Cell Subset Analysis Percentage, Blood

Overview

Useful For

Screening for humoral or combined immunodeficiencies, including common variable immunodeficiency, hyper IgM syndrome, among others, where B-cell subset distribution information is desired

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

Assessing B-cell subset reconstitution following recovery of B cells after B-cell-depleting immunotherapy

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

This test **should not be used to** monitor B-cell counts to assess B-cell depletion in patients on B-cell-depleting therapies.

Method Name

Flow Cytometry

NY State Available

Yes

Specimen

Specimen Type

Whole Blood EDTA

Ordering Guidance

This test should be ordered **only** when percentages are needed for the reportable B-cell subsets. If **both** percentages and absolute counts are needed for the reportable B-cell subsets, order IABCS / B-Cell Phenotyping Profile for Immunodeficiency and Immune Competence Assessment, Blood.

Shipping Instructions

Testing performed Monday through-Friday. Specimens not received by 4 p.m. Central time on Fridays may be canceled.

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

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

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: 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 as blood for RBCS.

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

Specimen Minimum Volume

< or =14 years: 3 mL; >14 years: 5 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

The adaptive immune response includes both cell-mediated (mediated by T cells and natural killer 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 of 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 1:25,000 to 1:50,000.(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. Various 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 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: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-depleting therapies, such as Rituxan (rituximab) and Zevalin (ibritumomab tiuxetan).

Reference Values

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

Interpretation

The assay provides semiquantitative information on the various B-cell subsets. 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-negative B cells
CD19+ CD21+=CD21-positive 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, total CD19+ B cell quantitation may not be identical to those performed on whole blood (eg, TBBS / Quantitative Lymphocyte Subsets: T, B, and Natural Killer (NK) Cells, Blood).

This test **should not be used** to monitor B-cell counts to assess B-cell depletion in patients on B-cell-depleting therapies; order CD20B / CD20 on B Cells, Blood for that purpose; this test is meant to be used specifically for assessing the relative distribution of B-cell subsets within the total B-cell pool.

Timing and consistency in timing of blood collection is critical when serially monitoring patients for lymphocyte subsets.

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;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;30(9):2516-2520
3. Wehr C, Kivioja T, Schmitt C, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood. 2008;111(1):77-85
4. Alachkar H, Taubenheim N, Haeney MR, Durandy A, Arkwright PD. Memory switched B-cell percentage and not serum immunoglobulin concentration is associated with clinical complications in children and adults with specific antibody deficiency and common variable immunodeficiency. Clin Immunol. 2006;120(3):310-318
5. Lee WI, Torgerson TR, Schumacher MJ, Yel L, Zhu Q, Ochs HD. Molecular analysis of a large cohort of patients with hyper immunoglobulin M (hyper IgM) syndrome. Blood. 2005;105(5):1881-1890
6. Ramirez NJ, Posadas-Cantera S, Caballero-Oteyza A, Camacho-Ordonez N, Grimbacher B. There is no gene for CVID - novel monogenetic causes for primary antibody deficiency. Curr Opin Immunol. 202172:176-185. doi:10.1016/j.coi.2021.05.010
7. 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
8. Sadighi Akha AA, Csomos K, Ujhazi B, Walter JE, Kumanovics A. Evolving approach to clinical cytometry for immunodeficiencies and other immune disorders. Clin Lab Med. 2023;43(3):467-483. doi:10.1016/j.cll.2023.05.002

Performance

Method Description

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 BD FACSCanto II 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+).(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
3 to 4 days

Specimen Retention Time
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
86356 x7

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
RBCS	Relative B Cell Subset Analysis %	90416-9

Result ID	Test Result Name	Result LOINC® Value
BCD19	CD19+ % of total Lymphocytes	8117-4
BCD20	CD20+ % of total Lymphocytes	8119-0
BCD27	CD27+ % of CD19+ B Cells	89358-6
B27MD	CD27+ IgM+ IgD+ % of CD19+ B Cells	89352-9
B27N	CD27+ IgM- IgD- % of CD19+ B Cells	89350-3
B27M	CD27+ IgM+ IgD- % of CD19+ B Cells	89348-7
BIGM	IgM+ % of CD19+ B Cells	89346-1

B38MN	CD38+ IgM- % of CD19+ B Cells	89344-6
B38MP	CD38+ IgM+ % of CD19+ B Cells	89341-2
B21P	CD21+ % of CD19+ B Cells	89356-0
B21N	CD21- % of CD19+ B Cells	89355-2
RBCSI	Interpretation	69048-7