MAYO CLINIC LABORATORIES

Test Definition: RBCS

Relative B Cell Subset Analysis %

Overview

Useful For

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

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

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

Testing Algorithm

This test should be ordered **only** when percentages (relative distribution of B cell subsets within the total B-cell population) 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.

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CVID	CVID Confirmation Flow Panel	Yes	No

Method Name

Fluorescent Flow Cytometry

NY State Available

Yes

Specimen

Specimen Type

Whole Blood EDTA

Shipping Instructions

Specimens are required to be received in the laboratory on weekdays and by 4 p.m. on Friday. No weekend processing. Draw and package specimens as close to shipping time as possible. Ship specimens overnight.

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

Necessary Information

Ordering physician's name and phone number are required.

Specimen Required

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

Specimen Type: Whole 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 RBCS / Relative B Cell Subset Analysis Percentage.

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject

Specimen Minimum Volume

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

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole Blood EDTA	Refrigerated (preferred)		PURPLE OR PINK TOP/EDTA

Clinical & Interpretive

Clinical Information

The adaptive immune response includes both cell-mediated (mediated by T cells and 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 of 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 1:25,000 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 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: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 defectsâ€”mutations in the *CD40L*, *CD40*, *AID* (activation-induced cytidine deaminase), *UNG* (uracil DNA glycosylase), and *NEMO* (NF-kappa B essential modulator) genes.(5) 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 increase 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-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 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 quantitative defect in memory subsets 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, if appropriate.

Cautions

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.

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 / T- and B-Cell Quantitation by Flow Cytometry).

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 (use CD20B / CD20 on B Cells 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;99:1544-1551

2. Brouet JC, Chedeville A, Fermand JP, Royer B: Study of the B cell memory compartment in common variable immunodeficiency. Eur J Immunol 2000;30:2516-2520

3. Wehr C, Kivioja T, Schmitt C, et al: The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood 2008;111:77-85

4. Alachkar H, Taubenheim N, Haeney MR, et al: 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;120:310-318

5. Lee WI, Torgerson TR, Schumacher MJ, et al: Molecular analysis of a large cohort of patients with hyper immunoglobulin M (hyper IgM) syndrome. Blood 2005;105:1881-1890

Performance

Method Description

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 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+).(Package insert: GE Healthcare Life Sciences Ficoll-Hypaque Plus; unpublished Mayo method)

PDF Report
No

Specimen Retention Time
PBMC's are stored for 7 days at -70 degrees C

Performing Laboratory Location
Rochester

Fees & Codes

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	Reporting Name	LOINC®
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