Overview

Useful For
Evaluation of CD19 deficiency in patients with a suspected CD19 deficiency (humoral immunodeficiency)

Confirming complete absence of B cells in suspected primary humoral immunodeficiencies using both CD19 and CD20 markers

Assessing therapeutic B-cell depletion quantitatively (absolute counts of cells/mcL) in any clinical context, including malignancies, autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and membranous glomerulonephritis among others, and treatment or prevention of acute humoral rejection in positive crossmatch renal transplant recipients

This test is not useful for assessing whether B cells express the target molecule (CD20) in the context of initiating therapeutic monoclonal anti-CD20 antibody therapy (rituximab, ofatumumab, and tositumomab) for any of the hematological malignancies, or in other clinical contexts, such as autoimmunity, instead order CEE20 / CD20 Cell Expression Evaluation, Varies.

Method Name
FlowCytometry

NY State Available
Yes

Specimen

Specimen Type
Whole Blood EDTA

Advisory Information
This is the correct test to order if specifically confirming the absence of B cells due to suspected primary humoral or combined immunodeficiency or evaluating for CD19 deficiency.

If desirous of only quantitatively measuring total CD19 or CD20+ B cells, order TBBS / Quantitative Lymphocyte Subsets: T, B, and Natural Killer (NK) or CD20B / CD20 on B Cells, respectively. Do not order the detailed analysis of B cell subsets for this purpose.

This test should not be ordered for a comprehensive evaluation of peripheral B-cell subsets. For evaluation of memory B-cell subsets, transitional B cells, mature and immature B cells, order IABCS / B-Cell Phenotyping Profile for Immunodeficiency and Immune Competence Assessment, Blood.

This test should not be used for evaluating presence of CD20 on malignant or nonmalignant B cells. The following test should be used instead, CEE20 / CD20 Cell Expression Evaluation, Varies, which provides percent of B cells expressing CD19 or CD20, but does not provide absolute cell counts (cells/mcL).

Shipping Instructions
Draw and package specimens as close to shipping time as possible. It is recommended that specimens arrive within 24 hours of draw.
Test Definition: CD20B
CD20, B-Cells

Necessary Information
Date of draw is required.

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

Container/Tube: Lavender top (EDTA)

Specimen Volume: 3 mL

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

Additional Information:
1. Secondary aliquot tubes will be rejected.
2. Testing will be canceled if the specimen is not received ambient.

Specimen Minimum Volume
1 mL

Reject Due To

<table>
<thead>
<tr>
<th>Gross hemolysis</th>
<th>Reject</th>
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</thead>
<tbody>
<tr>
<td>Gross lipemia</td>
<td>Reject</td>
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<tr>
<td>Other</td>
<td>Secondary aliquot tube</td>
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Specimen Stability Information

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood EDTA</td>
<td>Ambient</td>
<td>4 days</td>
<td>PURPLE OR PINK TOP/EDTA</td>
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</table>

Clinical and Interpretive

Clinical Information
CD20 is a protein that is expressed on the surface of B cells, starting at the pre-B cell stage and also on mature B cells in the bone marrow and in the periphery. CD20 is not expressed on hematopoietic stem cells, pro-B cells, or normal plasma cells.(1) Plasmablasts and stimulated plasma cells may express CD20.(2) CD20 is generally coexpressed on B cells with CD19, another B-cell differentiation marker. CD20 appears to play a role in B-cell development, differentiation, B-cell receptor (BCR) signaling, and cell-cycle initiation events.(3) CD20 is not shed from the surface of B cells and does not internalize on binding with anti-CD20 antibody, nor is it typically present as a soluble free antigen in circulation.(3) Certain primary humoral immunodeficiencies, such as X-linked agammaglobulinemia and autosomal recessive agammaglobulinemia, are characterized by a complete absence or profound reduction of peripheral B cells, expressing both CD20 and CD19 (another B-cell differentiation marker).

Mutations in the CD19 gene have been shown to be associated with a primary humoral immunodeficiency,
sometimes classified as common variable immunodeficiency (CVID). This defect accounts for less than 1% to 2% of CVID patients and appears to be inherited as an autosomal recessive defect. Since these patients have normal numbers of B cells with absent CD19 expression on the cell surface, CD20 can be used as a marker to help identify these patients.

A contrasting situation exists for patients receiving rituximab, ofatumumab, and other anti-CD20 monoclonal antibodies that are used to treat certain cancers, autoimmune diseases, or for B-cell depletion to prevent humoral rejection in positive crossmatch renal transplantation. These agents block available CD20-binding sites and, therefore, the antibody used for this flow cytometric assay cannot recognize the CD20 molecule on B cells. The concomitant use of the CD19 marker provides information on the extent of B-cell depletion when using this particular treatment strategy.

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 am and noon, with no change between noon and afternoon. Natural killer cell counts, on the other hand, are constant throughout the day. Circadian variations in circulating T-cell counts have been shown to be negatively correlated with plasma cortisol concentration. In fact, cortisol and catecholamine concentrations control distribution and, therefore, numbers of naive versus effector CD4 and CD8 T cells. It is generally accepted that lower CD4 T-cell counts are seen in the morning compared with the evening, and during summer compared to winter. These data, therefore, indicate that timing and consistency in timing of blood collection is critical when serially monitoring patients for lymphocyte subsets.

**Reference Values**

<table>
<thead>
<tr>
<th>%CD19 B CELLS</th>
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<tbody>
<tr>
<td>&gt; or =19 years: 4.6-22.1%</td>
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<table>
<thead>
<tr>
<th>CD19 ABSOLUTE</th>
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<table>
<thead>
<tr>
<th>%CD20 B CELLS</th>
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<tbody>
<tr>
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</table>

<table>
<thead>
<tr>
<th>CD20 ABSOLUTE</th>
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<tbody>
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<td>&gt; or =19 years: 74.4-441.1 cells/mcL</td>
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<table>
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<tr>
<td>18-55 years: 0.99-3.15 thou/mcL</td>
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<tr>
<td>&gt;55 years: 1.00-3.33 thou/mcL</td>
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**Interpretation**

The presence of CD20+ B cells with corresponding absence of CD19 staining in individuals not receiving anti-CD20 monoclonal antibody treatment or with clinical features of variable primary humoral immunodeficiency may suggest an underlying CD19 deficiency, which should be further evaluated.

Absence of both CD20 and CD19 markers on B cells in blood from individuals not on anti-CD20 monoclonal antibody
treatment is consistent with complete mature and immature peripheral B-cell depletion, which may be due to an underlying primary immunodeficiency.

Patients receiving B-cell depleting therapy with anti-CD20 antibodies can show unusual populations of B cells on reconstitution that express either CD19 or CD20 due to a phenomenon known as trogocytosis.

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

**Clinical Reference**


**Performance**

**Method Description**
This test is performed using flow cytometry and is a single-tube, whole-blood assay incorporating CD45, CD19, and CD20 antibodies. After staining with specific antibodies, the red blood cells are lysed and the sample is analyzed by flow cytometry on a BD FACS Canto flow cytometer. Absolute counts are obtained using BD Trucount.(BD BioSciences) tubes. Both percent and absolute count will be reported for CD19 and CD20+ B cells. An absolute count will be reported for CD45.(Unpublished Mayo method)

**PDF Report**
No

**Day(s) and Time(s) Test Performed**
Monday through Sunday; Continuously to 2 p.m.

**Analytic Time**
Same day/1 day

**Maximum Laboratory Time**
2 days

**Specimen Retention Time**
4 days

**Performing Laboratory Location**
Rochester

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### 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
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 U.S. Food and Drug Administration.

#### CPT Code Information
- 86355
- 86356

#### LOINC® Information

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<th>Test Order Name</th>
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