Test Definition: CD20B

CD20 on B Cells, Blood

Overview

Useful For

Evaluation of 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.

Method Name

Flow Cytometry

NY State Available

Yes

Specimen

Specimen Type

Whole Blood EDTA

Ordering Guidance

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) Cells, Blood or CD20B / CD20 on B Cells, Blood, 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 ordered instead, CEE20 / CD20 Cell Expression Evaluation, Varies.

Shipping Instructions

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

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

Necessary Information

Date and time of collection is required.

Specimen Required

Container/Tube: Lavender top (EDTA)
Specimen Volume: 3 mL
Collection Instructions: Send whole blood 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.
3. For serial monitoring, it is recommended that specimens are collected at the same time of day.

Forms

If not ordering electronically, complete, print, and send a Kidney Transplant Test Request with the specimen.

Specimen Minimum Volume

1 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Secondary aliquot tube	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole Blood EDTA	Ambient	4 days	PURPLE OR PINK TOP/EDTA

Clinical & Interpretive

Clinical Information

CD20 (cluster of differentiation 20) is a protein that is expressed on the surface of B cells, starting at the pre-B cell stage and 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.

Variants in the *CD19* gene have been shown to be associated with a primary humoral immunodeficiency, sometimes classified as common variable immunodeficiency (CVID).(4) This defect accounts for less than 1% of CVID patients and appears to be inherited as an autosomal recessive defect.(4) Since these patients have normal numbers of B cells with absent CD19 expression on the cell surface (4), CD20 can be used as a marker to help identify these patients. Genetic CD20 deficiency (autosomal recessive) is also associated with a primary humoral immunodeficiency. In this disease, B cells can be identified by CD19 expression.(5) Similarly, patients receiving anti-CD19 monoclonal antibody therapeutics, such as inebilizumab (6) may show loss of CD19 staining as well.

A contrasting situation exists for patients with genetic defects in CD20 (5) or 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 kidney 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 a.m. and noon, with no change between noon and afternoon. Natural killer cell counts, on the other hand, are constant throughout the day.(6) Circadian variations in circulating T-cell counts have been shown to be negatively correlated with plasma cortisol concentration.(7-9) In fact, cortisol and catecholamine concentrations control distribution and, therefore, numbers of naive versus effector CD4 and CD8 T cells.(7) It is generally accepted that lower CD4 T-cell counts are seen in the morning compared with the evening(10), and during summer compared to winter.(11) These data, therefore, indicate that timing and consistency in timing of blood collection is critical when serially monitoring patients for lymphocyte subsets.

Reference Values

%CD19 B Cells
> or =19 years: 4.6-22.1%

CD19 Absolute
> or =19 years: 56.6-417.4 cells/mcL

%CD20 B Cells
> or =19 years: 5.0-22.3%

CD20 Absolute
> or =19 years: 74.4-441.1 cells/mcL

CD45 Absolute
18-55 years: 0.99-3.15 thou/mcL
>55 years: 1.00-3.33 thou/mcL

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

1. Nadler LM, Ritz J, Hardy R, Pesando JM, Schlossman SF, Stashenko P. A unique cell-surface antigen identifying lymphoid malignancies of B-cell origin. J Clin Invest. 1981;67(1):134
2. Robillard N, Avet-Loiseau H, Garand R, et al. CD20 is associated with a small mature plasma cell morphology and t(11;14) in multiple myeloma. Blood. 2003;102(3):1070-1071
3. Pescovitz MD. Rituximab, an anti-CD20 monoclonal antibody: history and mechanism of action. Am J Transplant. 2006;6(5 Pt 1):859-866
4. van Zelm MC, Reisli I, van der Burg M, et al. An antibody-deficiency syndrome due to mutations in the CD19 gene. N Engl J Med. 2006;354(18):1901-1912
5. Kuijpers TW, Bende RJ, Baars PA, et al. CD20 deficiency in humans results in impaired T cell-independent antibody responses. J Clin Invest. 2010;120(1):214-222. doi:10.1172/JCI40231
6.Carmichael KF, Abayomi A. Analysis of diurnal variation of lymphocyte subsets in healthy subjects and its implication in HIV monitoring and treatment. 15th International Conference on AIDS, Bangkok, Thailand, 2004, Abstract B11052
7. Dimitrov S, Benedict C, Heutling D, Westermann J, Born J, Lange T. Cortisol and epinephrine control opposing circadian rhythms in T-cell subsets. Blood. 2009;113(21):5134-5143
8. Dimitrov S, Lange T, Nohroudi K, Born J. Number and function of circulating antigen presenting cells regulated by sleep. Sleep. 2007;30(4):401-411
9. Kronfol Z, Nair M, Zhang Q, Hill EE, Brown MB. Circadian immune measures in healthy volunteers: relationship to hypothalamic-pituitary-adrenal axis hormones and sympathetic neurotransmitters. Psychosom Med. 1997;59(1):42-50
10. Malone JL, Simms TE, Gray GC, Wagner KF, Burge JR, Burke DS. Sources of variability in repeated T-helper lymphocyte counts from HIV 1-infected patients: total lymphocyte count fluctuations and diurnal cycle are important. J Acquir Immune Defic Syndr (1988). 1990;3(2):144-151
11. Paglieroni TG, Holland PV. Circannual variation in lymphocyte subsets, revisited. Transfusion. 1994;34(6):512-516
12. Engel ER, Walter JE. Rituximab and eculizumab when treating nonmalignant hematologic disorders: infection risk, immunization recommendations, and antimicrobial prophylaxis needs. Hematology Am Soc Hematol Educ Program. 2020;2020(1):312-318. doi:10.1182/hematology.2020000171

13. Cree BAC, Kim HJ, Weinshenker BG, et al. Safety and efficacy of inebilizumab for the treatment of neuromyelitis optica spectrum disorder: end-of-study results from the open-label period of the N-MOmentum trial Lancet Neurol. 2024;23(6):588-602. doi:10.1016/S1474-4422(24)00077-2

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. 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 is reported for CD45.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Sunday
Resulted Monday through Friday

Report Available

Same day/1 to 3 days

Specimen Retention Time

4 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

86356

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
CD20B	CD20, B-Cells	100994-3

Result ID	Test Result Name	Result LOINC® Value
29579	%CD19 B-Cells	8117-4
29581	CD19 Absolute	8116-6
29580	%CD20 B-Cells	8119-0
29582	CD20 Absolute	9558-8
89584	CD45 Absolute	27071-0
29583	Comment	48767-8