Overview

Useful For
Serial monitoring of CD4 T-cell count in HIV-positive patients
Follow-up and diagnostic evaluation of primary immunodeficiencies, including severe combined immunodeficiency
Immune monitoring following immunosuppressive therapy for transplantation, autoimmunity, and other immunological conditions where such treatment is utilized
Assessment of immune reconstitution post hematopoietic cell transplantation
Early screening of gross quantitative anomalies in lymphocyte subsets in infection or malignancies
Absolute quantitation of circulating B cells for diagnosis of chronic lymphocytic leukemia patients as indicated in the 2008 International Workshop on Chronic Lymphocytic Leukemia guidelines

Testing Algorithm
See Newborn Screen Follow-up for Severe Combined Immunodeficiency Syndrome (SCID) in Special Instructions.

Special Instructions
• Newborn Screen Follow-up for Severe Combined Immunodeficiency Syndrome (SCID)

Method Name
FlowCytometry

NY State Available
Yes

Specimen

Specimen Type
Whole Blood EDTA

Ordering Guidance
This assay should not be used for diagnosing lymphocytic malignancies or evaluation of lymphocytosis of unknown etiology, though the latter may be identified through this assay in a screening assessment. In such cases, LCMS / Leukemia/Lymphoma Immunophenotyping, Flow Cytometry, Varies will be recommended, which includes a hematopathology review. However, this assay can be used for absolute quantitation of B cells in CLL patients as indicated above.

Shipping Instructions
It is recommended that specimens arrive within 24 hours of draw. Draw and package specimen as close to shipping time as possible.

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.
Test Definition: TBBS
QN Lymphocyte Subsets: T, B, and NK

Container/Tube: 4 mL Lavender top (EDTA)
Specimen Volume: 3 mL
Collection Instructions: Send specimen in original tube. Do not aliquot.

Reject Due To
- Gross hemolysis: Reject
- Gross lipemia: Reject
- Other: Specimen in aliquot tube

Specimen Minimum Volume
1 mL

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 (preferred)</td>
<td></td>
<td>PURPLE OR PINK TOP/EDTA</td>
</tr>
</tbody>
</table>

Clinical & Interpretive

Clinical Information
Lymphocytes in peripheral blood (circulation) are heterogeneous and can be broadly classified into T cells, B cells, and natural killer (NK) cells. There are various subsets of each of these individual populations with specific cell-surface markers and function. This assay provides absolute (cells/mcL) and relative (%) quantitation for the main categories of T cells, B cells, and NK cells, in addition to a total lymphocyte count (CD45+). Each of these lymphocyte subpopulations have distinct effector and regulatory functions and are maintained in homeostasis under normal physiological conditions. Each of these lymphocyte subsets can be identified by a combination of one or more cell surface markers. The CD3 antigen is a pan-T cell marker, and T cells can be further divided into 2 broad categories, based on the expression of CD4 or CD8 coreceptors. B cells can be identified by expression of CD19, while NK cells are typically identified by the coexpression of CD16 and CD56.

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. NK-cell counts, on the other hand, are constant throughout the day. (1) Circadian variations in circulating T-cell counts have been shown to be negatively correlated with plasma cortisol concentration. (2-4) In fact, cortisol and catecholamine concentrations control distribution and, therefore, numbers of naive versus effector CD4 and CD8 T cells. (2) It is generally accepted that lower CD4 T-cell counts are seen in the morning compared to the evening (5) and during summer compared to winter. (6) These data, therefore, indicate that timing and consistency in timing of blood collection is critical when serially monitoring patients for lymphocyte subsets.

Abnormalities in the number and percent of T (CD3, CD4, CD8), B (CD19), and NK (CD16+CD56) lymphocytes have been described in a number of different disease conditions. In patients who are infected with HIV, the CD4 count is measured for AIDS diagnosis and for initiation of antiviral therapy. The progressive loss of CD4 T-lymphocytes inpatients infected with HIV is associated with increased infections and complications. The Public Health Service has recommended that all HIV-positive patients be tested every 3 to 6 months for the level of CD4 T lymphocytes. Lymphocyte subset quantitation is also very useful in the evaluation of patients with primary immunodeficiencies of all
Test Definition: TBBS
QN Lymphocyte Subsets: T, B, and NK

ages, including follow-up for newborn screening for severe combined immunodeficiency (SCID) and immune monitoring following immunosuppressive therapy for transplantation, autoimmunity, or any other relevant clinical condition where immunomodulatory treatment is used.

It is also helpful as a preliminary screening assay for gross quantitative anomalies in any lymphocyte subset, whether related to malignancies or infection.

The 2008 guidelines for diagnosis and treatment of Chronic Lymphocytic Leukemia (CLL) from the International Workshop on Chronic Lymphocytic Leukemia recommends changing the diagnostic criteria for CLL from an absolute lymphocyte count (ALC) greater than 5 x 10^9/L to a circulating B-cell count greater than 5 x 10^9/L previously defined in the 1996 National Cancer Institute (NCI) guidelines for CLL. This flow cytometric assay enables accurate quantitation of circulating B cells using a single platform technology with absolute quantitation through the use of flow cytometry beads.

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

Interpretation
When the CD4 count falls below 500 cells/mcL, HIV-positive patients 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.

Cautions
Lymphocyte subset counts should be appropriately interpreted in context of the clinical presentation and other immunological parameters and relevant laboratory test results.

For serial monitoring of lymphocyte subsets it is recommended that the patient be evaluated at the same time of the day to account for diurnal variation.

For follow-up of infants identified by newborn screening for severe combined immunodeficiency (SCID) and severe T-cell lymphopenia, SCID should be considered as a potential diagnosis in infants with fewer than 300 autologous CD3 T cells/mcL. Infants with 300 to 1,500 autologous CD3 T cells/mcL may have leaky SCID, Omenn syndrome, or variant SCID, depending on other clinical and molecular features.

T-cell lymphopenia in infants identified by newborn screening for SCID is defined as having up to 1,500 autologous CD3T cells/mcL.

While this assay can be used to follow patients on B-cell-depleting therapy, like Rituximab or Ofatumumab, it may be more reasonable and financially viable to use CD20B / CD20 on B Cells (includes CD45, CD19 and CD20 markers).

This assay should not be used for diagnosing lymphocytic malignancies or evaluation of lymphocytosis of unknown etiology, though the latter may be identified through this assay in a screening assessment. In such cases, LCMS / Leukemia/Lymphoma Immunophenotyping, Flow Cytometry, Varies will be recommended, which includes a hematopathology review. However, this assay can be used for absolute quantitation of B cells in CLL patients as indicated above.

Clinical Reference
1-infected patients: total lymphocyte count fluctuations and diurnal cycle are important. J AIDS 1990;3:144-151
report from the International Workshop on CLL updating the National Cancer Institute Working Group 1996 guidelines.
Blood 2008;111:5446-5456
8. Hanson CA, Kurtin PJ, Dogan A: The proposed diagnostic criteria change for chronic lymphocytic leukemia: unintended
consequences? Blood 2009;113:6495-6496
and adolescents. Available at http://aidsinfo.nih.gov/guidelines
International Antiviral Society-USA panel. JAMA 2012;308:387-402

Method Description

Quantitative Lymphocyte Subsets: T, B, and Natural Killer (NK)
The T, B, and 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, natural killer
(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, Goedstien G: Simple and
rapid measurement of human T lymphocytes and their subclasses in peripheral blood. Proc Natl Acad Sci USA,
determinations in persons with human immunodeficiency virus infection. MMWR Morb Mortal Wkly Rep 1997;46 no.
RR-2 pp 1-29)

PDF Report
No

Specimen Retention Time
4 days

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
86355-B cells, total count
86357-Natural killer (NK) cells, total count
86359-T cells, total count
86360-Absolute CD4/CD8 count with ratio