Overview

Useful For
Diagnosing autoimmune lymphoproliferative syndrome, primarily in patients <45 years of age

Method Name
Flow Cytometry

NY State Available
Yes

Specimen

Specimen Type
Whole Blood EDTA

Shipping Instructions
Specimens are required to be received in the laboratory weekdays and by 4 p.m. on Friday. Draw and package specimen as close to shipping time as possible.

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

Samples arriving on the weekend may be canceled.

Necessary Information
Ordering physician name and phone number are 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.

Forms
If not ordering electronically, complete, print, and send a Benign Hematology Test Request Form (T755) with the specimen.

Specimen Minimum Volume
0.5 mL

Reject Due To

<table>
<thead>
<tr>
<th>Condition</th>
<th>Acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolysis</td>
<td>Mild OK; Gross reject</td>
</tr>
<tr>
<td>Lipemia</td>
<td>Mild OK; Gross reject</td>
</tr>
<tr>
<td>Icterus</td>
<td>NA</td>
</tr>
<tr>
<td>Other</td>
<td>NA</td>
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Autoimmune lymphoproliferative syndrome (ALPS) (also known as Canale-Smith syndrome) is a complex clinical disorder of dysregulated lymphocyte homeostasis that is characterized by lymphoproliferative disease, autoimmune cytopenias, splenomegaly, and lymphadenopathy with an increased susceptibility to malignancy.(1) Typically, ALPS is diagnosed by childhood or young adulthood.

Genetic defects in the apoptosis (programmed cell death) pathway have been determined for most cases of ALPS. Apoptosis plays a role in normal immune homeostasis by limiting lymphocyte accumulation and autoimmune reactivity. The interaction of the surface receptor CD95 (FAS) and its ligand (CD95L;FASL) triggers the apoptotic pathway in lymphocytes.

The following molecular ALPS classification has been established:

<table>
<thead>
<tr>
<th>ALPS Classification</th>
<th>Molecular/Genetic Defect in Apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type Ia</td>
<td>CD95 (FAS) mutations(1)</td>
</tr>
<tr>
<td>Type Ib</td>
<td>Heterozygous CD95L (FASLG) mutations(1)</td>
</tr>
<tr>
<td>Type Ic</td>
<td>Homozygous CD95L (FASLG) mutation(2)</td>
</tr>
<tr>
<td>Type II</td>
<td>CASP8 or CASP10 mutations(1,3)</td>
</tr>
<tr>
<td>Type III</td>
<td>Unknown(1,3)</td>
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Patients with ALPS have an increase in a normally rare population of T cells (typically <1%) that are alpha beta T-cell receptor (TCR)-positive, as well as negative for both CD4 and CD8 coreceptors (double-negative T cells: DNT).(1) The alpha beta TCR+DNT cells from ALPS patients also express an unusual B-cell-specific CD45R isoform, called B220.(4,5) B220 expression on alpha beta TCR+DNT cells has been demonstrated to be a sensitive and specific marker for ALPS and is associated with FAS mutations.(4)

Several other diseases can present with an ALPS-like phenotype, including independent conditions like Evans syndrome (a combination of autoimmune hemolytic anemia and autoimmune thrombocytopenic purpura), Rosai-Dorfman disease (massive painless cervical lymphadenopathy that may be accompanied by leukocytosis, elevated erythrocyte sedimentation rate, and hypergammaglobulinemia), and nodular lymphocyte-predominant Hodgkin disease.(1)
Reference Values
Alpha beta TCR+DNT cells
2-18 years: <2% CD3 T cells
19-70+ years: <3% CD3 T cells
Reference values have not been established for patients that are less than 24 months of age.

Alpha beta TCR+DNT cells
2-18 years: <35 cells/mcL
19-70+ years: <35 cells/mcL
Reference values have not been established for patients that are less than 24 months of age.

Alpha beta TCR+DNT B220+ cells
2-18 years: <0.4% CD3 T cells
19-70+ years: <0.3% CD3 T cells
Reference values have not been established for patients that are less than 24 months of age.

Alpha beta TCR+DNT B220+ cells
2-18 years: <7 cells/mcL
19-70+ years: <6 cells/mcL
Reference values have not been established for patients that are less than 24 months of age.

Interpretation

The presence of increased circulating T cells (CD3+) that are negative for CD4 and CD8 (double-negative T cells: DNT) and positive for the alpha/beta T-cell receptor (TCR) is required for the diagnosis of autoimmune lymphoproliferative syndrome (ALPS).

The laboratory finding of increased alpha beta TCR+DNT cells is consistent with ALPS only with the appropriate clinical picture (nonmalignant lymphadenopathy, splenomegaly, and autoimmune cytopenias). Conversely, there are other immunological disorders, including common variable immunodeficiency (CVID), which have subsets for patients with this clinical picture, but no increase in alpha beta TCR+DNT cells.

If the percent of the absolute count of either the alpha beta TCR+DNT cells or alpha beta TCR+DNT B220+ cells is abnormal, additional testing is indicated. All abnormal alpha beta TCR+DNT cell results should be confirmed (for ALPS) with additional testing for defective in vitro lymphocyte apoptosis, followed by confirmatory genetic testing for FAS mutations (call 800-533-1710 for test forwarding information).

Cautions

This test is typically not indicated in older adults. For questions about appropriate test selection, call 800-533-1710.
The sole presence of increased alpha beta TCR+DNT B220+ cells is not sufficient for a diagnosis of autoimmune lymphoproliferative syndrome (ALPS); additional testing is required to confirm a diagnosis of ALPS.

Clinical Reference


Performance

Method Description
This assay uses a 5-color, single-platform method with a 2-tube panel stained for the following antibodies: CD3, CD4, CD8, CD45, alpha beta TCR, and B220. The sample is stained with the antibody cocktail and incubated in the dark at room temperature for 20 minutes. Following incubation, the samples are treated with BD lysis solution to lyse the RBCs, followed with a wash step using BD FACS wash buffer. The cells are resuspended in 1% paraformaldehyde and analyzed by flow cytometry. The different subsets are expressed as a percent of CD3 T cells, and the absolute counts of all subsets are expressed as cells/mcL.(Unpublished Mayo method; Blessing JJH, Brown MR, Dale JK, et al: TCR alpha beta + CD4-CD8- T-cells in humans with the autoimmune lymphoproliferative syndrome express a novel CD45 isoform that is analogous to murine B220 and represents a marker of altered O-glycan biosynthesis. Clin Immunol 2001;100[3]:314-324)

PDF Report
No

Day(s) and Time(s) Test Performed
Monday through Friday

Specimens are required to be received in the lab weekdays and by 4 p.m. on Friday. No weekend processing.

Analytic Time
3 days

Maximum Laboratory Time
4 days

Specimen Retention Time
4 days
Performing Laboratory Location
Rochester

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
86356 x2
86359

LOINC® Information

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<th>Order LOINC Value</th>
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<tr>
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<td>ALPS Screen</td>
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<table>
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<th>Test Result Name</th>
<th>Result LOINC Value</th>
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<tbody>
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<td>alpha/beta-TCR DNT</td>
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<td>% TCR+DNT B220+</td>
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<td>Absolute TCR+DNT B220+</td>
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