Test Definition: ALPS

Alpha Beta Double-Negative T Cells for Autoimmune Lymphoproliferative Syndrome, Blood

Overview

Useful For
Diagnosing autoimmune lymphoproliferative syndrome, primarily in patients younger than 45 years

Method Name
Flow Cytometry

NY State Available
Yes

Specimen

Specimen Type
Whole Blood EDTA

Shipping Instructions
Testing is performed Monday through Friday. Specimens not received by 4pm (CST) on Friday may be canceled.

Collect and package specimen as close to shipping time as possible. It is recommended that specimens arrive within 24 hours of collection.

Samples arriving on the weekend and observed holidays may be canceled.

Necessary Information
Ordering healthcare professional name and phone number are 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: 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 Benign Hematology Test Request Form (T755) with the specimen.

Specimen Minimum Volume
0.5 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole Blood EDTA	Ambient	72 hours	PURPLE OR PINK TOP/EDTA

Clinical & Interpretive

Clinical Information

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. Germline variants in CD95 (*FAS*) are the most common cause (60-75%) of ALPS(2), followed by somatic mutations in CD95 (*FAS*). Variants in CD95L (*FASL*), *CASP10*, and others are rare causes. Currently up to 20% of patients do not have an identifiable genetic variant (ALPS-U).

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 may also express an unusual B-cell-specific CD45R isoform, called B220.(3,4)

Several other diseases can present with an ALPS-like phenotype, including other inborn errors of immunity, like CTLA4 and LRBA deficiency, and gain-of function variants in *STAT3* and *CARD11* genes(2,5), as well as 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, among others.(1,2,5) B220 expression on double negative T cells has also been described in large granular lymphocyte leukemias.(4)

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 younger 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 younger 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 younger 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 younger than 24 months of age.

TCR = T-cell receptor
DNT = Double negative T cell

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 (ALPSG / Autoimmune Lymphoproliferative Syndrome [ALPS] Gene Panel, Varies).

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 T-cell receptor + double-negative T cells 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

1. Oliveira JB, Bleesing JJ, Dianzani U, et al. Revised diagnostic criteria and classification for the autoimmune lymphoproliferative syndrome (ALPS): report from the 2009 NIH International Workshop. Blood. 2010;116(14):e35-40
2. Consonni F, Gambineri E, Favre C. ALPS, FAS, and beyond: from inborn errors of immunity to acquired immunodeficiencies. Ann Hematol. 2022;101(3):469-484. doi:10.1007/s00277-022-04761-7
3. Bleesing JJ, 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 urine B220 and represents a marker of altered O-glycan biosynthesis. Clin Immunol. 2001;100(3):314-324
4. Bleesing JJ, Janik JE, Fleisher TA. Common expression of an unusual CD45 isoform on T-cells from patients with large granular lymphocyte leukemia and autoimmune lymphoproliferative syndrome. Br J Haematol. 2003;120(1):93-96
5. Lopez-Nevado M, Gonzalez-Granado LI, Ruiz-Garcia R, et al. Primary immune regulatory disorders with an autoimmune lymphoproliferative syndrome-like phenotype: Immunologic evaluation, early diagnosis and management. Front Immunol. 2021;12:671755. doi:10.3389/fimmu.2021.671755

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 T-cell receptor, 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 lysing solution to lyse the red blood cells 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)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

3 to 4 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

86356 x2
86359

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
ALPS	ALPS Screen	101414-1

Result ID	Test Result Name	Result LOINC® Value
28905	Absolute TCR+DNT B220+	88053-4
23974	alpha/beta-TCR DNT	34963-9
28904	% TCR+DNT B220+	88052-6
23973	%alpha/beta-TCR DNT	34962-1
23975	Interpretation	69052-9