MAYO CLINIC LABORATORIES

Test Definition: ALPSG

Autoimmune Lymphoproliferative Syndrome (ALPS) Gene Panel, Varies

Overview

Useful For

Providing a comprehensive genetic evaluation for patients with a personal or family history suggestive of autoimmune lymphoproliferative syndrome (ALPS) or related disorders

Establishing a diagnosis of ALPS or a related disorder, allowing for appropriate management and surveillance for disease features based on the gene or variant involved

Identifying variants within genes known to be associated with ALPS or a related disorder, allowing for predictive testing of at-risk family members

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CULFB	Fibroblast Culture for Genetic Test	Yes	No

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in 26 genes associated with autoimmune lymphoproliferative syndrome (ALPS) and ALPS-like disorders: *ADA2, CARD11, CASP10, CASP8, CTLA4, DEF6, FADD, FAS, FASLG, IL2RA, IL2RB, ITK, LRBA, MAGT1, PIK3CD, PIK3R1, PRKCD, RASGRP1, SH2D1A, STAT3, STK4, TET2, TNFAIP3, TNFRSF9, TPP2,* and *XIAP*. See Targeted Genes and Methodology Details for Autoimmune Lymphoproliferative Syndrome (ALPS) Gene Panel and Method Description for additional details.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, recurrence risk assessment, familial screening, and genetic counseling for ALPS or an ALPS-like disorder.

Testing Algorithm

For skin biopsy or cultured fibroblast specimens, fibroblast culture will be performed at an additional charge. If viable cells are not obtained, the client will be notified.

Special Instructions

- Informed Consent for Genetic Testing
- Informed Consent for Genetic Testing (Spanish)
- Targeted Genes and Methodology Details for Autoimmune Lymphoproliferative Syndrome (ALPS) Gene Panel
- Inborn Errors of Immunity, Autoimmunity, and Autoinflammatory Disease Patient Information

Method Name

Sequence Capture and Targeted Next-Generation Sequencing (NGS)

NY State Available

Yes

Specimen

Specimen Type
Varies

Ordering Guidance
Targeted testing for familial variants (also called site-specific or known variants testing) is available for the genes on this panel. See FMTT / Familial Variant, Targeted Testing, Varies. To obtain more information about this testing option, call 800-533-1710.

Shipping Instructions
Specimen preferred to arrive within 96 hours of collection.

Specimen Required
Patient Preparation: A previous bone marrow transplant from an allogenic donor will interfere with testing. Call 800-533-1710 for instructions for testing patients who have received a bone marrow transplant.

Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender top (EDTA) or yellow top (ACD)
Acceptable: Any anticoagulant
Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot.**
Specimen Stability Information: Ambient (preferred)/Refrigerated

Specimen Type: Skin biopsy
Supplies: Fibroblast Biopsy Transport Media (T115)
Container/Tube: Sterile container with any standard cell culture media (eg, minimal essential media, RPMI 1640). The solution should be supplemented with 1% penicillin and streptomycin.
Specimen Volume: 4-mm punch
Specimen Stability Information: Refrigerated (preferred)/Ambient
Additional Information: A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

Specimen Type: Cultured fibroblasts
Container/Tube: T-25 flask

Specimen Volume: 2 Flasks
Collection Instructions: Submit confluent cultured fibroblast cells from a skin biopsy from another laboratory. Cultured cells from a prenatal specimen will not be accepted.
Specimen Stability Information: Ambient (preferred)/Refrigerated (<24 hours)
Additional Information: A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

Forms

1. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing (Spanish) (T826)
2. Molecular Genetics: Congenital Inherited Diseases Patient Information (T521)
3. Inborn Errors of Immunity, Autoimmunity, and Autoinflammatory Disease Patient Information

Specimen Minimum Volume

Blood: 1 mL; Skin biopsy or cultured fibroblasts: See Specimen Required

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information

Autoimmune lymphoproliferative syndrome (ALPS) is a complex clinical disorder of dysregulated lymphocyte homeostasis characterized by chronic nonmalignant lymphoproliferative disease, splenomegaly, lymphadenopathy, and autoimmunity (mainly autoimmune cytopenias), with an increased susceptibility to lymphomas. Typically, ALPS is diagnosed by childhood or young adulthood. Lymphoproliferation and autoimmunity are usually the first presentations. Lymphomas (Hodgkin and non-Hodgkin) can occur at any age but are usually late complications. ALPS is reported worldwide in various racial and ethnic backgrounds but affects more men than women (approximately 2.2 affected men per 1.6 affected women).

Laboratory investigations showed that ALPS patients have an increase in a normally rare population of T cells (typically <1%) that are alpha beta T-cell receptor-positive, as well as negative for both CD4 and CD8 coreceptors (double-negative T cells). In addition, there are elevated peripheral blood interleukin (IL)-10, IL-18, vitamin B12, and soluble FAS ligand (FASL) levels. Defective FAS-mediated apoptosis on in vitro assays is another main characteristic of ALPS.

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 (FASL or CD95L) triggers the apoptotic pathway in lymphocytes. Germline variants in CD95 (*FAS*) are the most common cause (60%-75%) of ALPS (ALPS-FAS), followed by somatic mutations in CD95 (ALPS-sFAS). Variants in CD95L (ALPS-FASL), CASP10 (ALPS-CAS10), and others are rare causes. Currently, up to 20% of patients do not have an identifiable genetic variant (ALPS-U).

All these forms present with the main clinical features of ALPS, but there are differences in the results of laboratory tests used to evaluate ALPS patients. Genotype-phenotype correlations are noted in ALPS-FAS, which is the only form common enough for these studies. Both mono- and bi-allelic variants in *FAS* can cause disease. Dominant negative, haploinsufficient mechanisms are invoked to explain the disease mechanism. It appears that biallelic disease-causing *FAS* variants cause a more severe clinical phenotype than the monoallelic forms. Lymphomas are mostly associated with disease-causing variants in the intracellular domain of *FAS*. Penetrance of the clinical phenotype is reduced and varies based on the location and type of causative variant (30%-90%).

The latest diagnostic criteria for ALPS were published in 2010.(1) A definitive diagnosis is based on the presence of both required criteria and one primary accessory criterion. A probable diagnosis is based on the presence of both required criteria plus one secondary accessory criterion.

Several other diseases can present with an ALPS-like phenotype, including other inborn errors of immunity, like *CTLA4* deficiency (also known as *CTLA4* haploinsufficiency or *CTLA4* haploinsufficiency with autoimmune infiltration [CHAI]) and LRBA (lipopolysaccharide-responsive and beige-like anchor protein) deficiency, gain-of-function variants in *STAT3* and *CARD11* genes, as well as conditions like Evans syndrome (a combination of autoimmune hemolytic anemia and autoimmune thrombocytopenic purpura) and malignant conditions like Hodgkin disease and large granular lymphocyte leukemias. Genes associated with several ALPS-like disorders are also included on this panel.

Reference Values

An interpretive report will be provided.

Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(2) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Cautions

Clinical Correlations:
Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If testing was performed because of a clinically significant family history, it is often useful to first test an affected family member. Detection of a reportable variant in an affected family member would allow for more informative testing of at-risk individuals.

To discuss the availability of additional testing options or for assistance in the interpretation of these results, contact Mayo Clinic Laboratories genetic counselors at 800-533-1710.

Technical Limitations:
Next-generation sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some target regions; assay performance below the minimum acceptable criteria or for failed regions will be noted. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is validated to detect 95% of deletions up to 75 base pairs (bp) and insertions up to 47 bp. Deletions-insertions (delins) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller delins.

Deletion/Duplication Analysis:
This analysis targets single and multi-exon deletions/duplications; however, in some instances, single exon resolution cannot be achieved due to isolated reduction in sequence coverage or inherent genomic complexity. Balanced structural rearrangements (such as translocations and inversions) may not be detected.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results.

Genes may be added or removed based on updated clinical relevance. Refer to the Targeted Genes and Methodology Details for Autoimmune Lymphoproliferative Syndrome (ALPS) Gene Panel for the most up to date list of genes included in this test. For detailed information regarding gene-specific performance and technical limitations, see Method Description or contact a laboratory genetic counselor.

If the patient has had an allogeneic hematopoietic stem cell transplant or a recent nonleukoreduced blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.

Reclassification of Variants:
Currently, it is not standard practice for the laboratory to systematically review previously classified variants on a regular basis. The laboratory encourages healthcare providers to contact the laboratory at any time to learn how the classification of a particular variant may have changed over time. Due to broadening genetic knowledge, it is possible that the laboratory may discover new information of relevance to the patient. Should that occur, the laboratory may issue an amended report.

Variant Evaluation:
Evaluation and categorization of variants are performed using published American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations as a guideline.(2) Other gene-specific guidelines may also be considered. Variants are classified based on known, predicted, or possible pathogenicity and

reported with interpretive comments detailing their potential or known significance. Variants classified as benign or likely benign are not reported.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions made by in silico evaluation tools is highly dependent upon the data available for a given gene, and periodic updates to these tools may cause predictions to change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

Rarely, incidental or secondary findings may implicate another predisposition or presence of active disease. These findings will be carefully reviewed to determine whether they will be reported.

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 Oct 7;116(14):e35-40
2. Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee: Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-424
3. Consonni F, Gambineri E, Favre C: ALPS, FAS, and beyond: from inborn errors of immunity to acquired immunodeficiencies. Ann Hematol. 2022 Mar;101(3):469-484. doi: 10.1007/s00277-022-04761-7
4. 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 Aug 10;12:671755. doi:10.3389/fimmu.2021.671755
5. Molnar E, Radwan N, Kovacs G, et al: Key diagnostic markers for autoimmune lymphoproliferative syndrome with molecular genetic diagnosis. Blood. 2020 Oct 22;136(17):1933-1945. doi: 10.1182/blood.2020005486
6. Price S, Shaw PA, Seitz A, et al: Natural history of autoimmune lymphoproliferative syndrome associated with FAS gene mutations. Blood. 2014 Mar 27;123(13):1989-1999. doi: 10.1182/blood-2013-10-535393

Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing are performed to test for the presence of variants in coding regions and intron/exon boundaries of the genes analyzed, as well as some other regions that have known disease-causing variants. The human genome reference GRCh37/hg19 build was used for sequence read alignment. At least 99% of the bases are covered at a read depth over 30X. Sensitivity is estimated at above 99% for single nucleotide variants, above 94% for deletions/insertions (delins) less than 40 base pairs (bp), and above 95% for deletions up to 75 bp and insertions up to 47 bp. NGS and/or a polymerase chain reaction-based quantitative method is performed to test for the presence of deletions and duplications in the genes analyzed.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and

repetitive sequences.(Unpublished Mayo method)

See Targeted Genes and Methodology Details for Autoimmune Lymphoproliferative Syndrome (ALPS) Gene Panel for details regarding the targeted genes analyzed for each test and specific gene regions not routinely covered.

Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.

Genes analyzed: *ADA2, CARD11, CASP10, CASP8, CTLA4, DEF6, FADD, FAS, FASLG, IL2RA, IL2RB, ITK, LRBA, MAGT1, PIK3CD, PIK3R1, PRKCD, RASGRP1, SH2D1A, STAT3, STK4, TET2, TNFAIP3, TNFRSF9, TPP2,* and *XIAP*

PDF Report
Supplemental

Day(s) Performed
Varies

Report Available
28 to 42 days

Specimen Retention Time
Whole blood: 2 weeks (if available); Extracted DNA: 3 months; Cultured fibroblasts, skin biopsy: 1 month

Performing Laboratory Location
Rochester

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 and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
81443
88233-Tissue culture, skin, solid tissue biopsy (if appropriate)
88240-Cryopreservation (if appropriate)

LOINC® Information

MAYO CLINIC LABORATORIES

Test Definition: ALPSG

Autoimmune Lymphoproliferative Syndrome (ALPS) Gene Panel, Varies

Test ID	Test Order Name	Order LOINC® Value
ALPSG	ALPS Gene Panel	103743-1

Result ID	Test Result Name	Result LOINC® Value
619747	Test Description	62364-5
619748	Specimen	31208-2
619749	Source	31208-2
619750	Result Summary	50397-9
619751	Result	82939-0
619752	Interpretation	69047-9
619753	Additional Results	82939-0
619754	Resources	99622-3
619755	Additional Information	48767-8
619756	Method	85069-3
619757	Genes Analyzed	82939-0
619758	Disclaimer	62364-5
619759	Released By	18771-6