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 of 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
CULAF	Amniotic Fluid Culture/Genetic Test	Yes	No
_STR1	Comp Analysis using STR (Bill only)	No, (Bill only)	No
_STR2	Add'l comp analysis w/STR (Bill Only)	No, (Bill only)	No
CULFB	Fibroblast Culture for Genetic Test	Yes	No
MATCC	Maternal Cell Contamination, B	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 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

Skin biopsy or cultured fibroblast specimens:

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

Cord blood:

For cord blood specimens that have an accompanying maternal blood specimen, maternal cell contamination studies will be performed at an additional charge.

Special Instructions

- Informed Consent for Genetic Testing
- Informed Consent for Genetic Testing (Spanish)
- Targeted Genes and Methodology Details for Autoimmune Lymphoproliferative Syndrome 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

Customization of this panel and single gene analysis for any gene present on this panel are available. For more information see CGPH / Custom Gene Panel, Hereditary, Next-Generation Sequencing, Varies. To modify this panel via CGPH, use the Inborn Errors of Immunity/Bone Marrow Failure/Telomeropathy/Pulmonary Fibrosis/Very Early Onset IBD/Pancreatitis disease state for step 1 on the Custom Gene Ordering Tool.

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.

Specimen Required

Patient Preparation: A previous hematopoietic stem cell transplant from an allogenic donor will interfere with testing. For information about testing patients who have received a hematopoietic stem cell transplant, call 800-533-1710.

Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender top (EDTA) or yellow top (ACD)
Acceptable: Green top (sodium heparin)
Specimen Volume: 3 mL
Collection Instructions:

1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot**.
3. Whole blood collected postnatal from an umbilical cord is also acceptable. See Additional Information

Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated 4 days/Frozen 4 days

Additional Information:

1. Specimens are preferred to be received within 4 days of collection. Extraction will be attempted for specimens received after 4 days, and DNA yield will be evaluated to determine if testing may proceed.
2. To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.
3. For postnatal umbilical cord whole blood specimens, maternal cell contamination studies are recommended to ensure test results reflect that of the patient tested. A maternal blood specimen is required to complete maternal cell contamination studies. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on both the cord blood and maternal blood specimens under separate order numbers.

Specimen Type: Saliva

Patient Preparation: Patient should not eat, drink, smoke, or chew gum 30 minutes prior to collection.

Supplies:

DNA Saliva Kit High Yield (T1007)
Saliva Swab Collection Kit (T786)

Container/Tube:

Preferred: High-yield DNA saliva kit
Acceptable: Saliva swab

Specimen Volume: 1 Tube if using T1007 or 2 swabs if using T786

Collection Instructions: Collect and send specimen per kit instructions.

Specimen Stability Information: Ambient (preferred) 30 days/Refrigerated 30 days

Additional Information: Saliva specimens are acceptable but not recommended. Due to lower quantity/quality of DNA yielded from saliva, some aspects of the test may not perform as well as DNA extracted from a whole blood sample. When applicable, specific gene regions that were unable to be interrogated will be noted in the report. Alternatively, additional specimen may be required to complete testing.

Specimen Type: Blood spot

Supplies: Card-Blood Spot Collection (Filter Paper) (T493)

Container/Tube:

Preferred: Collection card (Whatman Protein Saver 903 Paper)
Acceptable: PerkinElmer 226 filter paper or blood spot collection card

Specimen Volume: 2 to 5 Blood spots

Collection Instructions:

1. An alternative blood collection option for a patient older than 1 year is a fingerstick. For detailed instructions, see How to Collect a Dried Blood Spot Sample.
2. Let blood dry on the filter paper at ambient temperature in a horizontal position for a minimum of 3 hours.
3. Do not expose specimen to heat or direct sunlight.
4. Do not stack wet specimens.
5. Keep specimen dry.

Specimen Stability Information: Ambient (preferred)/Refrigerated

Additional Information:
1. Blood spot specimens are acceptable but not recommended. Multiple extractions will be required to obtain sufficient yield for supplemental analysis, and there is significant risk for test failure due to insufficient DNA.
2. Due to lower concentration of DNA yielded from blood spot, some aspects of the test may not perform as well as DNA extracted from a whole blood sample. When applicable, specific gene regions that were unable to be interrogated will be noted in the report. Alternatively, additional specimen may be required to complete testing.
3. For collection instructions, see Blood Spot Collection Instructions
4. For collection instructions in Spanish, see Blood Spot Collection Card-Spanish Instructions (T777)
5. For collection instructions in Chinese, see Blood Spot Collection Card-Chinese Instructions (T800)

Specimen Type: Bone marrow aspirate
Container/Tube:
Preferred: Lavender top (EDTA)
Acceptable: Yellow top (ACD)
Specimen Volume: 2 mL
Collection Instructions:
1. Invert several times to mix bone marrow.
2. Label specimen as bone marrow.
3. Send bone marrow specimen in original tube. **Do not aliquot.**
Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated 4 days/Frozen 4 days
Additional Information:
1. Specimens are preferred to be received within 4 days of collection. Extraction will be attempted for specimens received after 4 days, and DNA yield will be evaluated to determine if testing may proceed.
2. To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.

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: Ambient (preferred) <24 hours/Refrigerated <24 hours
Additional Information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

Specimen Type: Cultured fibroblasts
Source: Skin or tissue
Container/Tube: T-25 flask
Specimen Volume: 2 Flasks
Collection Instructions: Submit confluent cultured fibroblast cells from a skin or tissue biopsy. Cultured cells from a prenatal specimen **will not be accepted**.

Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours
Additional Information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

Specimen Type: Extracted DNA
Container/Tube:
Preferred: Screw Cap Micro Tube, 2 mL with skirted conical base
Acceptable: Matrix tube, 1 mL
Collection Instructions:
1. The preferred volume is at least 100 mcL at a concentration of 75 ng/mcL.
2. Include concentration and volume on tube.
Specimen Stability Information: Frozen (preferred) 1 year/Ambient/Refrigerated
Additional Information: DNA must be extracted in a CLIA-certified laboratory or equivalent and must be extracted from a specimen type listed as acceptable for this test (including applicable anticoagulants). Our laboratory has experience with Chemagic, Puregene, Autopure, MagnaPure, and EZ1 extraction platforms and cannot guarantee that all extraction methods are compatible with this test. If testing fails, one repeat will be attempted, and if unsuccessful, the test will be reported as failed and a charge will be applied. If applicable, specific gene regions that were unable to be interrogated due to DNA quality will be noted in the report.

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

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 monoallelic and biallelic 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.

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.

Deletion/duplication events that extend past the genes included on the panel may occur. In these instances, genes included in the ordered test are provided on the report and interpreted, and genomic breakpoints are reported if they are confirmed. However, copy number variants for genes not listed in the Method Description are typically not reported or interpreted for haploinsufficiency/triplosensitivity. CMACB / Chromosomal Microarray, Congenital, Blood; WESPR / Panel to Whole Exome Sequencing Reflex Test, Varies; or WGSDX / Whole Genome Sequencing for Hereditary Disorders, Varies is recommended for a full interpretation of deletions/duplications predicted to extend past the genes included on the panel.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic mutations 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. For the most up to date list of genes included in this test and 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 non-leukocyte reduced 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 professionals 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;116(14):e35-e40
2. Richards S, Aziz N, Bale S, et al. 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;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;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;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;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;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. 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.(Unpublished Mayo method)

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

No

Day(s) Performed

Varies

Report Available

21 to 28 days

Specimen Retention Time

Whole blood: 25 days (if available); Saliva: 30 days (if available); Hair root bulb (if available)/Extracted DNA: 3 months; Blood spots: 1 year (if available)

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

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

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