

Autoimmune Lymphoproliferative Syndrome Gene Sequencing Panel

Genes Tested:

<i>ADA2 (CECR1)</i>	<i>CASP8</i>	<i>CASP10</i>	<i>CTLA4</i>	<i>FADD</i>
<i>FAS</i>	<i>FASLG</i>	<i>ITK</i>	<i>KRAS</i>	<i>LRBA</i>
<i>MAGT1</i>	<i>NRAS</i>	<i>PRKCD</i>	<i>RASGRP1</i>	<i>STAT3</i>

Description:

Autoimmune Lymphoproliferative Syndrome (ALPS) is a primary immunodeficiency disorder of defective FAS-mediated apoptosis (restimulation-induced cell death). Patients with ALPS develop chronic/recurrent lymphadenopathy, [hepato] splenomegaly, and autoimmune disease affecting blood cells and other tissues. There is a highly increased risk of lymphoma in ALPS patients. The presence of additional, unidentified genetic or environmental modifiers may be necessary to effect the development of the ALPS phenotype in individuals with pathogenic variants.

According to the latest diagnostic criteria*, a diagnosis of ALPS is based on the presence of:

1. Chronic, nonmalignant, noninfectious lymphadenopathy, splenomegaly or both

and

2. Elevated CD3+TCR $\alpha\beta$ +CD4-CD8- double-negative T cells (DNTCs)

Additionally, at least one of the following must be present:

- Defective lymphocyte apoptosis (determined by 2 separate assays)
- Identified pathogenic variants in *FAS*, *FASLG*, or *CASP10*

Secondary diagnostic criteria, which include biomarkers (plasma/ serum sFASL, interleukin-10, interleukin-18, and/or vitamin B12), immunohistological findings, cytopenias/elevated immunoglobulin G, and/or positive family history, may help determine if an individual has a “probable” ALPS diagnosis.

This panel includes genes associated with ALPS as well as top differential diagnosis of lymphoproliferative disorders:

Gene	Inheritance	Condition
<i>ADA2 (CECR1)</i>	AR	Vasculitis, autoinflammation, immunodeficiency, and hematologic defects syndrome
<i>CASP8</i>	AR	Autoimmune lymphoproliferative syndrome 2B
<i>CASP10</i>	AD	Autoimmune lymphoproliferative syndrome 2
<i>CTLA4</i>	AD	Autoimmune lymphoproliferative syndrome 5
<i>FADD</i>	AR	Recurrent infections with encephalopathy, hepatic dysfunction, and cardiovascular malformations
<i>FAS</i>	AD, Somatic	Autoimmune lymphoproliferative syndrome 1A, ALPS due to somatic FAS (ALPS-sFAS)
<i>FASLG</i>	AD/AR	Autoimmune lymphoproliferative syndrome 1B
<i>ITK</i>	AR	Lymphoproliferative syndrome 1
<i>KRAS</i>	AD	RAS-associated autoimmune leukoproliferative disorder
<i>LRBA</i>	AR	Common variable immunodeficiency 8, with autoimmunity
<i>MAGT1</i>	XR	X-linked immunodeficiency with magnesium defect, Epstein-Barr virus infection and neoplasia (XMEN)
<i>NRAS</i>	AD, Somatic	RAS-associated autoimmune lymphoproliferative syndrome 4, Noonan syndrome (AD)
<i>PRKCD</i>	AR	Autoimmune lymphoproliferative syndrome 3
<i>RASGRP1</i>	AR	RASGRP1 deficiency
<i>STAT3</i>	AD	Infantile-onset multisystem autoimmune disease 1

Somatic pathogenic variants in *FAS* will be detected if they are present in over 5% of the alleles in the specimen provided for testing. In ALPS-sFAS patients, somatic *FAS* pathogenic variants are mainly restricted to double negative T-cells. Confirmation of the presence of the variant in double negative T-cells is recommended.

Indications:

ALPS Gene Sequencing Panel:

- Confirmation of genetic diagnosis in a patient with a clinical diagnosis of ALPS
- Genetic diagnosis of ALPS in an asymptomatic individual with a family history of ALPS of unknown genetic basis.

Gene Specific Sequencing:

- Confirmation of genetic diagnosis in a patient with ALPS when a specific gene is suspected.

Variant Specific Analysis:

- Carrier testing of parents and other relatives for recurrence risk assessment
- Prenatal diagnosis of an at-risk fetus, after confirmation of variant(s) in the parent(s) and by prior arrangement only..

Specimen:

At least 3 mLs whole blood in a lavender top (EDTA) tube or saliva in an Oragene saliva kit. Please call 513-636-4474 for a free saliva collection kit.

Note: For post-transplant patients, we accept pre-transplant samples or post-transplant skin fibroblasts ONLY (blood, saliva, and cytobrushes are not accepted). Culturing of skin fibroblasts is done at an additional charge.

FAS somatic mutation study in double negative T cells:

Please contact the Diagnostic Immunology Laboratory at 513-636-4685 to schedule this testing.

Testing Methodology:

ALPS NGS Panel: This test is performed by enrichment of the coding exons, flanking intronic and untranslated regions (5' and 3'), as well as known pathogenic variants (HGMD 2018.4) in the promoter and deep intronic regions of the genes specified above using oligonucleotide probe

hybridization followed by next-generation sequencing with >50X coverage at every target base. All pathogenic and likely pathogenic variants, as well as variants of unknown (indeterminate) significance, as determined bioinformatically, are confirmed by Sanger sequencing. Regions with <50X will be filled in by Sanger sequencing. A detailed non-coding variant list is available upon request.

Gene specific sequencing: PCR-based sequencing of the entire coding region and intron/ exon boundaries of the specified gene.

Variant specific analysis: Sanger sequencing following PCR amplification of the targeted variant(s) of the specified gene.

Test Sensitivity:

Clinical Sensitivity: Approximately 75% of patients with ALPS have a germline pathogenic variant in *FAS*. The next largest group (10%) have somatic *FAS* pathogenic variants in double negative T cells. Sorting of double negative T cells and *FAS* somatic variant testing is available at our lab, please refer to our website or contact us for questions regarding this test. Pathogenic variants in *CASPI0* (2-3%) and *FASLG* (<1%) are rare. More recently, one case of an ALPS-like disorder was reported due to pathogenic variants in *PRKCD*. Other genes on this panel are associated with differential diagnoses for ALPS, and the clinical sensitivity of these genes is dependent on the patient's features.

Analytical Sensitivity: The sensitivity of DNA sequencing is over 99% for the detection of nucleotide base changes, small deletions and insertions in the regions analyzed. Somatic variants in *FAS* are expected to be identifiable when they are present at a variant allele frequency greater than 5%.

Limitations: Variants in the regulatory regions and non-reported variants in the untranslated regions may not be detected by this test. Large deletions/ duplications, large insertions and other complex genetic events will not be identified using sequencing methodology.

Note: Deletion/duplication is available for many of the genes on this panel. For further details, visit: www.cincinnatichildrens.org/deldup.

Turn-Around Time:

- ALPS gene sequencing panel: up to 6 weeks
- Single Gene Sequencing: 28 days

CPT Codes:

- **ALPS gene sequencing panel: 81443**
- **Single gene sequencing, targeted variant analysis, and deletion/duplication:** call for information.

Please call 1-866-450-4198 for current pricing, insurance preauthorization or with any billing questions.

Shipping Instructions:

Please enclose **test requisition** with sample.

All information must be completed before sample can be processed.

Place samples in styrofoam mailer and ship at room temperature by overnight Federal Express to arrive Monday through Friday.

Ship to:

Cytogenetics and Molecular Genetics Laboratories
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

*Oliveira JB, Bleesing JJ, 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

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