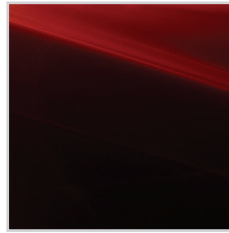
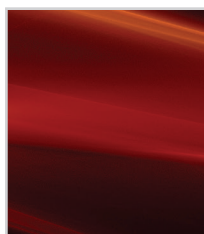
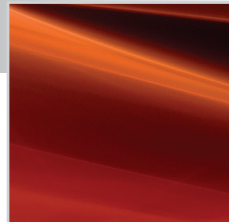


# Autoimmune Lymphoproliferative Syndrome Panel by next-generation sequencing (NGS)



## Genes Tested

<i>CASP8</i>	<i>CASP10</i>	<i>FADD</i>	<i>FAS</i>	
<i>FASLG</i>	<i>ITK</i>	<i>KRAS</i>	<i>MAGT1</i>	<i>NRAS</i>

**Description:** 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 auto-immune 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 disease-causing mutations.

ALPS is generally inherited as an autosomal dominant disease with high variability and expressivity among affected family members. Once an ALPS related mutation is identified in a family, genetic testing of other at-risk family members is critical for their appropriate medical management.

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 mutation(s) in *FAS*, *FASLG*, or *CASP10*

\*Oliveira JB, Bleasing 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.

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.

Mutations in *FADD* are associated with a rare primary immunodeficiency in which patients have many of the biochemical markers of ALPS including increased numbers of DNTCs and impaired FAS-mediated apoptosis, but lack the characteristic clinical features of lymphadenopathy and splenomegaly. Biallelic mutations in *CASP8* result in a rare immunodeficiency characterized by lymphadenopathy and splenomegaly, marginal elevation of DNTCs, defective FAS-mediated apoptosis, in addition to frequent bacterial and viral infections. Somatic mutations in *NRAS* and *KRAS* are rare causes of an ALPS-like condition known as RAS-associated lymphoproliferative disease (RALD). Mutations in *ITK* and *MAGT1* are not associated with ALPS or RALD but are included in this panel as part of the differential diagnosis of lymphoproliferative disorders.



Human Genetics

Cytogenetics and Molecular Genetics Laboratories  
CLIA#: 36D0656333  
Phone: (513) 636-4474  
Fax: (513) 636-4373  
[www.cchmc.org/genetics](http://www.cchmc.org/genetics)



## Indications:

### ALPS Panel by NGS:

- 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:

- Mutation analysis of sorted double negative T cells for *FAS* somatic mutation.

### Mutation Specific Analysis:

- Identification of at-risk relatives for future medical management
- Prenatal diagnosis of an at-risk fetus.

## Specimen:

**ALPS Panel by NGS:** At least 5 mLs whole blood in a lavender top (EDTA) tube.

### Gene Specific Sequencing or Mutation Specific

**Analysis:** At least 3 mLs whole blood in a lavender top (EDTA) tube.

***FAS* somatic mutation study:** Please contact the Diagnostic Immunology Laboratory at 513-636-4685 to schedule this testing.

## Testing Methodology:

**ALPS Panel by NGS:** This test is performed by enrichment of the exons, flanking intronic and untranslated regions (5' and 3') of the genes specified above using microdroplet PCR technology followed by next-generation sequencing with > 20 fold coverage at every target base. All pathogenic and novel variants, as well as variants of unknown (indeterminate) significance, as determined bioinformatically, are confirmed by Sanger sequencing.

### Gene Specific Sequencing/ Mutation Specific

**Analysis:** This test is performed by Sanger sequencing following PCR amplification of the specified coding and exon/intron boundaries of the specified gene. Single gene sequencing is available for every gene in the panel.

## Sensitivity:

**Clinical Sensitivity:** Approximately 75% of patients with ALPS have a germline mutation in *FAS*, while mutations in *CASP10*, *FADD* and *FASLG* have been reported in < 5% of patients with ALPS or ALPS-like disorders. PCR-based sequencing detects the majority of reported mutations in these genes. Gross deletions and rearrangements are reported in less than 10% of patients with ALPS and are not detected by this test methodology. Similarly, somatic *FAS* mutations in double negative T cells have been reported in approximately 20% of patients with ALPS and are not routinely detected. For patients with classic ALPS and a normal ALPS Panel by NGS test result or normal *FAS* gene sequencing, somatic mutation analysis is available on a fresh sample and at an additional charge.

**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. Mutations in regulatory regions or other untranslated regions are not detected by this test. Somatic mutations in *FAS* are a significant cause of ALPS and are not detected by this test. The limit of detection of somatic mutations in *KRAS* and *NRAS* is 20% mutation load. Large deletions involving entire single exons or multiple exons, large insertions and other complex genetic events have been reported in *FAS*, *FASLG* and *CASP10* and will not be identified using this test methodology. Rare primer site variants may lead to erroneous results.

**Note:** Targeted deletion and duplication analysis of each gene on this panel is clinically available at an additional charge.

## Turn-Around Time:

- ALPS Panel by NGS: up to 12 weeks
- Single Gene Sequencing: 4 weeks



## CPT Codes:

- ALPS Panel by NGS: 81479x8 and 81405
- Single gene sequencing of any gene on this panel except *KRAS* 81479
- Single gene sequencing of *KRAS* 81405
- Targeted mutation analysis 81403

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

**Results:** Results will be reported to the referring physician or health care provider as specified on the test requisition.

## 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

## References:

Blesing, J. J. H., J. Johnson, et al. (1993). *Autoimmune Lymphoproliferative Syndrome*. GeneReviews. R. A. Pagon, M. P. Adam, T. D. Bird et al. Seattle (WA).

Bolze, A., M. Byun, et al. (2010). "Whole-exome-sequencing-based discovery of human FADD deficiency." *Am J Hum Genet* 87(6): 873-881. Cerutti, E., et al. (2007). *BMC Immunol*, 8, 28.

Chun, H. J., L. Zheng, et al. (2002). "Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency." *Nature* 419(6905): 395-399.

Del-Rey, M., J. Ruiz-Contreras, et al. (2006). "A homozygous Fas ligand gene mutation in a patient causes a new type of autoimmune lymphoproliferative syndrome." *Blood* 108(4): 1306-1312.

Dowdell, K. C., J. E. Niemela, et al. (2010). "Somatic FAS mutations are common in patients with genetically undefined autoimmune lymphoproliferative syndrome." *Blood* 115(25): 5164-5169.

Hsu, A. P., K. C. Dowdell, et al. (2012). "Autoimmune lymphoproliferative syndrome due to FAS mutations outside the signal-transducing death domain: molecular mechanisms and clinical penetrance." *Genet Med* 14(1): 81-89.

Kuehn, H. S., I. Caminha, et al. (2011). "FAS haploinsufficiency is a common disease mechanism in the human autoimmune lymphoproliferative syndrome." *J Immunol* 186(10): 6035-6043.

Li, F. Y., M. J. Lenardo, et al. (2011). "Loss of MAGT1 abrogates the Mg<sup>2+</sup> flux required for T cell signaling and leads to a novel human primary immunodeficiency." *Magn Res* 24(3): S109-114.

Madkaikar, M., S. Mhatre, et al. (2011). "Advances in autoimmune lymphoproliferative syndromes." *Eur J Haematol* 87(1): 1-9.

Magerus-Chatinet, A., B. Neven, et al. (2011). "Onset of autoimmune lymphoproliferative syndrome (ALPS) in humans as a consequence of genetic defect accumulation." *J Clin Invest* 121(1): 106-112.

Neven, B., A. Magerus-Chatinet, et al. (2011). "A survey of 90 patients with autoimmune lymphoproliferative syndrome related to TNFRSF6 mutation." *Blood* 118(18): 4798-4807.

Niemela, J. E., L. Lu, et al. (2011). "Somatic KRAS mutations associated with a human nonmalignant syndrome of autoimmunity and abnormal leukocyte homeostasis." *Blood* 117(10): 2883-2886.

Oliveira, J. B. (2013). "The expanding spectrum of the autoimmune lymphoproliferative syndromes." *Curr Opin Pediatr* 25(6): 722-729.

Oliveira, J. B., J. J. Blesing, et al. (2010). "Revised diagnostic criteria and classification for the autoimmune lymphoproliferative syndrome (ALPS): report from the 2009 NIH International Workshop." *Blood* 116(14): e35-40.

Oliveira, J. B., N. Bidere, et al. (2007). "NRAS mutation causes a human autoimmune lymphoproliferative syndrome." *Proc Natl Acad Sci U S A* 104(21): 8953-8958.

Rao, V. K. and J. B. Oliveira (2011). "How I treat autoimmune lymphoproliferative syndrome." *Blood* 118(22): 5741-5751.

Takagi, M., K. Shinoda, et al. (2011). "Autoimmune lymphoproliferative syndrome-like disease with somatic KRAS mutation." *Blood* 117(10): 2887-2890.

Tewhey, R., J. B. Warner, et al. (2009). "Microdroplet-based PCR enrichment for large-scale targeted sequencing." *Nat Biotechnol* 27(11): 1025-1031.

Wang, J., L. Zheng, et al. (1999). "Inherited human Caspase 10 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome type II." *Cell* 98(1): 47-58.

Zhu, S., A. P. Hsu, et al. (2006). "Genetic alterations in caspase-10 may be causative or protective in autoimmune lymphoproliferative syndrome." *Hum Genet* 119(3): 284-294.

# ALPS Diagnostic Testing Algorithm

