**Genes Tested**

<table>
<thead>
<tr>
<th>CASP8</th>
<th>CASP10</th>
<th>FADD</th>
<th>FAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FASLG</td>
<td>ITK</td>
<td>KRAS</td>
<td>MAGT1</td>
</tr>
</tbody>
</table>

**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+TCRαβ+CD4-CD8- double-negative T cells (DNNTCs)

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*


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 DNNTCs 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 DNNTCs, 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.
**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 513-636-2731 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: 42 days
- Single Gene Sequencing: 28 days
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: