Hemophagocytic Lymphohistiocytosis Panel by next generation sequencing (NGS)

**Genes Tested:**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
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<tbody>
<tr>
<td>AP3B1</td>
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<tr>
<td>BLOC1S6</td>
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<tr>
<td>CD27</td>
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<tr>
<td>GATA2</td>
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<td>ITK</td>
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<td>LYST</td>
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<td>MAGT1</td>
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<tr>
<td>PRF1</td>
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<tr>
<td>RAB27A</td>
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<tr>
<td>SH2D1A</td>
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<tr>
<td>SLC7A7</td>
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<tr>
<td>STX11</td>
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<td>STXB2</td>
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<tr>
<td>UNC13D (MUNC13-4)</td>
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<td>XIAP (BIRC4)</td>
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</tbody>
</table>

**Description:**
This panel detects most known genetic causes of HLH: familial hemophagocytic lymphohistiocytosis (PRF1, UNC13-D, STX11, STXB2), X-linked lymphoproliferative (XLP) syndromes 1 and 2 (SH2D1A and XIAP), ITK deficiency (ITK), Hermansky-Pudlak syndrome types 2 and 9 (AP3B1 and BLOC1S6), Chediak-Higashi syndrome (LYST), CD27 deficiency (CD27), XLMEN syndrome, lysinuric protein intolerance (SLC7A7) and GATA2 deficiency (GATA2). All inherited as autosomal recessive conditions except for X-linked XLMEN syndrome and XLP1/2 and autosomal dominant GATA2 deficiency. Mutations in GATA2 have been reported in patients with GATA2 deficiency who have developed HLH in the setting of marked EBV viremia and an EBV-driven T-cell lymphoproliferative disorder or herpesvirus infections. Mutations in MAGT1 have not been associated with HLH to date, but it is included in this panel to gain knowledge about its association.

**Hemophagocytic lymphohistiocytosis (HLH)** is a disorder of widespread accumulation of lymphocytes and mature macrophages, sometimes with hemophagocytosis, primarily involving the spleen, lymph nodes, bone marrow, liver, and cerebral spinal fluid. HLH can either occur sporadically (secondary HLH), or be result of an underlying genetic defect in any one of several genes.

The diagnostic criteria for HLH, based on the recommendations of the Histiocyte Society, includes the presence of at least five of the eight following findings:
- Fever
- Splenomegaly
- Cytopenias affecting at least two of three cell lineages in peripheral blood
- Hypertriglyceridemia and/or hypofibrinogemia
- Hemophagocytosis in bone marrow, spleen or lymph nodes
- Low or absent natural killer (NK) cell function activity
- Hyperferritinemia
- High levels of soluble IL-2r

**Indications:**

**HLH Panel by NGS:**
- Confirmation of genetic diagnosis in a patient with a clinical diagnosis of HLH or associated syndrome
- Carrier identification in individuals with a family history of HLH of unknown genetic basis.

**Gene Specific Sequencing:**
- Confirmation of genetic diagnosis in a patient with HLH and in whom ancillary testing suggests a specific genetic diagnosis.

**Mutation Specific Analysis:**
- Presymptomatic testing of at-risk siblings for medical management and prior to bone marrow donation
- Carrier identification in individuals in whom specific mutation(s) have been identified in the proband with HLH
- Prenatal diagnosis of an at-risk fetus, after confirmation of mutation in the parent(s) (by prior arrangement only).

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Laboratory of Genetics and Genomics  
CLIA#: 36D0656333  
Phone: (513) 636-4474  
Fax: (513) 636-4373  
Email: LabGeneticCounselors@cchmc.org  
www.cincinnatichildrens.org/genetics
**Specimen:**

**HLH 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.

**Note:** Saliva samples are required for analysis in patients who have undergone bone marrow transplantation and may facilitate DNA isolation in patients undergoing chemotherapy or in individuals with leukopenia. Please call 513-636-4474 for a free saliva collection kit.

**Testing Methodology:**

**HLH Panel by NGS:** This test is performed by enrichment of the exons, flanking intronic and un-translated 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.

**Test Sensitivity:**

**Clinical Sensitivity:** Approximately 70% of patients with FHLH have identifiable mutation(s) in a gene on this panel. Approximately 90% of patients with a clinical diagnosis of CHS will have identifiable biallelic mutations in the LYST gene. Approximately 95% of Finnish patients with suspected LPI will have identifiable mutations in the SLC7A7 gene; the detection rate in non-Finnish patients is approximately 80%. Approximately 75% of males with XLP1 and 85% of males with XIAP deficiency (XLP2) will have identifiable mutations. Clinical sensitivity has not been determined for CD27 deficiency, HPS2, HPS9 XMEN syndrome, and ITK deficiency, due to the rarity of these conditions. Deletion/duplication analysis may be indicated as a follow-up test in symptomatic patients with a normal NGS sequencing result or a single (heterozygous) mutation.

**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.

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

**Turn-Around Time:**

- HLH Next Generation Sequencing Panel: 42 days
- Single Gene Sequencing: up to 42 days

**CPT Codes:**

- HLH Next Generation Sequencing Panel: 81479x13, 81404
- Single gene sequencing of any gene on this panel except SH2D1A: 81479
- Single gene sequencing of SH2D1A: 81404
- Mutation specific analysis: 81403

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

**Results:**

Each test report includes a detailed interpretation of the genetic findings, the clinical significance of the result, and specific recommendations for the clinical management and additional testing, if warranted. Results will be reported to the referring physician or health care provider as specified on the test requisition form.
References:


