Hemophagocytic Lymphohistiocytosis Gene Sequencing Panel

Genes Tested:

AP3B1	*AP3D1*	*CD27*	*CD70*
CDC42	*CTPS1*	*CYBA*	*CYBB*
CYBC1	*GATA2*	*ITK*	*LYST*
MAGT1	*NCF2*	*NCF4*	*NLRC4*
PRF1	*RAB27A*	*RASGRP1*	*RC3H1*
RHOG	*SH2D1A*	*SLC7A7*	*STX11*
STXBP2	*UNC13D*	*XIAP*	

Description:

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

Gene	Inheritance	Condition
AP3B1	AR	Hermansky-Pudlak syndrome 2
AP3D1	AR	Hermansky-Pudlak syndrome 10
CD27	AR	Lymphoproliferative syndrome 2
CD70	AR	Lymphoproliferative syndrome 3
CDC42	AD	Takenouchi-Kosaki syndrome
CTPS1	AR	Immunodeficiency 24
CYBA	AR	Chronic granulomatous disease 4, autosomal recessive
CYBB	XL	Chronic granulomatous disease, X-linked; Immunodeficiency 34, mycobacteriosis, X-linked
CYBC1	AR	Chronic granulomatous disease 5, autosomal recessive
GATA2	AD	Immunodeficiency 21
ITK	AR	Lymphoproliferative syndrome 1
LYST	AR	Chediak-Higashi syndrome
MAGT1	XL	X-linked immunodeficiency with magnesium defect, Epstein-Barr virus infection and neoplasia (XMEN)
NCF2	AR	Chronic granulomatous disease 2, autosomal recessive
NCF4	AR	Chronic granulomatous disease 3, autosomal recessive
NLRC4	AD	Autoinflammation with infantile enterocolitis
PRF1	AR	Familial hemophagocytic lymphohistiocytosis 2
RAB27A	AR	Griscelli syndrome 2
RASGRP1	AR	Immunodeficiency 64
RC3H1	AR	Immune dysregulation and systemic hyperinflammation syndrome
RHOG	AR	Hemophagocytic lymphohistiocytosis (HLH)
SH2D1A	XL	X-linked Lymphoproliferative syndrome 1
SLC7A7	AR	Lysinuric protein intolerance
STX11	AR	Familial hemophagocytic lymphohistiocytosis 4

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Gene	Inheritance	Condition
STXBP2	AR	Familial hemophagocytic lymphohistiocytosis 5
UNC13D	AR	Familial hemophagocytic lymphohistiocytosis 3
XIAP	XL	X-linked Lymphoproliferative syndrome 2

Note: Single gene sequencing and targeted variant analysis is available for all genes on the HLH Panel. Deletion/ duplication is also available for genes on this panel except *CD70, CDC42, CYBA, CYBB, CYBC1, NCF2, NCF4, NLRC4, RASGRP1, RC3H1* and *RHOG*. For further details visit: www.cincinnatichildrens.org/deldup.

Indications:

HLH Gene Sequencing Panel:

- 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 when a specific gene is suspected

Variant Specific Analysis:

- Presymptomatic testing of at-risk family members for medical management and prior to bone marrow donation
- 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.

Testing Methodology:

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 2021.2) in the promoter and deep intronic regions of the genes specified above using oligonucleotide probe hybridization followed by next-generation sequencing with >20X 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 <20X will be filled in by Sanger sequencing. A detailed non-coding variant list is available upon request. Allele specific analysis for the 253kb inversion as well as targeted analysis of the c.118-308 region in UNC13D are performed.

Gene Specific Sequencing: PCR-based sequencing of the entire coding region and intron/exon boundaries of the specified gene and selected known pathogenic variants in the promoter and deep intronic regions.

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

Test Sensitivity:

Clinical Sensitivity: Approximately 70% of individuals with familial hemophagocytic lymphohistiocytosis (FHL) have pathogenic variants in *PRF1, UNC13D, STX11* or *STXBP2*. About 2-3% of individuals with FHL have pathogenic variants in *RAB27A*. Other genes on this panel are associated with differential diagnoses for FHL, and the clinical sensitivity for these genes depend 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.

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.

Turn-Around Time:

- HLH panel: 28 days
- Single gene sequencing: up to 28 days

CPT Codes:

- **HLH 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 Saturday.

Ship to:

Genetics and Genomics Diagnostic Laboratory
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

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