Test Definition: HLHGP

Primary Hemophagocytic Lymphohistiocytosis Gene Panel, Varies

Overview

Useful For

Providing a comprehensive genetic evaluation for patients with a personal or family history suggestive of familial hemophagocytic lymphohistiocytosis (F-HLH)

Establishing a diagnosis of F-HLH, allowing for appropriate management and surveillance for disease features based on the gene and/or variant involved

Identifying variants within genes known to be associated with F-HLH, allowing for predictive testing of at-risk family members

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CULAF	Amniotic Fluid Culture/Genetic Test	Yes	No
_STR1	Comp Analysis using STR (Bill only)	No, (Bill only)	No
_STR2	Add'l comp analysis w/STR (Bill Only)	No, (Bill only)	No
CULFB	Fibroblast Culture for Genetic Test	Yes	No
MATCC	Maternal Cell Contamination, B	Yes	No

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in 23 genes associated with primary hemophagocytic lymphohistiocytosis (HLH, also known as familial HLH or F-HLH): *ADA, AP3B1, AP3D1, BLOC1S6, CD27, CD70, CDC42, CORO1A, CTPS1, IFNAR2, ITK, LYST, MAGT1, MVK, NLRC4, PRF1, RAB27A, SH2D1A, SLC7A7, STX11, STXBP2, UNC13D,* and *XIAP*.

This test may aid in the diagnosis of primary hemophagocytic lymphohistiocytosis (HLH) or a related disorder. This test is not intended or validated for detection of somatic mutations and cannot distinguish between germline variants associated with primary HLH versus somatic (oncogenic, nongermline) mutations, which may be associated with hematologic neoplasms. Therefore, this test does not provide diagnostic, prognostic, or therapeutic information for somatic mutations. Variants detected by this test are interpreted as germline unless otherwise noted in the interpretation.

If a patient has active hematological malignancy, skin biopsy is recommended (instead of whole blood) for detection of germline variants.

See Targeted Genes and Methodology Details for Primary Hemophagocytic Lymphohistiocytosis Gene Panel and Method Description for additional details.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, recurrence risk assessment, familial screening, and genetic counseling for HLH.

Testing Algorithm

Skin biopsy:

For skin biopsy or cultured fibroblast specimens, fibroblast culture will be performed at an additional charge. If viable cells are not obtained, the client will be notified.

Cord blood:

For cord blood specimens that have an accompanying maternal blood specimen, maternal cell contamination studies will be performed at an additional charge.

Special Instructions

- Informed Consent for Genetic Testing
- Blood Spot Collection Card-Spanish Instructions
- Blood Spot Collection Card-Chinese Instructions
- Informed Consent for Genetic Testing (Spanish)
- Blood Spot Collection Instructions
- Targeted Genes and Methodology Details for Primary Hemophagocytic Lymphohistiocytosis (HLH) Gene Panel
- Viral Susceptibility, Lymphoproliferation, and Hemophagocytic Lymphohistiocytosis Patient Information

Method Name

Sequence Capture Next-Generation Sequencing (NGS) followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

Patients who have had a previous bone marrow transplant from an allogenic donor should not have testing performed on blood, bone marrow, or saliva because any results generated will reflect the genome of the donor rather than the recipient. Testing on patients who have an active hematologic malignancy or hematologic disorder with clonal proliferation may identify both somatic mutations and germline variants, which may result in test failure or necessitate follow-up testing to determine whether the detected variant is germline or somatic. For these patients, testing a skin

biopsy or cultured fibroblasts is recommended. For instructions for testing patients who have received a bone marrow transplant or have an active hematologic disorder, call 800-533-1710. For more information see Cautions.

Upon request and after initial testing is complete, WESPR / Panel to Whole Exome Sequencing Reflex Test, Varies may be added to this test. To obtain more information about this option or add WESPR testing, call 800-533-1710.

Customization of this panel and single gene analysis for any gene present on this panel are available. For more information see CGPH / Custom Gene Panel, Hereditary, Next-Generation Sequencing, Varies. To modify this panel via CGPH, please use the Inborn Errors of Immunity/Bone Marrow Failure/Telomeropathy/Pulmonary Fibrosis/Very Early Onset IBD/Pancreatitis disease state for step 1 on the Custom Gene Ordering Tool.

Targeted testing for familial variants (also called site-specific or known variants testing) is available for the genes on this panel. See FMTT / Familial Variant, Targeted Testing, Varies. To obtain more information about this testing option, call 800-533-1710.

Additional Testing Requirements

For cord blood specimens: Maternal cell contamination (MCC) studies are available. **Order MATCC /** Maternal Cell Contamination, Molecular Analysis, Varies **on both the cord blood and maternal specimens under separate order numbers**. Cord blood testing will proceed without MCC studies, but results may be compromised if MCC is present.

Specimen Required

Patient Preparation: A previous hematopoietic stem cell transplant from an allogenic donor will interfere with testing. Call 800-533-1710 for instructions for testing patients who have received a hematopoietic stem cell transplant.

Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube: Lavender top (EDTA) or yellow top (ACD)
Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot.**
3. Whole blood collected postnatal from an umbilical cord is also acceptable. See Additional Information
Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated 4 days/Frozen 4 days
Additional Information:
1. Specimens are preferred to be received within 4 days of collection. Extraction will be attempted for specimens received after 4 days, and DNA yield will be evaluated to determine if testing may proceed.
2. To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.
3. For postnatal umbilical cord whole blood specimens, maternal cell contamination studies are recommended to ensure test results reflect that of the patient tested. A maternal blood specimen is required to complete maternal cell contamination studies. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on both the cord blood and maternal blood specimens under separate order numbers.

Specimen Type: Skin biopsy
Supplies: Fibroblast Biopsy Transport Media (T115)
Container/Tube: Sterile container with any standard cell culture media (eg, minimal essential media, RPMI 1640). The solution should be supplemented with 1% penicillin and streptomycin.
Specimen Volume: 4-mm Punch
Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours
Additional Information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

Specimen Type: Cultured fibroblasts
Source: Skin
Container/Tube: T-25 flask
Specimen Volume: 2 Flasks
Collection Instructions: Submit confluent cultured fibroblast cells from a skin biopsy from another laboratory. Cultured cells from a prenatal specimen will not be accepted.
Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours
Additional Information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical and Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

Specimen Type: Extracted DNA
Container/Tube:
Preferred: Screw Cap Micro Tube, 2 mL with skirted conical base
Acceptable: Matrix tube, 1 mL
Collection Instructions:
1. The preferred volume is at least 100 mcL at a concentration of 75 ng/mcL.
2. Include concentration and volume on tube.
Specimen Stability Information: Frozen (preferred) 1 year/Ambient/Refrigerated
Additional Information: DNA must be extracted in a CLIA-certified laboratory, or equivalent, and must be extracted from a specimen type listed as acceptable for this test (including applicable anticoagulants). Our laboratory has experience with Chemagic, Puregene, Autopure, MagnaPure, and EZ1 extraction platforms and cannot guarantee that all extraction methods are compatible with this test. If testing fails, one repeat will be attempted, and if unsuccessful, the test will be reported as failed and a charge will be applied. If applicable, specific gene regions that were unable to be interrogated due to DNA quality will be noted in the report.

Specimen Type: Blood spot
Supplies: Card-Blood Spot Collection (Filter Paper) (T493)
Container/Tube:
Preferred: Collection card (Whatman Protein Saver 903 Paper)

Acceptable: PerkinElmer 226 filter paper or blood spot collection card
Specimen Volume: 2 to 5 Blood spots
Collection Instructions:
1. An alternative blood collection option for a patient older than 1 year is a fingerstick. For detailed instructions, see How to Collect a Dried Blood Spot Sample.
2. Let blood dry on the filter paper at ambient temperature in a horizontal position for a minimum of 3 hours.
3. Do not expose specimen to heat or direct sunlight.
4. Do not stack wet specimens.
5. Keep specimen dry
Specimen Stability Information: Ambient (preferred)/Refrigerated
Additional Information:
1. Blood spot specimens are acceptable but not recommended. Multiple extractions will be required to obtain sufficient yield for supplemental analysis, and there is significant risk for test failure due to insufficient DNA.
2. Due to lower concentration of DNA yielded from blood spot, some aspects of the test may not perform as well as DNA extracted from a whole blood sample. When applicable, specific gene regions that were unable to be interrogated will be noted in the report. Alternatively, additional specimen may be required to complete testing.
3. For collection instructions, see Blood Spot Collection Instructions
4. For collection instructions in Spanish, see Blood Spot Collection Card-Spanish Instructions (T777)
5. For collection instructions in Chinese, see Blood Spot Collection Card-Chinese Instructions (T800)

Specimen Type: Saliva
Patient Preparation: Patient should not eat, drink, smoke, or chew gum 30 minutes prior to collection.
Supplies:
DNA Saliva Kit High Yield (T1007)
Saliva Swab Collection Kit (T786)
Container/Tube:
Preferred: High-yield DNA saliva kit
Acceptable: Saliva swab
Specimen Volume: 1 Tube if using T1007 or 2 swabs if using T786
Collection Instructions: Collect and send specimen per kit instructions.
Specimen Stability Information: Ambient (preferred) 30 days/Refrigerated 30 days
Additional Information: Saliva specimens are acceptable but not recommended. Due to lower quantity/quality of DNA yielded from saliva, some aspects of the test may not perform as well as DNA extracted from a whole blood sample. When applicable, specific gene regions that were unable to be interrogated will be noted in the report. Alternatively, additional specimen may be required to complete testing.

Forms

1. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing (Spanish) (T826)
2. Molecular Genetics: Congenital Inherited Diseases Patient Information (T521)
3. Viral Susceptibility, Lymphoproliferation, and Hemophagocytic Lymphohistiocytosis Patient Information

Specimen Minimum Volume
See Specimen Required

Reject Due To
All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information
Hemophagocytic lymphohistiocytosis (HLH) is a rare and life-threatening disorder characterized by fever, cytopenias, coagulopathy, hepatosplenomegaly, neurologic symptoms, and hemophagocytosis in the bone marrow, spleen, lymph nodes, or liver. Patients often have elevated ferritin and soluble interleukin-2 receptor concentrations, as well as low fibrinogen levels. The Histiocyte Society established criteria for HLH for the HLH-2004 clinical trial, and these criteria are often referred to by physicians considering a diagnosis of HLH.(1) Primary HLH, also known as familial HLH (F-HLH), is caused by disease-causing variants in several genes. Secondary or acquired HLH can be triggered by infection, malignancy, transplant, autoimmune disorders, or drugs. While the terms "primary" and "secondary" have been in use for some time, the North American Consortium for Histiocytosis recommended a new classification system that divides HLH into forms that respond to immunosuppressive treatment, which are referred to as "HLH disease" and forms that do not respond to immunosuppressives, which are referred to as "HLH mimics."(2)

In the pediatric population, the incidence of HLH is thought to range from 1 to 225 per 300,000 live births, equally distributed between male and female infants, with the mean age of occurrence of 1.8 years. The epidemiology among adults is less well-studied; however, the incidence is estimated to be 1 of every 2000 adult admissions to tertiary medical centers, with the mean age at presentation of approximately 50 years.

Many genes have been identified in association with F-HLH. In a pediatric population, genetic variants in *PRF1* account for approximately 25% of cases, while *STXBP2* and *UNC13D* are each responsible for approximately 20% of cases, and *XIAP* accounts for 10% of cases. Disease-causing variants in *PRF1*, *UNC13D, STX11,* and *STXBP2* prevent the release of cytotoxic granules into the immunological synapse, resulting in an inability to kill target cells. Pigment disorders, including Griscelli syndrome type 2, Chediak-Higashi syndrome, and Hermansky-Pudlak syndrome type 2 (due to variants in *RAB27A, LYST,* and *AP3B1,* respectively*)* also are associated with HLH. Due to significant granule trafficking defects, patients may also have bleeding tendencies, neutropenia, and neurological symptoms. X-linked lymphoproliferative disorders and Epstein-Barr virus susceptibility disorders are also associated with HLH. While most forms of F-HLH are inherited in an autosomal recessive pattern, there are autosomal dominant and X-linked forms.

Reference Values
An interpretive report will be provided

Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(3) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Cautions

Clinical Correlations:
Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If testing was performed because of a clinically significant family history, it is often useful to first test an affected family member. Detection of a reportable variant in an affected family member would allow for more informative testing of at-risk individuals.

To discuss the availability of additional testing options or for assistance in the interpretation of these results, contact Mayo Clinic Laboratories genetic counselors at 800-533-1710.

Technical Limitations:
Next-generation sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some target regions; assay performance below the minimum acceptable criteria or for failed regions will be noted. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is validated to detect 95% of deletions up to 75 base pairs (bp) and insertions up to 47 bp. Deletions-insertions (delins) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller delins.

Deletion/Duplication Analysis:
This analysis targets single and multi-exon deletions/duplications; however, in some instances, single exon resolution cannot be achieved due to isolated reduction in sequence coverage or inherent genomic complexity. Balanced structural rearrangements (such as translocations and inversions) may not be detected.

Deletion/duplication events that extend past the genes included on the panel may occur. In these instances, genes included in the ordered test are provided on the report and interpreted, and genomic breakpoints are reported if they are confirmed. However, copy number variants for genes not listed in the Method Description are typically not reported or interpreted for haploinsufficiency/triplosensitivity. CMACB / Chromosomal Microarray, Congenital, Blood; WESPR / Panel to Whole Exome Sequencing Reflex Test, Varies; or WGSDX / Whole Genome Sequencing for Hereditary Disorders, Varies is recommended for a full interpretation of deletions/duplications predicted to extend past the genes included on the panel.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic mutations and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results.

Genes may be added or removed based on updated clinical relevance. For the most up to date list of genes included in this test and detailed information regarding gene specific performance and technical limitations, see Method Description or contact a laboratory genetic counselor.

If the patient has had an allogeneic hematopoietic stem cell transplant or a recent non-leukoreduced blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.

Reclassification of Variants**:**
Currently, it is not standard practice for the laboratory to systematically review previously classified variants on a regular basis. The laboratory encourages healthcare professionals to contact the laboratory at any time to learn how the classification of a particular variant may have changed over time. Due to broadening genetic knowledge, it is possible that the laboratory may discover new information of relevance to the patient. Should that occur, the laboratory may issue an amended report.

Variant Evaluation:
Evaluation and categorization of variants are performed using published American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations as a guideline.(3) Other gene-specific guidelines may also be considered. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. Variants classified as benign or likely benign are not reported.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions made by in silico evaluation tools is highly dependent upon the data available for a given gene, and periodic updates to these tools may cause predictions to change over time. Results from in silico evaluation tools are interpreted with caution and professional clinical judgment.

Rarely, incidental or secondary findings may implicate another predisposition or presence of active disease. These findings will be carefully reviewed to determine whether they will be reported.

Clinical Reference

1. Henter JI, Horne A, Arico M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;48(2):124-131. doi:10.1002/pbc.21039
2. Jordan MB, Allen CE, Greenberg J, et al. Challenges in the diagnosis of hemophagocytic lymphohistiocytosis: Recommendations from the North American Consortium for Histiocytosis (NACHO). Pediatr Blood Cancer. 2019;66(11):e27929. doi:10.1002/pbc.27929
3. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424
4. Gadoury-Levesque V, Dong L, Su R, et al. Frequency and spectrum of disease-causing variants in 1892 patients with

suspected genetic HLH disorders. Blood Adv. 2020;4(12):2578-2594
5. Canna SW, Marsh RA. Pediatric hemophagocytic lymphohistiocytosis. Blood. 2020;135(16):1332-1343
6. Ponnatt TS, Lilley CM, Mirza KM. Hemophagocytic lymphohistiocytosis. Arch Pathol Lab Med. 2022;146(4):507-519
7. Tangye SG, Al-Herz W, Bousfiha A, et al. Human Inborn Errors of Immunity: 2022 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol. 2022;42(7):1473-1507. doi:10.1007/s10875-022-01289-3

Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing are performed to test for the presence of variants in coding regions and intron/exon boundaries of the genes analyzed, as well as some other regions that have known disease-causing variants. The human genome reference GRCh37/hg19 build was used for sequence read alignment. At least 99% of the bases are covered at a read depth over 30X. Sensitivity is estimated at above 99% for single nucleotide variants, above 94% for deletions/insertions (delins) less than 40 base pairs (bp), and above 95% for deletions up to 75 bp and insertions up to 47 bp. NGS and/or a polymerase chain reaction (PCR)-based quantitative method is performed to test for the presence of deletions and duplications in the genes analyzed. Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria. A supplemental PCR-based method is used to detect an inversion in *UNC13D*.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. See Targeted Genes and Methodology Details for Primary Hemophagocytic Lymphohistiocytosis Gene Panel for details regarding the targeted genes analyzed for each test and specific gene regions not routinely covered.(Unpublished Mayo method)

Genes analyzed: *ADA, AP3B1, AP3D1, BLOC1S6, CD27, CD70, CDC42, CORO1A, CTPS1, IFNAR2, ITK, LYST, MAGT1, MVK, NLRC4, PRF1, RAB27A, SH2D1A, SLC7A7, STX11, STXBP2, UNC13D,* and *XIAP*

PDF Report

No

Day(s) Performed

Varies

Report Available

28 to 42 days

Specimen Retention Time

Whole blood: 28 days (if available); Saliva: 30 days (if available); Extracted DNA: 3 months; Blood spots: 1 year (if available)

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

Fees & Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

81443
88233- Tissue culture, skin, solid tissue biopsy (if appropriate)
88240- Cryopreservation (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
HLHGP	HLH Gene Panel	99971-4

Result ID	Test Result Name	Result LOINC® Value
619831	Test Description	62364-5
619832	Specimen	31208-2
619833	Source	31208-2
619834	Result Summary	50397-9
619835	Result	82939-0
619836	Interpretation	69047-9
619837	Additional Results	82939-0
619838	Resources	99622-3
619839	Additional Information	48767-8
619840	Method	85069-3
619841	Genes Analyzed	82939-0
619842	Disclaimer	62364-5
619843	Released By	18771-6