Test Definition: PMHLH

Postmortem Primary Hemophagocytic Lymphohistiocytosis (HLH) Gene Panel, Tissue

Overview

Useful For

Providing a comprehensive postmortem genetic evaluation in the setting of a death attributed to primary hemophagocytic lymphohistiocytosis

Identifying a disease-causing variant in the decedent, which may assist with risk assessment and predictive testing of at-risk family members

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide variants and deletions-insertions (delins) 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*. See Method Description for additional details.

Identification of a disease-causing variant may assist with familial risk assessment, screening, and genetic counseling for primary hemophagocytic lymphohistiocytosis.

Special Instructions

- Informed Consent for Genetic Testing for Deceased Individuals
- Viral Susceptibility, Lymphoproliferation, and Hemophagocytic Lymphohistiocytosis Patient Information

Method Name

Sequence Capture and Targeted Next-Generation Sequencing (NGS)

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

This test is intended for use when whole blood is not available, and formalin-fixed, paraffin-embedded (FFPE) tissue is the only available specimen. If whole blood is available, consider HLHGP / Primary Hemophagocytic Lymphohistiocytosis Gene Panel, Varies.

Specimen Required

Specimen Type: Tissue (FFPE)

Container/Tube: Tissue block
Collection Instructions: Submit a formalin-fixed, paraffin-embedded tissue block
Additional Information: Testing will be attempted on blocks of any age but may be canceled if adequate DNA concentration cannot be obtained.

Forms

1. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file or submit the Informed Consent for Genetic Testing for Deceased Individuals (T782).
2. 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	Ambient (preferred)		
	Refrigerated		

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 as well as low fibrinogen. 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. Primary HLH, also known as familial HLH (F-HLH), is attributed to 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 into forms that do not respond to immunosuppressive treatment, which are referred to as "HLH mimics."

In the pediatric population, the incidence of HLH is thought to range from 1 to 225 per 300,000 live births, be 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 a mean age at presentation of approximately 50 years.

Many genes have now 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.(1) 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 (NGS) 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 as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of NGS results by Sanger sequencing is typically not performed for this test.

Deletions-insertions (delins) of 40 or more base pairs, including mobile element insertions, may be less reliably detected than smaller delins.

Deletion/duplication analysis is not performed due to technical limitations of the formalin-fixed paraffin-embedded specimen type.

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 detailed information regarding gene specific performance and technical limitations, see Method Description or contact a laboratory genetic counselor.

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.(1) 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 should be interpreted with caution and professional clinical judgment.

Rarely, incidental or secondary findings may implicate another predisposition or presence of active disease. Incidental findings may include, but are not limited to, results related to the sex chromosomes. These findings will be carefully reviewed to determine whether they will be reported.

Clinical Reference

1. 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. doi:10.1038/gim.2015.30
2. 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. doi:10.1182/bloodadvances.2020001605
3. Canna SW, Marsh RA. Pediatric hemophagocytic lymphohistiocytosis. Blood. 2020;135(16):1332-1343. doi:10.1182/blood.2019000936
4. Ponnatt TS, Lilley CM, Mirza KM. Hemophagocytic lymphohistiocytosis. Arch Pathol Lab Med. 2022;146(4):507-519. doi:10.5858/arpa.2020-0802-RA
5. 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) is 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 20X. Sensitivity is estimated at above 99% for single nucleotide variants and above 94% for deletions-insertions (delins) less than 40 base pairs.

There may be regions of genes that cannot be effectively evaluated by sequencing as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of NGS results by Sanger sequencing is typically not performed for this test.(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

FFPE tissue block: Client provided paraffin blocks (FFPE) will be returned to client after testing is complete; Extracted DNA: 3 months.

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

LOINC® Information

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

Result ID	Test Result Name	Result LOINC® Value
620625	Test Description	62364-5
620626	Specimen	31208-2
620627	Source	31208-2
620628	Result Summary	50397-9
620629	Result	82939-0
620630	Interpretation	69047-9
620631	Additional Results	82939-0
620632	Resources	99622-3
620633	Additional Information	48767-8
620634	Method	85069-3
620635	Genes Analyzed	82939-0
620636	Disclaimer	62364-5
620637	Released By	18771-6