MAYO CLINIC LABORATORIES

Test Definition: THEV1

Thalassemia and Hemoglobinopathy Evaluation, Blood and Serum

Overview

Useful For

Evaluation of microcytosis

Extensive and economical diagnosis and classification of hemoglobinopathies or thalassemia, including complex disorders

Diagnosis of hereditary persistence of hemoglobin

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
THEVI	Hemoglobinopathy Interpretation	No	Yes
HGBCE	Hb Variant, A2 and F Quantitation,B	Yes	Yes
HPLC	HPLC Hb Variant, B	No	Yes
FERR1	Ferritin, S	Yes	Yes

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
HPFH	Hb F Distribution, B	No	No
SDEX	Sickle Solubility, B	Yes	No
IEF	Isoelectric Focusing, B	No	No
UNHB	Hb Stability, B	No	No
MASS	Hb Variant by Mass Spec, B	No	No
WASQR	Alpha Globin Gene Sequencing, B	Yes, (Order WASEQ)	No
WBSQR	Beta Globin Gene Sequencing, B	Yes, (Order WBSEQ)	No
WGSQR	Gamma Globin Full Gene Sequencing	Yes, (Order WGSEQ)	No
THEV0	Thalassemia Summary Interpretation	No	No
WAGDR	Alpha Globin Clustr Locus Del/Dup,B	Yes, (Order AGDD)	No
WBGDR	Beta Globin Gene Cluster, Del/Dup,B	Yes, (Order WBGDD)	No

Testing Algorithm

This is a consultative evaluation in which the case will be evaluated at Mayo Clinic Laboratories, the appropriate tests performed at an additional charge, and the results interpreted.

This evaluation will always include hemoglobins (Hb) A2 and F and hemoglobin electrophoresis utilizing cation exchange high-performance liquid chromatography (HPLC) and capillary electrophoresis methods.

If a serum sample is received, a serum ferritin will always be performed to allow incorporation of possible iron deficiency into profile interpretation and economical test utilization. If the ferritin component is not needed, do not send a serum sample, and the ferritin test will not be performed. **Note:** If a ferritin is not performed or provided, and if microcytosis is present and no other abnormalities are found (beta thalassemia, a hemoglobin variant that is associated with microcytosis), the case will be reflexed to alpha-globin gene analysis unless otherwise requested not to be performed.

Hemoglobin electrophoresis reflex testing, performed at additional charge, may include any or all of the following as indicated to identify rare hemoglobin variants present: sickle solubility (hemoglobin S screen), hemoglobin heat and isopropanol stability studies, isoelectric focusing, HbF distribution by flow cytometry, cation exchange HPLC, DNA (Sanger) testing for beta-chain variants and the most common beta thalassemias (beta-globin gene sequencing), multiplex ligation-dependent probe amplification testing for beta-cluster locus large deletions and duplications, including large deletional hereditary persistence of fetal hemoglobin (HPFH), delta-beta, delta thalassemias, gamma-delta-beta, and epsilon-gamma-delta-beta thalassemias (beta-globin cluster locus deletion/duplication), large deletional alpha thalassemias and alpha-gene duplications (alpha-globin gene analysis), alpha-chain variants and nondeletional alpha thalassemias (alpha-globin gene sequencing), and gamma-chain variants and nondeletional HPFH (gamma-globin full gene sequencing).

An additional consultative interpretation that summarizes all testing will be provided after test completion to incorporate subsequent results into overall evaluation if any of the following molecular tests are reflexed on this test.
-WAGDR / Alpha Globin Cluster Locus Deletion/Duplication, Blood
-WASQR / Alpha-Globin Gene Sequencing, Blood
-WBSQR / Beta-Globin Gene Sequencing, Blood
-WBGDR / Beta-Globin Gene Cluster Deletion/Duplication, Blood
-WGSQR / Gamma-Globin Full Gene Sequencing, Varies
The results of the individual protein and molecular tests will be released as they are completed; with a final summary interpretation report correlating all performed testing with any clinical information or complete blood cell count results received.

For more information see Benign Hematology Evaluation Comparison

Special Instructions

- Informed Consent for Genetic Testing
- Metabolic Hematology Patient Information
- Benign Hematology Evaluation Comparison
- Informed Consent for Genetic Testing (Spanish)

Method Name

THEVI, THEV0: Medical Interpretation
HGBCE: Capillary Electrophoresis
HPLC: Cation Exchange/High-Performance Liquid Chromatography (HPLC)
FERR1: Electrochemiluminescence Immunoassay
IEF: Isoelectric Focusing
MASS: Mass Spectrometry (MS)
HPFH: Flow Cytometry
UNHB: Isopropanol and Heat Stability

NY State Available
Yes

Specimen

Specimen Type
Serum
Whole Blood EDTA

Ordering Guidance
Multiple hematology evaluations are available. For information on testing that is performed with each evaluation, see Benign Hematology Evaluation Comparison.

If monitoring treatment with HbA-T87Q (Lyfgenia for sickle cell disorders or Zynteglo for thalassemia), complete Metabolic Hematology Patient Information (T810) to notify the laboratory and request mass spectrometry testing.

Necessary Information
Include the following information with the specimen:
Recent transfusion information
-Most recent complete blood cell count results
-If not sending serum, include ferritin results.

Metabolic Hematology Patient Information (T810) is strongly recommended. Testing may proceed without this information, however if the information requested is received, any pertinent reported clinical features and data will drive the focus of the evaluation and be considered in the interpretation.

The laboratory has extensive experience in hemoglobin variant identification and many cases can be confidently classified without molecular testing. However, molecular confirmation is always available, subject to sufficient sample quantity (eg, MLPA testing requires at least 2 mL of sample in addition to protein testing requirements). If no molecular testing or specific molecular tests are desired, utilize the appropriate check boxes on the form. If the form or other communication is not received, the reviewing hematopathologist will select appropriate tests to sufficiently explain the protein findings which may or may not include molecular testing.

Specimen Required

Blood and serum are required.

Specimen Type: Whole blood
Container/Tube: Lavender top (EDTA)
Specimen Volume: 15 mL
Collection Instructions: Send whole blood specimen in original tube. **Do not aliquot.**

Specimen Type: Serum
Patient Preparation: For 12 hour before specimen collection, patient **should not** take multivitamins or dietary supplements (eg, hair, skin, and nail supplements) containing biotin (vitamin B7).
Collection Container/Tube:
Preferred: Serum gel
Acceptable: Red top
Submission Container/Tube: Plastic vial
Specimen Volume: 0.6 mL
Collection Instructions:
1. Within 2 hours of collection, serum gel tubes should be centrifuged.
2. Within 2 hours of collection, red-top tubes should be centrifuged and the serum aliquoted into a plastic vial.
3. Label specimen as serum.

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. Metabolic Hematology Patient Information (T810)
3. If not ordering electronically, complete, print, and send a Benign Hematology Test Request (T755) with the specimen

Specimen Minimum Volume

Blood: 2.5 mL
Serum: 0.5 mL

Reject Due To

Gross hemolysis	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Refrigerated	7 days	
Whole Blood EDTA	Refrigerated	7 days	

Clinical & Interpretive

Clinical Information

This consultative study is primarily designed for the evaluation of microcytosis but also has the ability to test for the detection of almost all known hemoglobin disorders in an economical manner. Because this can include multiple tests for alpha thalassemias, beta thalassemias, delta-beta thalassemia, hereditary persistence of fetal hemoglobin (HPFH), and for all known hemoglobin (Hb) variants, an expert in these disorders can guide testing to explain the clinical question or reported complete blood cell count values. This evaluation is particularly useful for complete classification of compound combinations of Hb S with alpha or beta thalassemia, Hb E/beta-0-thalassemia, and many other complex alpha and beta thalassemia disorders. Since iron deficiency can mimic thalassemias, ferritin levels are measured to evaluate this possibility if a serum sample is received.

Hb disorders include those associated with thalassemias (decreased protein quantity) and Hb variants (abnormal protein production). Many are clinically harmless, and others cause symptoms, including microcytosis, sickling disorders, hemolysis, erythrocytosis, cyanosis/hypoxia, long-standing or familial anemia, compensated or episodic anemia, and increased methemoglobin or sulfhemoglobin results. Hb disorders can show patterns of either autosomal recessive or autosomal dominant inheritance.

The thalassemias are a group of disorders of Hb synthesis. Normal adult Hb consists of 2 alpha-globin chains (encoded by 2 pairs of alpha-globin genes, each pair located on chromosome 16), and 2 beta-globin chains (encoded by 2 beta-globin genes, each located on chromosome 11). Thalassemia syndromes result from an underproduction of 1 or 2 types of globin chains and are characterized by the type (alpha, beta, delta, gamma) and magnitude of underproduction (number of defective genes) and the severity of clinical symptoms (minor, intermedia, major). The severity of the clinical and hematologic effects is directly related to the imbalance of alpha-like to beta-like chains.

The most common form of thalassemia is alpha thalassemia. Alpha thalassemia usually involves deletion of entire alpha genes and varies in severity depending on the number of alpha chains deleted (or rendered nonfunctional). Alpha thalassemia trait usually results from the deletion of 2 alpha genes. The most common form of Hb H disease, results from dysfunction of 3 alpha chains, and shows a variable phenotype with most showing moderate anemia. The deletion of all 4 alpha genes (Barts hydrops fetalis) is incompatible with life without significant medical intervention. Nondeletional alpha thalassemia alterations can also result in either thalassemia trait or Hb H disease and are less common than deletional forms.

Conversely, most beta thalassemia alterations are due to single nucleotide substitutions that can occur anywhere in the beta-globin gene. Large deletions of the beta-globin gene complex can result in elevations in Hb F, such as HPFH or delta-beta thalassemia. While the presence of a single beta-gene variants (beta thalassemia trait) results primarily in red blood cells microcytosis, cases with 2 beta-gene abnormalities show a wide range in clinical severity, and many cases require molecular testing to understand the phenotype.

Reference Values

Definitive results and an interpretive report will be provided.

Interpretation

A hematopathologist expert in these disorders evaluates the case, appropriate tests are performed, and an interpretive report is issued.

Cautions

DNA probe studies reveal deletional alterations that include most, but not all, alpha thalassemias.

Clinical Reference

1. Hoyer JD, Hoffman DR. The thalassemia and hemoglobinopathy syndromes. In: McClatchey KD, Amin HM, Curry JL, eds. Clinical Laboratory Medicine. 2nd ed. Lippencott Williams and Wilkins; 2002:866-892
2. Brancaleoni V, Di Pierro E, Motta I, Cappellini MD. Laboratory diagnosis of thalassemia. Int J Lab Hematol. 2016;38 Suppl 1:32-40
3. Hartveld C. State of the art and new developments in molecular diagnostics for hemoglobinopathies in multiethnic societies. Int J Lab Hematol. 2014;36:1-12

Performance

Method Description

Hemoglobin Electrophoresis:
The CAPILLARYS System is an automated system that uses capillary electrophoresis to separate charged molecules by their electrophoretic mobility in an alkaline buffer. Separation occurs according to the electrolyte pH and electro-osmotic flow. A sample dilution with hemolyzing solution is injected by aspiration. A high voltage protein separation occurs, and direct detection of the hemoglobin protein fractions is at 415 nm, which is specific to hemoglobins. The resulting electropherogram peaks are evaluated for pattern abnormalities and are quantified as a percentage of the total hemoglobin present. Examples of position of commonly found hemoglobin fractions are, from cathode to anode: HbA2', C, A2/O-Arab, E, S, D, G-Philadelphia, F, A, Hope, Bart, J, N-Baltimore, and H.(Louahabi A, Philippe M, Lali S, Wallemacq P, Maisin D. Evaluation of a new Sebia kit for analysis of hemoglobin fractions and variants on the Capillarys system. Clin Chem Lab Med. 2006;44[3]:340-345; Instruction manual: CAPI 3 HEMOGLOBIN(E) Phoresis vs $\geq$9.15. Sebia; 12/2020)

High-Performance Liquid Chromatography Hemoglobin Variant:
Hemolysate of whole blood is injected into an analysis stream passing through a cation exchange column using high-performance liquid chromatography. A preprogrammed gradient controls the elution buffer mixture that also passes through the analytical cartridge. The ionic strength of the elution buffer is raised by increasing the percentage of a second buffer. As the ionic strength of the buffer increases the more strongly retained hemoglobins elute from the cartridge. Absorbance changes are detected by a dual-wavelength filter photometer. Changes in absorbance are displayed as a chromatogram of absorbance versus time.(Huismann TH, Scroeder WA, Brodie AN, Mayson SM, Jakway J. Microchromotography of hemoglobins. III. A simplified procedure for the determination of hemoglobin A2. J Lab Clin Med. 1975;86:700-702; Ou CN, Buffone GJ, Reimer GL, Alpert AJ. High-performance liquid chromatography of human hemoglobins on a new cation exchanger. J Chromatogr. 1983;266:197-205; Szuberski J, Oliveira JL, Hoyer JD. A comprehensive analysis of hemoglobin variants by high-performance liquid chromatography (HPLC). Int J Lab Hematol. 2012; 34(6):594-604; Instruction manual: Bio-Rad Variant II Beta-thalassemia Short Program Instructions for Use, L70203705. Bio-Rad Laboratories, Inc; 11/2011)

Ferritin:
The Roche ferritin method employs monoclonal antibodies specifically directed against ferritin. A biotinylated monoclonal antibody and a second monoclonal antibody labeled with a ruthenium complex react to form a sandwich complex. After the addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Application of a voltage to the electrode then induces chemiluminescent emission, which is measured by a photo multiplier.(Package insert: Elecsys Ferritin. Roche Diagnostics; 10/2022)

PDF Report
No

Day(s) Performed
Monday through Thursday

Report Available
2 to 25 days

Specimen Retention Time
Blood: 7 days; Abnormal samples: 14 days

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
83020-26-Hemoglobinopathy Interpretation
83020-Hb Variant, A2 and F Quantitation
83021
82728
82664 (if appropriate)
83068 (if appropriate)

83789 (if appropriate)
88184 (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
THEV1	Thalassemia and Hemoglobinopathy Ev	In Process

Result ID	Test Result Name	Result LOINC® Value
41927	Hb A	20572-4
41928	Hb F	32682-7
41929	Hb A2	4552-6
41930	Variant 1	24469-9
41931	Variant 2	24469-9
41932	Variant 3	24469-9
41933	HGBCE Interpretation	78748-1
FERR1	Ferritin, S	20567-4
65615	HPLC Hb Variant, B	No LOINC Needed
608425	Hemoglobinopathy Interpretation	13514-5
608868	Reviewed By	18771-6