

Overview

Useful For

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

Evaluation of microcytosis

Diagnosis of hereditary persistence of hemoglobin (HPFH)

Profile Information

| Test ID | Reporting Name | Available Separately | Always Performed |
|---------|---------------------------------|----------------------|------------------|
| THEV | Hemoglobinopathy Interpretation | No | Yes |
| A2F | Hemoglobin A2 and F | No | Yes |
| HBEL | Hemoglobin Electrophoresis, B | No | Yes |
| FERR | Ferritin, S | Yes | Yes |

Reflex Tests

| Test ID | Reporting Name | Available Separately | Always Performed |
|---------|--------------------------------------|----------------------|------------------|
| SDEX | Hemoglobin S, Scrn, B | Yes | No |
| IEF | IEF Confirms | No | No |
| MASS | Hb Variant by Mass Spec, B | No | No |
| UNHB | Unstable Hemoglobin, B | No | No |
| HPFH | Hemoglobin F, Red Cell Distrib, B | No | No |
| ATHAL | Alpha-Globin Gene Analysis | Yes | No |
| WASQR | Alpha Globin Gene Sequencing, B | Yes, (WASEQ) | No |
| WBSQR | Beta Globin Gene Sequencing, B | Yes, (WBSEQ) | No |
| WBDDR | Beta Globin Cluster Locus Del/Dup, B | Yes, (WBDD) | No |
| WGSQR | Gamma Globin Full Gene Sequencing | Yes, (WGSEQ) | No |
| THEVA | Thalassemia Summary Interpretation | No | 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 hemoglobin A(2) and F and hemoglobin electrophoresis utilizing cation exchange 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 desired, do not send a serum sample and none will be performed or charged. Fill out the [Thalassemia/Hemoglobinopathy Patient Information](#) sheet (T358) and indicate the CBC values and ferritin levels for a more complete evaluation.

Note: If a ferritin is not done or provided, and if microcytosis is present and no other abnormalities are found, the case will be reflexed to alpha-globin gene analysis.

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 (unstable hemoglobin), isoelectric focusing, intact globin chain mass spectrometry (hemoglobin variant by mass spectrometry), Hb F distribution by flow cytometry (hemoglobin F red cell distribution), DNA (Sanger) testing for beta chain variants and the most common beta thalassemias (beta-globin gene sequencing), multiplex ligation-dependent probe amplification (MLPA) testing for beta cluster locus large deletions and duplications, including large deletional hereditary persistence of fetal hemoglobin (HPFH), delta-beta (DBT), delta thalassemias, gamma-delta-beta (GDBT), and epsilon-gamma-delta-beta (EGDBT) thalassemias (beta globin cluster locus del/dup), large deletional alpha thalassemias and alpha gene duplications (alpha-globin gene analysis), alpha chain variants and non-deletional alpha thalassemias (alpha-globin gene sequencing), and gamma chain variants and non-deletional HPFH (gamma-globin full gene sequencing).

If a [Thalassemia/Hemoglobinopathy Patient Information](#) sheet (T358) is received with the sample, the reported clinical features or clinical impression will be considered in the interpretation and focus of the evaluation. Our laboratory has extensive experience in hemoglobin variant identification and many cases can be confidently classified without molecular testing. However, molecular confirmation is always available. If no molecular testing or, conversely, specific molecular tests are desired, please utilize the appropriate check boxes in the information sheet. If the information sheet or other communication is not received, the reviewing hematopathologist will select appropriate tests to sufficiently explain the clinical impression or reported CBC results, which may or may not include molecular testing.

Thalassemia Summary Interpretation, 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 the Thalassemia and Hemoglobinopathy Evaluation:

- ATHAL / Alpha-Globin Gene Analysis
- WASQR / Alpha-Globin Gene Sequencing, Blood
- WBSQR / Beta-Globin Gene Sequencing, Blood
- WBDDR / Beta-Globin Cluster Locus Deletion/Duplication, Blood
- WGSQR / Gamma-Globin Full Gene Sequencing

See [Benign Hematology Evaluation Comparison](#) in Special Instructions.

Special Instructions

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

Method Name

THEV: Consultative Interpretation

A2F: Cation Exchange/High-Performance Liquid Chromatography (HPLC)

HBEL: Capillary Electrophoresis

FERR: Immunoenzymatic Assay

IEF: Electrophoresis

MASS: Mass Spectrometry (MS)

HPFH: Flow Cytometry

UNHB: Isopropanol and Heat Stability

THEVA: Consultative Interpretation

NY State Available

Yes

Specimen**Specimen Type**

Serum

Whole Blood EDTA

Necessary Information

1. Include recent transfusion information.
2. Include most recent CBC results.

Specimen Required

Both blood and serum are required.

Specimen Type: Whole blood

Container/Tube: Lavender top (EDTA)

Specimen Volume: 15 mL

Collection Instructions: Send specimen in original tube. **Do not aliquot.**

Specimen Type: Serum

Container/Tube: Red top or serum gel

Specimen Volume: 0.6 mL

Collection Instructions: 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 in Special Instructions:

[-Informed Consent for Genetic Testing](#) (T576)

[-Informed Consent for Genetic Testing-Spanish](#) (T826)

2. [Metabolic Hematology Patient Information](#) (T810) in Special Instructions

3. If not ordering electronically, complete, print, and send a [Benign Hematology Test Request Form](#) (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 |
|------------------|--------------|--------|
| Serum | Refrigerated | 7 days |
| Whole Blood EDTA | Refrigerated | 7 days |

Clinical and Interpretive

Clinical Information

This consultative study 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 Hb variants, an expert in these disorders can guide testing to explain the clinical question or CBC 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 thalassemic disorders. Since iron deficiency can mimic thalassemias, ferritin levels are measured to evaluate this possibility.

Hemoglobin disorders include those associated with thalassemias (decreased protein quantity) and hemoglobin 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. Hemoglobin disorders can show autosomal recessive or autosomal dominant inheritance patterns.

The thalassemias are a group of disorders of hemoglobin (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) 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. Hemoglobin H (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 chains is incompatible with life. Affected fetuses are hydropic and die in utero or shortly after premature birth. The blood smears show large hypochromic red cells, nucleated red cells, target cells, and red cell fragments. Hb Barts, Hb H, and Hb Portland are present in significant quantities. It is the most common cause of hydrops fetalis in Southeast Asia and southern China.

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 mutations that include most, but not all, alpha-thalassemias.

Clinical Reference

Hoyer JD, Hoffman DR: The thalassemia and hemoglobinopathy syndromes. In *Clinical Laboratory Medicine*. Second edition. Edited by KD McMillatchey. Philadelphia, Lippencott Williams and Wilkins, 2002, pp 866-892

Performance

Method Description

Hemoglobin A2 and F:

Hemolysate of whole blood is injected into an analysis stream passing through a cartridge containing diethylaminoethyl-resin using high-performance liquid chromatography (HPLC). 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 absorbances are displayed as a chromatogram of absorbances versus time. (Huisman TH, Schroeder WA, Brodie AN, et al: Microchromatography 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)

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 hemolysing 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 electrophoregrams 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: Hb A2', C, A2/O-Arab, E, S, D, G-Philadelphia, F, A, Hope, Bart, J, N-Baltimore, and H.(Louahabi A, Philippe M, et al: Evaluation of a new Sebia kit for analysis of hemoglobin fractions and variants on the Capillary system. Clin Chem Lab Med 2006;44[3]:340-345)

Ferritin, Serum:

The instrument used is a Beckman Coulter Unicel DXI 800. The Access Ferritin assay is a 2-site immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel with goat antiferritin-alkaline phosphatase conjugate, and paramagnetic particles coated with goat antimouse:mouse antiferritin complexes. Serum ferritin binds to the immobilized monoclonal antiferritin on the solid phase, while the goat antiferritin enzyme conjugate reacts with different antigenic sites on the ferritin molecules. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field, while unbound materials are washed away. Chemiluminescent substrate Lumi-Phos 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of ferritin in the sample. The amount of analyte in the sample is determined from a stored, multipoint calibration curve.(Package insert: Beckman Coulter Inc, Fullerton, CA 2009)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Saturday

Analytic Time

2 to 25 days if structural and/or molecular studies are required

Maximum Laboratory Time

25 days

Specimen Retention Time

7 days: abnormal kept for 14 days

Performing Laboratory Location

Rochester

Fees and 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 Regional Manager. For assistance, contact [Customer Service](#).

Test Classification

See Individual Test IDs

CPT Code Information

Thalassemia and Hemoglobinopathy Evaluation

82728-Ferritin

83020-Hemoglobin electrophoresis

83021-Hemoglobin A2 and F

IEF Confirms

82664 (if appropriate)

Hemoglobin, Unstable, Blood

83068 (if appropriate)

Hemoglobin Variant by Mass Spectrometry

83789 (if appropriate)

LOINC® Information

| Test ID | Test Order Name | Order LOINC Value |
|---------|-------------------------------------|-------------------|
| THEVP | Thalassemia and Hemoglobinopathy Ev | 14869-2 |

| Result ID | Test Result Name | Result LOINC Value |
|-----------|---------------------------------|--------------------|
| 2380 | Hemoglobin A | 20572-4 |
| FERR | Ferritin, S | 20567-4 |
| 2381 | Hemoglobin A2 | 42245-1 |
| 583 | Hemoglobinopathy Interpretation | 14869-2 |
| 2382 | Hemoglobin F | 42246-9 |
| 2383 | Variant | 32017-6 |
| 29224 | Variant 2 | 32017-6 |
| 29225 | Variant 3 | 32017-6 |
| 2101 | Interpretation | 78748-1 |