Overview

Useful For

Diagnosis of methemoglobinemia and sulfhemoglobinemia and possible hereditary (congenital) causes

Differentiation of methemoglobinemia and sulfhemoglobinemia from other causes of cyanosis (eg, congenital heart disease)

Profile Information

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEV</td>
<td>Methemoglobinemia Interpretation</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>A2F</td>
<td>Hemoglobin A2 and F</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>HBEL</td>
<td>Hemoglobin Electrophoresis, B</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>METH</td>
<td>Methemoglobin, B</td>
<td>Yes, (Order MET)</td>
<td>Yes</td>
</tr>
<tr>
<td>SULF</td>
<td>Sulfhemoglobin, B</td>
<td>Yes, (Order MET)</td>
<td>Yes</td>
</tr>
<tr>
<td>METR</td>
<td>Methemoglobin Reductase, B</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Reflex Tests

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDEX</td>
<td>Hemoglobin S, Scrn, B</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>IEF</td>
<td>IEF Confirms</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>MASS</td>
<td>Hb Variant by Mass Spec, B</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>UNHB</td>
<td>Unstable Hemoglobin, B</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>HPFH</td>
<td>Hemoglobin F, Red Cell Distrib, B</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>ATHAL</td>
<td>Alpha-Globin Gene Analysis</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>WASQR</td>
<td>Alpha Globin Gene Sequencing, B</td>
<td>Yes, (Order WASEQ)</td>
<td>No</td>
</tr>
<tr>
<td>WBSQR</td>
<td>Beta Globin Gene Sequencing, B</td>
<td>Yes, (Order WBSEQ)</td>
<td>No</td>
</tr>
<tr>
<td>WBDDR</td>
<td>Beta Globin Cluster Locus Del/Dup,B</td>
<td>Yes, (Order WBDD)</td>
<td>No</td>
</tr>
<tr>
<td>MEVA</td>
<td>Methemoglobin Summary Interp</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>WGSQR</td>
<td>Gamma Globin Full Gene Sequencing</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
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 is an evaluation for methemoglobin and sulfhemoglobin levels and possible hereditary causes. Methemoglobin, sulfhemoglobin levels, methemoglobin reductase (cytochrome-b5 reductase) activity and protein analysis screening for hemoglobin variants (cation exchange HPLC and capillary electrophoresis) will always be performed. If additional hemoglobin variant confirmatory testing is required, appropriate reflex testing will be performed. This will vary from additional protein analysis methods to molecular testing, as needed.

MEVA / Methemoglobin Summary Interpretation, an additional consultative interpretation that summarizes all testing, will be provided after additional testing is completed if any of the following molecular tests are reflexed on the MEVP / Methemoglobinemia 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

MEV: Consultative Interpretation

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

HBEL: Capillary Electrophoresis

METH, SULF: Spectrophotometry (SP)

METR: Kinetic Spectrophotometry (KS)

MASS: Mass Spectrometry (MS)

IEF: Electrophoresis

HPFH: Flow Cytometry

UNHB: Isopropanol and Heat Stability

MEVA: Consultative Interpretation
NY State Available
Yes

Specimen

Specimen Type
Whole Blood ACD-B
Whole Blood EDTA

Shipping Instructions
Specimen must arrive within 3 days (72 hours) of draw.

Necessary Information
Include recent transfusion information.
Include most recent CBC results.

Specimen Required
Both ACD-B and EDTA blood are required.

Container/Tube: Lavender top (EDTA) and yellow top (ACD [Solution B])

Specimen Volume:
EDTA: Two 4-mL tubes
ACD: One 6-mL tube

Collection Instructions: Send specimens in original tube. Do not aliquot.

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) is available in Special Instructions

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

Specimen Minimum Volume
EDTA Blood: 3 mL; ACD Blood: 2.7 mL

Reject Due To

<table>
<thead>
<tr>
<th>Gross hemolysis</th>
<th>Reject</th>
</tr>
</thead>
</table>

Document generated January 9, 2021 at 12:53am CST
Test Definition: MEVP
Methemoglobinemia Evaluation

Specimen Stability Information

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood ACD-B</td>
<td>Refrigerated</td>
<td>72 hours</td>
<td></td>
</tr>
<tr>
<td>Whole Blood EDTA</td>
<td>Refrigerated</td>
<td>72 hours</td>
<td></td>
</tr>
</tbody>
</table>

Clinical and Interpretive

Clinical Information

Methemoglobin:

Methemoglobin forms when the hemoglobin molecule iron is in the ferric (Fe3+) form instead of the functional ferrous (Fe2+) form. Methemoglobinemia can be hereditary or acquired and is present by definition when methemoglobin levels are greater than the normal range. Acquired methemoglobinemia results after toxic exposure to nitrates and nitrites/nitrates (fertilizer, nitric oxide), topical anesthetics ("caines"), dapsone, naphthalene (moth balls/toilet deodorant cakes) and industrial use of aromatic compounds (aniline dyes).

Congenital methemoglobinemias are rare. They are due either to:

- A deficiency of methemoglobin reductase (also called NADH-cytochrome b5 reductase 3 or diaphorase) in erythrocytes, an autosomal recessive disorder resulting from mutations in the CYB5R3 or the CYB5A genes.(1,2) Type IV is thought to be extraordinarily rare. Type III is no longer a category.

- One of several intrinsic structural disorders of hemoglobin, called M-hemoglobins (M-Hbs), all of which are inherited in an autosomal dominant manner.(3,4) Classically, M-Hbs result from histidine-to tyrosine substitutions at the proximal or distal histidine important in coordinating the oxygen molecule. These include alpha-, beta- and gamma-chain variants. Rarely, other substitutions outside the proximal and distal histidine location can cause Hb variants that increase methemoglobin or sulfhemoglobin levels. Most M-hemoglobin variants are readily identified by HPLC or mass spectrometry methods with characteristic electrophoresis patterns; however, some require more specialized techniques. Most are associated with increased methemoglobin with or without an increase in sulfhemoglobin. Alpha chain M-hemoglobin variants can be associated with increased sulfhemoglobin without an increase in methemoglobin.

Sulfhemoglobin:

Sulfhemoglobinemia often accompanies methemoglobinemia. Sulfhemoglobinemia can be due to exposure to trinitrotoluene, zinc ethylene bisdithiocarbamate (a fungicide), overexposure to paint or varnish fumes, metoclopramide, sulfonamides and some migraine medications. The formation of sulfhemoglobin cannot be reversed and there is no therapy for sulfhemoglobinemia aside from removal of the inciting agent. Because patients with sulfhemoglobinemia also often have methemoglobinemia, therapy is directed at reversing the methemoglobinemia present. Isolated increased sulfhemoglobin levels can also be associated with alpha chain M-hemoglobin variants.

Symptoms of both methemoglobinemia and sulfhemoglobinemia are characterized by cyanosis.

Reference Values

Definitive results and an interpretive report will be provided.
Test Definition: MEVP
Methemoglobinemia Evaluation

Interpretation
In congenital methemoglobinemia, the methemoglobin concentration in blood is about 15% to 20% of total hemoglobin. Such patients are mildly cyanotic and asymptomatic.

In acquired (toxic) methemoglobinemia, the concentration may be much higher. Symptoms may be severe when methemoglobin is greater than 40% of hemoglobin. Very high concentrations may be fatal.

This is a consultative evaluation in which the history and previous laboratory values are reviewed by a hematologist who is an expert on these disorders. Appropriate tests are performed and an interpretive report is issued.

Cautions
Sulfhemoglobin is exceedingly stable and does not change in stored or shipped specimens.

Methemoglobin is unstable and can degrade at a rate of about 40% per 24 hours.

A normal methemoglobin value obtained with stored or shipped specimens does not exclude prior methemoglobinemia of minimal degree. However, significant methemoglobinemia will still be demonstrable.

Clinical Reference

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

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 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, Lali S, et al: 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)

Methemoglobin:

The normal absorption spectrum of oxyhemoglobin has very little optical density above 600 nm. The absorption spectrum of methemoglobin exhibits a small, characteristic peak at 630 nm. This peak is abolished as methemoglobin is converted to cyanmethemoglobin upon addition of potassium cyanide, and the drop in optical density is proportional to methemoglobin concentration.(Evelyn KA, Malloy HT: Microdetermination of oxyhemoglobin, methemoglobin, and sulfhemoglobin in a single sample of blood. J Biol Chem 1938;126:655-662; Fairbanks VF, Klee GG: Biochemical aspects of hematology. In Teitz Textbook of Clinical Chemistry. Edited by CA Burtis, ER Ashwood, WB Saunders Company, 1999, pp 1676-1678)

Sulfhemoglobin:

The normal absorption spectrum of oxyhemoglobin has very little optical density above 600 nm. However, if certain poorly defined hemoglobin denaturation products are present in a hemolysate, there is a broad elevation of the absorption curve in the range of 600 to 620 nm. This sulfhemoglobin plateau is not affected by treatment with cyanide. Sulfhemoglobin is not available, nor can it be prepared, in a pure form for preparation of a sulfhemoglobin standard. In calculating sulfhemoglobin concentration, the factor for sulfhemoglobin quantitation is based on studies of Carrico et al.(Evelyn KA, Malloy HT: Microdetermination of oxyhemoglobin, methemoglobin, and sulfhemoglobin in a single sample of blood. J Biol Chem 1938;126:655-662; Carrico RJ, Peisach J, Alben JO: The preparation and some physical properties of sulfhemoglobin. J Analyt Biochem 1978;253:2386-2391; Fairbanks VF, Klee GG: Biochemical aspects of hematology. In Teitz Textbook of Clinical Chemistry. Edited by CA Burtis, ER Ashwood, WB Saunders Company, 1999, pp 1676-1678)

Methemoglobin Reductase:


PDF Report

No
Day(s) and Time(s) Test Performed
Monday through Saturday

Analytic Time
3-25 days if structural or molecular studies are required (not reported Saturday or Sunday)

Maximum Laboratory Time
25 days

Specimen Retention Time
30 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
Methemoglobinemia Evaluation

82657-Methemoglobin reductase
83020-Hemoglobin electrophoresis
83021-Hemoglobin A2 and F
83050-Methemoglobin, quantitative
83060-Sulfhemoglobin, quantitative

IEF Confirms

82664-Isoelectric focusing (if appropriate)

Hemoglobin, Unstable, Blood

83068 (if appropriate)

Hemoglobin Variant by Mass Spectrometry (MS), Blood

83789 (if appropriate)
Test Definition: MEVP  
Methemoglobinemia Evaluation

Hemoglobin F, Red Cell Distribution, Blood

88184 (if appropriate)

**LOINC® Information**

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Test Order Name</th>
<th>Order LOINC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEVP</td>
<td>Methemoglobinemia Evaluation</td>
<td>In Process</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Result ID</th>
<th>Test Result Name</th>
<th>Result LOINC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>8268</td>
<td>Methemoglobin, B</td>
<td>2614-6</td>
</tr>
<tr>
<td>9322</td>
<td>Methemoglobin Reductase, B</td>
<td>32703-1</td>
</tr>
<tr>
<td>8272</td>
<td>Sulfhemoglobin, B</td>
<td>4685-4</td>
</tr>
<tr>
<td>2381</td>
<td>Hemoglobin A2</td>
<td>42245-1</td>
</tr>
<tr>
<td>2380</td>
<td>Hemoglobin A</td>
<td>20572-4</td>
</tr>
<tr>
<td>586</td>
<td>Methemoglobinemia Interpretation</td>
<td>59465-5</td>
</tr>
<tr>
<td>2383</td>
<td>Variant</td>
<td>32017-6</td>
</tr>
<tr>
<td>2382</td>
<td>Hemoglobin F</td>
<td>42246-9</td>
</tr>
<tr>
<td>29224</td>
<td>Variant 2</td>
<td>32017-6</td>
</tr>
<tr>
<td>29225</td>
<td>Variant 3</td>
<td>32017-6</td>
</tr>
<tr>
<td>2101</td>
<td>Interpretation</td>
<td>78748-1</td>
</tr>
</tbody>
</table>