MAYO CLINIC LABORATORIES

Test Definition: MEV1

Methemoglobinemia Evaluation, Blood

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

Test Id	Reporting Name	Available Separately	Always Performed
MEVI	Methemoglobinemia Interpretation	No	Yes
HGBCE	Hb Variant, A2 and F Quantitation,B	Yes	Yes
HPLC	HPLC Hb Variant, B	No	Yes
METH	Methemoglobin, B	Yes, (Order MET)	Yes
SULF	Sulfhemoglobin, B	Yes, (Order MET)	Yes
METR1	Cytochrome b5 Reductase, B	Yes	Yes

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
SDEX	Sickle Solubility, B	Yes	No
IEF	Isoelectric Focusing, B	No	No
MASS	Hb Variant by Mass Spec, B	No	No
UNHB	Hb Stability, B	No	No
HPFH	Hb F Distribution, 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
MEV0	Methemoglobin Summary Interp	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 is an evaluation for methemoglobin and sulfhemoglobin levels and possible hereditary causes. Methemoglobin, sulfhemoglobin levels, cytochrome-b5 reductase (methemoglobin reductase) activity, and protein analysis screening for hemoglobin variants (capillary electrophoresis, cation exchange high performance liquid chromatography 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.

One or more of the following molecular tests may be reflexed:
-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

After all test results are finalized, an additional consultative interpretation that summarizes all testing and incorporates subsequent genetic results will be provided.

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

MEVI, MEV0: Medical Interpretation
HGBCE: Capillary Electrophoresis
HPLC: Cation Exchange/High-Performance Liquid Chromatography (HPLC)
METH, SULF: Spectrophotometry (SP)
METR1: Kinetic Spectrophotometry
IEF: Isoelectric Focusing
HPFH: Flow Cytometry
UNHB: Isopropanol and Heat Stability
MASS: Mass Spectrometry (MS)

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 collection.

Necessary Information

Include recent transfusion information.

Include most recent complete blood cell count 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, multiplex ligation-dependent probe amplification 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

The following specimens are required for testing:
Whole blood ACD-B specimen
2 Whole blood EDTA specimens

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 whole blood specimen 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:
-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

EDTA blood: 3 mL; ACD blood: 2.7 mL

Reject Due To

Gross hemolysis	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole Blood ACD-B	Refrigerated	72 hours	
Whole Blood EDTA	Refrigerated	72 hours	

Clinical & Interpretive

Clinical Information

Methemoglobin:
Methemoglobin forms when the hemoglobin (Hb) 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 cytochrome b5 reductase (methemoglobin reductase) in erythrocytes, an autosomal recessive disorder resulting from genetic variants in either *CYB5R3* or *CYB5A*.(1,2) Type IV is thought to be extraordinarily rare. Type III is no longer a category.

-One of several intrinsic structural disorders of Hb, called 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-Hb variants are readily identified by high performance liquid chromatography (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-Hb variants can be associated with increased sulfhemoglobin without an increase in methemoglobin.

Sulfhemoglobin:
Sulfhemoglobin cannot combine with oxygen. When acquired, sulfhemoglobinemia can be associated with cyanosis and often accompanies methemoglobinemia. Sulfhemoglobinemia has been associated with exposure to sumatriptan, sulfonamides, metoclopramide, paint or varnish vapors, dimethyl sulfoxide, acetanilide, phenacetin, trinitroluene, zinc ethylene bisdithiocarbamate (a fungicide), and flutamide. It is important to note that some Hb variants are known to interfere with this test (especially M-Hbs) and sulfhemoglobin absorbance can be increased due to the Hb variant. Hb evaluation that includes the HPLC method is recommended to exclude this possibility.

In contrast to methemoglobinemia, sulfhemoglobinemia persists until the erythrocytes containing it are destroyed. Therefore, blood level of sulfhemoglobin declines gradually over a period of weeks.

Reference Values

Definitive results and an interpretive report will be provided.

Interpretation

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

1. OMIM: 250800 Methemoglobinemia due to deficiency of methemoglobin reductase. Updated May 20, 2019. Accessed January 23, 2024. Available at www.omim.org/entry/250800?search=250800&highlight=250800
2. OMIM: 250790 Methemoglobinemia and ambiguous genitalia. Updated December 9, 2022. Accessed January 23, 2024. Available at www.omim.org/entry/250790?search=250790&highlight=250790
3. OMIM: 141800 Hemoglobin alpha locus 1; HBA1. Updated September 15, 2023. Accessed January 23, 2024. Available at www.omim.org/entry/141800?search=141800&highlight=141800
4. OMIM: 141900 Hemoglobin beta locus; HBB. Updated September 15, 2023. Accessed January 23, 2024. Available at www.omim.org/entry/141900?search=141900&highlight=141900
5. Haymond S, Cariappa R, Eby CS, Scott MG. Laboratory assessment of oxygenation in methemoglobinemia. Clin Chem. 2005;51(2):434-444
6. Noor M, Beutler E. Acquired sulfhemoglobinemia. An underreported diagnosis? West J Med. 1998;169(6):386-389
7. Thom CS, Dickson CF, Gell DA, Weiss MJ. Hemoglobin variants: biochemical properties and clinical correlates. Cold Spring Harb Perspect Med. 2013;3(3):a011858
8. Percy MJ, McFerran NV, Lappin TR. Disorders of oxidized haemoglobin. Blood Rev. 2005;19(2):61-68
9. Agarwal AM, Prchal JT. Methemoglobinemia and Other Dyshemoglobinemias. In: Kaushansky K, Lichtman MA, Prchal JT, Levi MM, Press OW, Burns LJ, Caligiuri M, eds. Williams Hematology. 9th ed. McGraw-Hill; 2016: 789-800

Performance

Method Description

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 (Hb) protein fractions is at 415 nm, which is specific to Hbs. The resulting electrophoregrams peaks are evaluated for pattern abnormalities and are quantified as a percentage of the total Hb present. Examples of position of commonly found Hb 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, 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: CAPILLARYS Hemoglobin(E) using the CAPILLARYS 2 flex-piercing instrument. Sebia; 06/2014)

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 Hbs 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, Schroeder 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; instruction manual: Bio-Rad Variant II Beta-thalassemia Short Program Instructions for Use, L70203705. Bio-Rad Laboratories, Inc; 11/2011)

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: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. WB Saunders Company; 1999:1676-1678; Robertson LD, Roper D. Laboratory methods used in the investigation of haemolytic anaemias. In: Bain BJ, Bates I, Laffan MA, eds. Dacie and Lewis Practical Haematology. 12th ed. Elsevier; 2017:214-227)

Sulfhemoglobin:
The normal absorption spectrum of oxyhemoglobin has very little optical density above 600 nm. However, if certain poorly defined Hb 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: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. WB Saunders Company; 1999:1676-1678; Robertson LD, Roper D. Laboratory methods used in the investigation of haemolytic anaemias. In: Bain BJ, Bates I, Laffan MA, eds. Dacie and Lewis Practical Haematology. 12th ed. Elsevier; 2017:214-227)

Cytochrome b5 Reductase:
Cytochrome B5 reductase (methemoglobin reductase) catalyzes the 1,4-dihydronicotinamide adenine dinucleotide (NADH)-linked reduction of several substrates, including ferricyanide. The activity at 30 degrees C is followed spectrophotometrically by measuring the oxidation of NADH at 340 nm.(Fairbank VF, Klee GG. Biochemical aspects of hematology. In: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 3rd ed. WB Saunders Company; 1999:1647-1648; van Solinge WW, van Wijk. Enzymes of the red blood cell. In: Rifai N, Horvath AR, Wittwer CT: eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier; 2018:chap 30)

PDF Report
No

Day(s) Performed
Monday through Saturday

Report Available
3 to 25 days

Specimen Retention Time
28 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-HPLC Hb Variant
82657-Methemoglobin reductase
83050-Methemoglobin, quantitative
83060-Sulfhemoglobin, quantitative
82664 (if appropriate)
83068 (if appropriate)
83789 (if appropriate)
88184 (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
MEV1	Methemoglobinemia Evaluation	In Process

Result ID	Test Result Name	Result LOINC® Value

8268	Methemoglobin, B	2614-6
8272	Sulfhemoglobin, B	4685-4
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
65615	HPLC Hb Variant, B	No LOINC Needed
METRB	Cytochrome b5 Reductase, B	32703-1
608086	Methemoglobinemia Interpretation	59465-5
608108	Reviewed By	18771-6