Test Definition: NCYB

Recessive Congenital Methemoglobinemia, CYB5 and CYB5 Reductase Genetic Analysis, Next-Generation Sequencing, Varies

Overview

Useful For

Providing a genetic evaluation for patients with a personal or family history suggestive of recessive congenital methemoglobinemia

Genotype confirmation of borderline cytochrome b5 reductase (methemoglobin reductase: METR) enzyme activity levels

Identifying variants within the CYB5 and CYB5 reductase genes (*CYB5A*, *CYB5R3*) allowing for further genetic counseling

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in the *CYB5A* and *CYB5R3* genes associated with autosomal recessive congenital methemoglobinemia. See Method Description for additional details.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, recurrence risk assessment, familial screening, and genetic counseling for congenital methemoglobinemia.

Testing Algorithm

Genetic testing for congenital methemoglobinemia is indicated if:
-There is cyanosis in the setting of clinically stable patient ("more blue than sick")
-Methemoglobin reductase (cytochrome b5 reductase) activity is decreased
-Hemoglobin electrophoresis, particularly high-performance liquid chromatography testing, is negative

Special Instructions

- Informed Consent for Genetic Testing
- Metabolic Hematology Next-Generation Sequencing (NGS) Patient Information
- Informed Consent for Genetic Testing (Spanish)
- Hereditary Erythrocytosis Gene Panel and Subpanel Comparison

Method Name

Sequence Capture and Targeted Next-Generation Sequencing (NGS) followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing.

NY State Available

Yes

Specimen

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Specimen Type

Varies

Ordering Guidance

This test should be performed after more common causes of methemoglobinemia have been eliminated. To assess for more common causes of methemoglobinemia, order MEV1 / Methemoglobinemia Evaluation, Blood.

Multiple gene panels are available. For more information see Hereditary Erythrocytosis Gene Panel and Subpanel Comparison.

Targeted testing for familial variants (also called site-specific or known variants testing) is available for the *CYB5A* and *CYB5R3* genes. See FMTT / Familial Variant, Targeted Testing, Varies. To obtain more information about this testing option, call 800-533-1710.

Additional Testing Requirements

This test is best interpreted in the context of protein functional findings by enzymatic assay and complete blood cell count analysis. This complete interpretation can be provided by also ordering the MEV1 / Methemoglobinemia Evaluation, Blood. Fill out the information sheet and indicate that a next-generation sequencing test was also ordered. Providing complete blood cell count data and clinical notes will also allow more precise interpretation of results.

Necessary Information

1. Metabolic Hematology Next-Generation Sequencing (NGS) Patient Information is strongly recommended but not required. Testing may proceed without the patient information; however, it aids in providing a more thorough interpretation. Ordering healthcare professionals are strongly encouraged to complete the form and send it with the specimen
2.If form not provided, include the following information with the test request: clinical diagnosis, pertinent clinical history (ie, complete blood cell count results and relevant clinical notes) and differentials based on any previous bone marrow studies, clinical or morphologic presentation.

Specimen Required

Patient Preparation: A previous bone marrow transplant from an allogenic donor will interfere with testing. For information about testing patients who have received a bone marrow transplant, call 800-533-1710.
Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender top (EDTA)
Acceptable: Yellow top (ACD)
Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot.**
Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated 4 days
Additional Information: To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.

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 Next-Generation Sequencing (NGS) Patient Information (T816)
3. If not ordering electronically, complete, print, and send a Benign Hematology Test Request (T755) with the specimen.

Specimen Minimum Volume

1 mL

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	Varies		

Clinical & Interpretive

Clinical Information

Methemoglobin forms when the hemoglobin (Hb) molecule contains iron in the ferric (Fe3[+]) form, which cannot carry oxygen. Methemoglobin is converted back to the functional ferrous state (Fe2[+]) by the enzyme cytochrome b5 reductase (methemoglobin reductase). Methemoglobinemia can be hereditary or acquired and is present when methemoglobin levels exceed 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 and are characterized by lifelong cyanosis. They are due either to an intrinsic structural disorder of hemoglobin, called M-Hbs (autosomal dominant inheritance)(1,2); or a deficiency of cytochrome b5 reductase (methemoglobin reductase) in erythrocytes (autosomal recessive inheritance). The hemoglobin genes, *HBA1/HBA2* and *HBB*, are not assessed in this assay.

Recessive congenital methemoglobinemia results from genetic variants in either *CYB5R3* or *CYB5A* and is described as type I, type II, or methemoglobinemia and ambiguous genitalia (previously type IV).(1-4) Methemoglobinemia type I results from alterations to *CYB5R3* with clinical impact exclusive to the red blood cells (erythrocytes) and typically presents as asymptomatic, well tolerated cyanosis. Methemoglobinemia type II also results from alterations to *CYB5R3*; however, it is due to alterations that lower activity or expression of the enzyme in all tissues. Methemoglobin type II is a more severe condition associated with cyanosis, neurological impairment, and altered lipid metabolism. Methemoglobinemia and ambiguous genitalia (previously type IV) is a very rare autosomal recessive condition caused by

deleterious alterations of the *CYB5A* gene that produce an isolated 17,20-lyase deficiency. In addition to mild to severe methemoglobinemia, individuals with this condition have been reported to have male under-masculinization/ambiguous genitalia and absent/disturbed pubertal development.

Reference Values

An interpretive report will be provided.

Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(5) 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 the Mayo Clinic Laboratories genetic counselors at 800-533-1710.

Technical Limitations:
Next-generation sequencing 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 or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is validated to detect 95% of deletions up to 75 base pairs (bp) and insertions up to 47 bp. Deletions-insertions (delins) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller delins.

Deletion/Duplication Analysis:
This analysis targets single and multi-exon deletions/duplications; however, in some instances, single exon resolution cannot be achieved due to isolated reduction in sequence coverage or inherent genomic complexity. Balanced structural rearrangements (such as translocations and inversions) may not be detected.

Deletion/duplication events that extend past the genes included on the panel may occur. In these instances, genes included in the ordered test are provided on the report and interpreted, and genomic breakpoints are reported if they are confirmed. However, copy number variants for genes not listed in the Method Description are typically not reported or interpreted for haploinsufficiency/triplosensitivity. CMACB / Chromosomal Microarray, Congenital, Blood; WESPR / Panel to Whole Exome Sequencing Reflex Test, Varies; or WGSDX / Whole Genome Sequencing for Hereditary Disorders, Varies is recommended for a full interpretation of deletions/duplications predicted to extend past the genes included on the panel.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic 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.

If the patient has had an allogeneic hematopoietic stem cell transplant or a recent blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.

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 health care 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.(5) 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. These findings will be carefully reviewed to determine whether they will be reported.

Clinical Reference

1. OMIM: 250800 Methemoglobinemia due to deficiency of methemoglobin reductase. Updated May 20, 2019. Accessed September 18, 2025. Available at www.omim.org/entry/250800?search=250800&highlight=250800
2. OMIM: 250790 Methemoglobinemia and ambiguous genitalia. Updated December 9, 2022. Accessed September 18, 2025. Available at www.omim.org/entry/250790?search=250790&highlight=250790
3. Iolascon A, Bianchi P, Andolfo I, et al. Recommendations for diagnosis and treatment of methemoglobinemia. Am J Hematol. 2021;96(12):1666-1678. doi:10.1002/ajh.26340
4. Percy MJ, Lappin TR. Recessive congenital methaemoglobinaemia: cytochrome b(5) reductase deficiency. Br J Haematol. 2008;141(3):298-308. doi:10.1111/j.1365-2141.2008.07017.x
5. Richards S, Aziz N, Bale S, et al. ACMG Laboratory Quality Assurance Committee: 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

Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing are performed to test for the presence of variants in coding regions and intron/exon boundaries of the *CYB5A* and *CYB5R3* genes, 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 30X. Sensitivity is estimated at above 99% for single nucleotide variants, above 94% for deletions-insertions (delins) less than 40 base pairs (bp), above 95% for deletions up to 75 bp and insertions up to 47 bp. NGS and/or a polymerase chain reaction based quantitative method is performed to test for the presence of deletions and duplications in the *CYB5A* and *CYB5R3* genes.

There may be regions of the genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences.(Unpublished Mayo method)

The reference transcript for *CYB5A* is NM_001914.4 and the reference transcript for *CYB5R3* is NM_000398.7. Reference transcript numbers may be updated due to transcript re-versioning. Always refer to the final patient report for gene transcript information referenced at the time of testing. Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.

PDF Report

No

Day(s) Performed

Varies

Report Available

28 to 42 days

Specimen Retention Time

Whole blood: 2 weeks (if available); 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

81479

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
NCYB	CYB5 and CYB5 Reductase, NGS	103737-3

Result ID	Test Result Name	Result LOINC® Value
618978	Test Description	62364-5
618979	Specimen	31208-2
618980	Source	31208-2
618981	Result Summary	50397-9
618982	Result	82939-0
618983	Interpretation	59465-5
618984	Additional Results	82939-0
618985	Resources	99622-3
618986	Additional Information	48767-8
618987	Method	85069-3
618988	Genes Analyzed	82939-0
618989	Disclaimer	62364-5
618990	Released By	18771-6