MAYO CLINIC LABORATORIES

Test Definition: NGCDA

CDA Sequencing, V

Overview

Useful For

Confirmation of the diagnosis or carrier mutation status of genes associated with congenital dyserythropoietic anemia
Identifying mutations within genes associated with phenotypic severity, allowing for predictive testing and further genetic counseling

Genetics Test Information

See Targeted Genes Interrogated by NGCDA Next-Generation Sequencing in Special Instructions for a list of the genes and exons targeted by this test.

Testing Algorithm

See NGHHA and Subpanel Comparison Gene List in Special Instructions.

Special Instructions

- Targeted Genes Interrogated by NGCDA Next-Generation Sequencing
- Metabolic Hematology Next-Generation Sequencing (NGS) Patient Information
- NGHHA and Subpanel Comparison Gene List

Method Name

Hereditary Mutation Detection by Next-Generation Sequencing (NGS)

NY State Available

Yes

Specimen

Specimen Type

Varies

Shipping Instructions

Peripheral blood specimens must arrive within 30 days of collection.

Necessary Information

1. Metabolic Hematology Next-Generation Sequencing (NGS) Patient Information **is required,** see Special Instructions. Testing may proceed without the patient information, however, the information aids in providing a more thorough interpretation. Ordering providers are strongly encouraged to fill out the form and send with the specimen.
2. If form not provided, include the following information with the test request: clinical diagnosis, pertinent clinical history (ie, CBC results and relevant clinical notes) and differentials based on clinical or morphologic presentation.

Specimen Required

Submit only 1 of the following specimens:
Specimen Type: Peripheral blood (preferred)
Container/Tube:
Preferred: Lavender top (EDTA) or Yellow top (ACD)

Acceptable: Green top (heparin)
Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send specimen in original tube.
3. Label specimen as blood.
Specimen Stability: Refrigerated < or =30 days
Specimen Type: Extracted DNA
Container/Tube: 1.5- to 2-mL
Specimen Volume: Entire specimen
Collection Instructions:
1. Indicate volume and concentration of the DNA.
2. Label specimen as extracted DNA and source of specimen.
Specimen Stability: Frozen/Refrigerated/Ambient < or =30 days

Forms

1. Metabolic Hematology Next-Generation Sequencing (NGS) Patient Information **is required**, see Special Instructions
2. If not ordering electronically, complete, print, and send a Benign Hematology Test Request (T755) with the specimen

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Other	Bone marrow biopsies Slides Paraffin shavings Frozen tissues Paraffin-embedded tissues Paraffin-embedded bone marrow aspirates

Specimen Minimum Volume

Blood: 1 mL; Extracted DNA: 100 mcL at 20 ng/mcL concentration

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies (preferred)		

Clinical & Interpretive

Clinical Information

Next-generation sequencing (NGS) is a methodology that can interrogate large regions of genomic DNA in a single assay. The presence and pattern of gene mutations can provide critical diagnostic, prognostic, and therapeutic information for managing physicians.

This panel aids in the diagnosis and genetic counseling of individuals with clinical or familial features of congenital dyserythropoietic anemia (CDA). CDA is a disorder of ineffective erythropoiesis clinically subdivided into subtypes with various phenotypic findings that segregate into different gene associations.(1-4) These disorders have distinctive cytopathologic findings consisting of nuclear abnormalities in bone marrow erythroid precursors. Types I and II CDA are

inherited in an autosomal recessive pattern whereas types III and IV are autosomal dominant.

Reference Values

An interpretive report will be provided.

Interpretation

Evaluation and categorization of variants is performed using the most recent published American College of Medical Genetics recommendations as a guideline.(5,6) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.
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 predictions made by these tools may change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

Cautions

Clinical:
Some individuals may have a mutation that is not identified by the methods performed. The absence of a mutation, therefore, does not eliminate the possibility of hereditary hemolytic anemia or a related disorder. This assay does not distinguish between germline and somatic alterations, particularly with variant allele frequencies (VAF) significantly lower than 50%. Test results should be interpreted in context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.
If there is a family history of hereditary hemolytic anemia or a related disorder, it is often useful to test first-degree family members to help establish the clinical significance of variants of unknown significance.
At this time, it is not standard practice for the laboratory to systematically review likely pathogenic variants or variants of uncertain significance that have been previously detected and reported. The laboratory encourages health care providers to contact the laboratory at any time to learn how the status of a particular variant may have changed over time.
Technical:
Some genetic or genomic alterations, such as large insertion/deletion (indel) events, copy number alterations and gene translocation events are not detected by this assay. The depth of coverage may be variable for some target regions, but assay performance below the minimum acceptable criteria or for failed regions will be noted. Additionally, rare polymorphisms may be present that could lead to false-negative or false-positive results. If results do not match clinical findings, consider alternative methods for analyzing these genes, such as Sanger sequencing or large deletion/duplication analysis. If the patient has had an allogenic blood transfusion, these results may be inaccurate due to the presence of donor DNA

Clinical Reference

1. Nathan and Oskiâ€™s Hematology of Infancy and Childhood. Edited by SH Orkin, DG Nathan, D Ginsburg, et al. Seventh edition. Philadelphia, Saunders Elsevier, 2009, pp 360-364
2. Iolascon A, Heimpel H, Wahlin A, Tamary H: Congenital dyserythropoietic anemias: molecular insights and diagnostic approach. Blood 2013 Sep 26;122(13):2162-2166
3. Arnaud L, Saison C, Helias V, et al. A dominant mutation in the gene encoding the erythroid transcription factor KLF1 causes a congenital dyserythropoietic anemia. Am J Hum Genet 2010 Nov 12;87(5):721-727
4. Iolascon A, Andolfo I, Barcellini W, et al: Recommendations for splenectomy in hereditary hemolytic anemias. Haematologica 2017 May 26. PMID: 28550188. doi: 10.3324/haematol.2016.161166
5. Richards S, Aziz N, Bale S, et al: 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 May;17(5):405-424

Performance

Method Description

This next-generation sequencing assay is performed to test for the presence of a mutation in targeted regions of the following 10 genes: *C15ORF41, CDAN1, GATA1, HBB, HBD, KIF23, KLF1, RPS19, SEC23B*, and *UGT1A*. See Targeted Genes Interrogated by NGCDA Next-Generation Sequencing in Special Instructions for details regarding the targeted gene regions identified by this test. This is a laboratory-developed test using Research Use Only reagents.
Next-generation sequencing (NGS) is performed using the Illumina MiSeq instrument with paired-end, 151-base pair (bp) reads. The DNA is prepared for NGS using a custom Agilent SureSelect Target Enrichment System. Data is analyzed with the CLC Genomics Server Program. Supplemental or confirmatory Sanger sequencing is performed when necessary.(Unpublished Mayo method)

PDF Report

No

Specimen Retention Time

DNA 3 months

Performing Laboratory Location

Rochester

Fees & Codes

Test Classification

This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

81364
81479