Hemolytic Anemia Panel by NGS

ABCG5	*ABCG8*	*AK1*	*ALAS2*
ALDOA	*ANK1*	*ATP11C*	*C15orf41*
CDAN1	*COL4A1*	*EPB41*	*EPB42*
G6PD	*GATA1*	*GCLC*	*GPI*
GPX1	*GSR*	*GSS*	*GYPC*
HK1	*KCNN4*	*KIF23*	*KLF1*
LPIN2	*NT5C3A*	*PFKM*	*PGK1*
PIEZO1	*PKLR*	*RHAG*	*SEC23B*
SLC2A1 (GLUT1)	*SLC4A1*	*SPTA1*	*SPTB*
TPI1	*XK*		

Description:

This panel is specifically designed to diagnose the most common genetic causes of hemolytic anemia. Hereditary hemolytic anemia (HHA) is caused by defects in the red blood cell membrane proteins, deficiencies in red blood cell enzymes, or hemoglobin disorders. Congenital dyserythropoietic anemias (CDAs) are caused by ineffective erythropoiesis and share some clinical characteristics with HHA. Hemolytic anemias are caused by variants in many different genes, and may be inherited in an autosomal dominant, autosomal recessive, or X- linked manner.

Tests Offered:

- Hemolytic Anemia 38 gene panel
- CDA 8 gene panel
- RBC Membrane Disorders 16 gene panel
- RBC Enzymopathies 14 gene panel
- Sanger sequencing of any gene on panel

Indications:

Hemolytic Anemia Panel by NGS

- Confirmation of genetic diagnosis in a patient with a clinical diagnosis of hemolytic anemia or associated syndrome
- Carrier or presymptomaic diagnosis identification in individuals with a family history of hemolytic anemia of unknown genetic basis.

Gene Specific or Sub-panel Sequencing:

- Confirmation of genetic diagnosis in a patient with hemolytic anemia and in whom a specific genetic diagnosis is suspected.

Variant Specific Analysis:

- Presymptomatic testing of at-risk siblings and parents for medical management and prior to bone marrow donation
- Carrier identification in individuals in whom specific variant(s) have been identified in the proband with hemolytic anemia
- Prenatal diagnosis of an at-risk fetus, after confirmation of variant(s) in the parent(s) and by prior arrangement only.

Congenital Dyserythropoietic Anemias

Congenital dyserythropoietic anemias (CDAs) are characterized by ineffective red blood cell production with distinct morphologic features in late bone marrow erythroblasts (dyserythropoiesis).
Symptoms of CDA include jaundice, anemia, splenomegaly, gallstones and secondary hemochromatosis. The peripheral blood smear reveals aniso-poikilocytosis and basophilic stippling.

Condition	Gene(s)	Inheritance
Sideroblastic anemia	*ALAS2*	XR
CDA1	*CDAN1, C15ORF41*	AR
CDA2	*SEC23B*	AR
CDA3	*KIF23*	AD
CDA4	*KLF1*	AD
GATA1-related cytopenia	*GATA1*	XR
Majeed syndrome	*LPIN2*	AR

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RBC Membrane Disorders

RBC membrane disorders are caused by quantitative or qualitative defects of the red cell cytoskeleton proteins and include hereditary spherocytosis (HS), elliptocytosis/ pyropoikilocytosis (HE/HPP), and stomatocytosis (HSt).

Symptoms can range from asymptomatic cases incidentally diagnosed after blood tests to severe cases presenting with hydrops fetalis which would require in utero blood transfusions.

Condition	Gene(s)	Inheritance	Associated Features
GLUT1 deficiency	*GLUT1*	AD	Seizures, intellectual disability, ataxia
Hemolytic anemia	*ATP11C*	XR	
Hereditary spherocytosis	*ANK1, SLC4A1, SPTB*	AD	
Hereditary spherocytosis	*ANK1, SPTA1, EPB42*	AR	
Hereditary elliptocytosis	*GYPC , SPTA, SPTB, EPB41*	AD	
Hereditary pyropoikilocytosis	*SPTA, SPTB, EPB41*	AR	
Hereditary stomatocytosis	*KCNN4, PIEZO1, RHAG*	AD	
Hereditary stomatocytosis	*ABCG5, ABCG8*	AR	Severe hypercholesterolemia and macrothrombocytopenia
McLeod Neuroacanthocytosis syndrome	*XK*	XR	Seizures, progressive chorea, myopathy and cardiac arrythmia
Porencephaly	*COL4A1*	AD	
Rh-null phenotype	*RHAG*	AR	Rh null blood group phenotype

RBC Enzymopathies

RBC Enzymopathies are caused by deficiencies in enzymes involved in glycolysis, the pentose phosphate pathway, or nucleotide clearance within RBCs.

Condition	Gene(s)	Inheritance	Associated Features
Adenylate kinase deficiency	*AK1, ALDOA*	AR	Exertional myopathy
G6PD deficiency	*G6PD*	XR	
Gamma-glutamylcysteine synthetase deficiency	*GCLC*	AR	
Glucose phosphate isomerase deficiency	*GPI*	AR	
Glutathione peroxidase deficiency	*GPX1*	AR	
Glutathione reductase deficiency	*GSR*	AR	
Glutathione synthetase deficiency	*GSS*	AR	5-oxoprolinuria, metabolic acidosis, CNS damage
Glycogen storage disease VII	*PFKM*	AR	Exertional myopathy
Hexokinase deficiency	*HK1*	AR	Neuropathy, Russe type
Phosphoglycerate kinase 1 deficiency	*PGK1*	XR	Myopathy, neurological involvement
Pyruvate kinase deficiency	*PKLR*	AR	
Triosephosphate isomerase deficiency	*TPI1*	AR	Myopathy
UMPH1 deficiency	*NT5C3A*	AR	Learning difficulties

Specimen:

At least 3 mLs whole blood in a lavender top (EDTA) tube.

Note: For post-transplant patients, we accept pre-transplant samples or post-transplant skin fibroblasts ONLY (blood, saliva, and cytobrushes are not accepted). Culturing of skin fibroblasts is done at an additional charge. **We are unable to accept blood samples that are collected within two (2) weeks of a transfusion.**

Testing Methodology:

Hemolytic Anemia Panel by NGS: This test is performed by enrichment of the coding exons, flanking intronic and untranslated regions (5' and 3') , as well as known pathogenic variants (HGMD 2017.3) in the promoter and deep intronic regions of the genes specified above using oligonucleotide probe hybridization followed by next-generation sequencing with >50X coverage at every target base. All pathogenic and novel variants, as well as variants of unknown (indeterminate) significance, as determined bioinformatically, are confirmed by Sanger sequencing.

Gene Specific Sequencing/ Variant Specific Analysis: Sanger sequencing following PCR amplification of the specified coding and exon/intron boundaries of the specified gene.

Sensitivities:

Clinical Sensitivity: The next generation sequencing panel detects 70-99% of the reported variants in these genes using this testing methodology. Many genes on this panel result in rare or overlapping phenotypes, and the clinical sensitivity of gene sequencing has not been determined. The clinical sensitivity of single gene testing is dependent on the test ordered. Large exonic deletions, duplications, or insertions have been reported in several of these genes. Deletion/duplication analysis may be indicated as a follow-up test in patients with a single variant in one of these genes, or in patients with normal Hemolytic Anemia Panel analysis.

Analytical Sensitivity: The sensitivity of DNA sequencing is over 98% for the detection of nucleotide base changes, small deletions and insertions in the regions analyzed.

Limitations: Variants in regulatory regions and non-reported variants in untranslated regions are not detected by this test. Large deletions involving entire single exons or multiple exons, large insertions and other complex genetic events have been reported in many of these genes and will not be identified using this test methodology. Rare primer site variants may lead to erroneous results.

Note: Single gene sequencing is available for all genes on the panel. Deletion/duplication analysis is available for all genes listed for an additional charge.

Turn-Around Time:

42 days for the next generation sequencing panel and up to 42 days for single gene sequencing.

CPT Codes:

- **Hemolytic Anemia 38 gene panel:** 81443
- **RBC Membrane Disorders 16 gene panel:** 81408
- **RBC Enzymopathies 14 gene panel:** 81479x3
- **CDA 8 gene panel:** 81479x3
- **Single gene testing of any gene on panel (except *COL4A1, SLC2A1 (GLUT1)):*** 81479
- **Single gene testing of *COL4A1:*** 81408
- **Single gene testing of *SLC2A1 (GLUT1):*** 81405
- **Deletion/Duplication analysis:** call for information

Please call 1-866-450-4198 for current pricing, insurance preauthorization or with any billing questions.

Results:

Results will be reported to the referring physician or health care provider as specified on the requisition form.

Shipping Instructions:

Please enclose **test requisition** with sample.
All information must be completed before sample can be processed.

Place samples in styrofoam mailer and ship at room temperature by overnight Federal Express to arrive Monday through Saturday.

Ship to:

Genetics and Genomics Diagnostic Laboratory
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

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