Hemolytic and Dyserythropoietic Anemia Genetic Testing

Genes Tested

RBC Membrane Disorders

ANK1	*EPB41*	*EPB42*	*PIEZO1*
RHAG	*SLC2A1*	*SLC4A1*	*SPTA1*
SPTB	*XK*		

RBC Enzymopathies

AK1	*ALDOA*	*G6PD*	*GCLC*
GPI	*GPX1*	*GSR*	*GSS*
HK1	*NT5C3A*	*PFKM*	*PGK1*
PKLR	*TPI1*		

Congenital Dyserythropoietic Anemia

CDAN1	*GATA1*	*KIF23*
KLF1	*SEC23B*	

Description:

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.

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.

- **Hereditary spherocytosis** is the most common RBC membrane disorder and is characterized by sphere-shaped red blood cells. This condition is inherited in an autosomal dominant manner in 75% of cases, where mutations of ankyrin *(ANK1),* band 3 *(SLC4A1),* and β-spectrin *(SPTB)* predominate. Recessive HS is usually caused by homozygous or compound heterozygous mutations in ankyrin *(ANK1)*, α-spectrin *(SPTA1),* or protein 4.2 *(EPB42).*
- **Hereditary elliptocytosis** is characterized by elliptically shaped red blood cells and is caused by mutations in *SPTA1, SPTB,* and protein 4.1 *(EPB41).* HE is caused by heterozygous mutations in these genes and is autosomal dominant while HPP is caused by homozygous or compound heterozygous mutations in these genes and is autosomal recessive.
- **Hereditary stomatocytosis** syndromes include xerocytosis/dehydrated hereditary stomatocytosis (DHS), which is caused by mutations in the *PIEZO1* gene, and overhydrated hereditary stomatocytosis (OHS), which is caused by mutations in the *RHAG* gene. Both are autosomal dominant and symptoms can range from asymptomatic to severe hemolytic anemia. Splenectomy is contraindicated in these patients because it predisposes to life-threatening thrombotic events.

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- **Rare RBC membrane defects**
  - **Rh-null phenotype** presents with hemolytic anemia with spherocytosis and stomatocytosis. *RhAG* mutations cause 80% of the cases (regulator type) and are inherited in an autosomal recessive manner.
  - **GLUT1 deficiency** can be caused by *SLC2A1* mutations and can present as classic GLUT1 deficiency with seizures, developmental delay, dysarthria, microcephaly, movement disorder (dyskinesia) or as non-epileptic GLUT1 deficiency, with intermittent ataxia, choreoathetosis, dystonia, and hemiplegia, both with or without hemolytic anemia with echinocytosis and altered erythrocyte ion concentrations. These conditions are inherited in an autosomal dominant manner. Sequencing of *SLC2A1* is expected to identify mutations in 81-89% of individuals with low glucose in cerebrospinal fluid. The remaining patients with GLUT1 deficiency have large or whole gene deletions, which are not detected by this test.
  - **McLeod Neuroacanthocytosis Syndrome (MLS)** is characterized by a specific blood group phenotype causing red blood cell acanthocytosis and compensated hemolysis. Symptoms may also include progressive chorea, seizures, sensorimotor axonopathy, neurogenic muscle atrophy, myopathy, dilated cardiomyopathy, and arrhythmias. This condition is inherited in an X linked manner and is caused by mutations in the *XK* gene which codes for the Kell blood group precursor substance Kx. *XK* sequencing is expected to identify mutations in greater than 95% of patients with MLS.

RBC Enzymopathies are caused by deficiencies in enzymes involved in glycolysis, the pentose phosphate pathway, or nucleotide clearance within RBCs. They are most commonly inherited in an autosomal recessive manner. G6PD deficiency and PGK1 deficiency are X-linked conditions. Patients primarily have symptoms of hemolytic anemia, but mutations in some genes have been also associated with other symptoms:

- *ALDOA* - Exertional myopathy
- *GSS* - 5-oxoprolinuria, metabolic acidosis, CNS damage
- *NT5C3A* - Learning difficulties
- *PFKM* - Exertional myopathy
- *PGK1* - Myopathy, neurological involvement
- *TPI1* - Myopathy

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.

- **CDA type I** is caused by autosomal recessive mutations in the *CDAN1* gene. This condition can be severe, presenting as hydrops fetalis. Patients may also have distal limb anomalies. Bone marrow examination reveals up to 10% binuclear erythroblasts with chromatin bridges between nuclei.
- **CDA type II** is the most common of the CDAs. Bone marrow aspirate shows more than 10% mature binuclear erythroblasts. This condition is caused by autosomal recessive mutations in the *SEC23B* gene.
- **CDA type III** is the rarest form of CDA and is caused by autosomal dominant mutations in the *KIF23* gene.

- **CDA type IV** is a rare CDA caused by autosomal dominant mutations in the *KLF1* gene.
- ***GATA1* related cytopenia** is characterized by thrombocytopenia and/or anemia with one or more of the following symptoms: playtelet dysfunction, mild beta thalassemia, neutropenia, and congenital erythropoietic porphyria. Symptoms can range from mild to severe, including hydrops fetalis. Female carriers may have mild to moderate symptoms. *GATA1* related cytopenia is inherited in an X-linked manner.

Test Offerings:

All 29 genes are included on a comprehensive next-generation sequencing panel:

- Hemolytic Anemia 29 gene panel

Sub-panels are available for specific conditions:

- RBC Membrane Disorders 10 gene panel
- RBC Enzymopathies 14 gene panel
- CDA 5 gene panel

Single gene sequencing is available for all genes on the panel.

Indications:

- Hereditary Anemia, isolated or with associated symptoms of hemolysis (jaundice, splenomegaly, gallstones) or along with symptoms of myopathy and neurological involvement
- Pregnancy with unexplained hydrops fetalis
- Family history of a hemolytic anemia syndrome.

Specimen:

Next Generation Sequencing Panel: At least 3 mLs whole blood in a lavender top (EDTA) tube.

Single Gene Sequencing: At least 3 mLs whole blood in a lavender top (EDTA) tube.

Label tube with patient's name, birth date, and date of collection.

Testing Methodology:

Next Generation Sequencing Panel: This test is performed by enrichment of the exons, flanking intronic and un-translated regions (5' and 3') of the genes specified above using oligonucleotide probe hybridization followed by next-generation sequencing with > 20 fold 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.

Single Gene Sequencing: Sanger sequencing following PCR amplification of the coding and exon/intron boundaries of the gene.

Sensitivity:

Clinical Sensitivity: The next generation sequencing panel detects 70-99% of the reported mutations 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 mutation 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: Mutations in regulatory regions and non-reported mutations 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.

Turn-Around Time: Four-eight weeks for the next generation sequencing panel or single gene sequencing.

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

CPT Codes:

Hemolytic Anemia 29 gene panel	81405, 81479x28
RBC Membrane Disorders 10 gene panel	81405, 81479x9
RBC Enzymopathies 14 gene panel	81479x14
CDA 5 gene panel	81479x5

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 Friday.

Ship to:

Cytogenetics and Molecular Genetics Laboratories
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

References:

Ciovacco, Wendy A, Wendy H Raskind, and Melissa A Kacena. "Human Phenotypes Associated with GATA-1 Mutations." Gene 427 (1–2): 1–6.

Da Costa, L., J. Galimand, O. Fenneteau and N. Mohandas (2013). "Hereditary spherocytosis, elliptocytosis, and other red cell membrane disorders." Blood Rev.

Iolascon, A., H. Heimpel, A. Wahlin, and H. Tamary (2013). "Congenital dyserythropoietic anemias: molecular insights and diagnostic approach." Blood 122(13): 2162-2166.

Jacobasch, G. and S. M. Rapoport (1996). "Hemolytic anemias due to erythrocyte enzyme deficiencies." Mol Aspects Med 17(2): 143-170.

Jung, H. H., A. Danek, R. H. Walker, B. M. Frey and C. Gassner (1993). McLeod Neuroacanthocytosis Syndrome. GeneReviews. R. A. Pagon, M. P. Adam, T. D. Bird et al. Seattle WA, University of Washington, Seattle.

Kacena, Melissa A, Stella T Chou, Mitchell J Weiss, and Wendy H Raskind. "GATA1-Related X-Linked Cytopenia." In GeneReviews(®), edited by Roberta A Pagon, Margaret P Adam, Thomas D Bird, Cynthia R Dolan, Chin-To Fong, Richard JH Smith, and Karen Stephens. Seattle (WA): University of Washington, Seattle, 1993.

Liljeholm, Maria, Andrew F Irvine, Ann-Louise Vikberg, Anna Norberg, Stacy Month, Herbert Sandström, Anders Wahlin, Masanori Mishima, and Irina Golovleva. "Congenital Dyserythropoietic Anemia Type III (CDA III) Is Caused by a Mutation in Kinesin Family Member, KIF23." Blood 121 (23): 4791–99.

Nichols, K E, J D Crispino, M Poncz, J G White, S H Orkin, J M Maris, and M J Weiss. "Familial Dyserythropoietic Anaemia and Thrombocytopenia due to an Inherited Mutation in GATA1." Nature Genetics 24(3): 266–70.

Noris, Patrizia, Alessandro Pecci, Filomena Di Bari, Maria Teresa Di Stazio, Michele Di Pumpo, Iride F Ceresa, Nicoletta Arezzi, Chiara Ambaglio, Anna Savoia, and Carlo L Balduini. "Application of a Diagnostic Algorithm for Inherited Thrombocytopenias to 46 Consecutive Patients." Haematologica 89 (10): 1219–25.

Sandström, H, and A Wahlin. "Congenital Dyserythropoietic Anemia Type III." Haematologica 85 (7): 753–57.

Tamary, H. and O. Dgany (1993). Congenital Dyserythropoietic Anemia Type I. GeneReviews. R. A. Pagon, M. P. Adam, T. D. Bird et al. Seattle WA, University of Washington, Seattle.

Wang, D., J. M. Pascual and D. De Vivo (1993). Glucose Transporter Type 1 Deficiency Syndrome. GeneReviews. R. A. Pagon, M. P. Adam, T. D. Bird et al. Seattle WA, University of Washington, Seattle.