Hereditary Hemolytic Anemia Panel, Sequencing

Hereditary hemolytic anemia (HHA) is characterized by premature red blood cell (RBC) destruction and anemia due to intrinsic RBC defects, and encompasses a diverse group of heterogeneous disorders. Genetic testing is indicated when initial test results do not explain clinical presentation or mode of inheritance.

Featured ARUP Testing

Hereditary Hemolytic Anemia Panel Sequencing 2012052

Method: Massively Parallel Sequencing

Use to determine etiology, elicit inheritance pattern, and assess recurrence risk in individuals with:

- Unexplained hemolytic anemia
- Unexplained hyperbilirubinemia (neonates)
- Family history of unexplained hemolytic anemia
- Pregnancy with hydrops fetalis of unknown etiology

If a familial sequence variant has been previously identified, targeted sequencing for that variant may be appropriate; refer to the Laboratory Test Directory for additional information.

Disease Overview

Clinical presentation is highly variable and may include anemia, fatigue, gallstones, hyperbilirubinemia/jaundice, pallor, scleral icterus, and splenomegaly. Laboratory findings include decreased hemoglobin concentration, hematocrit, and RBC count. Blood smear abnormalities such as spherocytes, acanthocytes, schistocytes, bite cells, stomatocytes, polychromasia, and target cells may be present.

Associated Disorders

RBC Membrane Disorders

Disorders characterized by weakened RBC membrane, resulting in irregular shape, increased fragility, and hemolysis:

- Hereditary spherocytosis (HS): *ANK1*, *EPB42*, *SLC4A1*, *SPTA1,* and *SPTB*
  - The most common RBC membrane disorder and is characterized by spherically shaped RBCs with decreased deformability
  - Approximately 75% of HS is autosomal dominant and 25% is autosomal recessive or de novo
- Hereditary elliptocytosis and hereditary pyropoikilocytosis (HE/HPP) are related disorders with a wide spectrum of clinical phenotypes.
  - HE is characterized by elliptically shaped RBCs.
  - Marked anisopoikilocytosis with elliptocytes, microspherocytes, and bizarrely shaped RBCs are characteristically seen in HPP.
  - HE is autosomal dominant and HPP is autosomal recessive. Variants in *SPTA1*, *SPTB*, and, less commonly, *EPB41* are causative.
  - Individuals with HE are generally asymptomatic but can have mild compensated hemolytic anemia.
  - HPP patients usually present with moderate to severe anemia.
- Dehydrated hereditary stomatocytosis (xerocytosis) is characterized by decreased intracellular potassium content, loss of cell water, increased cytoplasmic viscosity, and increased mean cell hemoglobin concentration.
  - Inheritance is autosomal dominant and the most commonly involved gene is *PIEZO1.*
  - Splenectomy should be avoided in patients with some forms of hereditary stomatocytosis as it may predispose the patient to life-threatening thrombotic events.

RBC Enzymopathies

More than 20 recognized disorders caused by deficiencies of enzymes involved with glycolysis, hexose monophosphate shunt, glutathione metabolism, and nucleotide metabolism:

- Common forms:
  - G6PD deficiency (*G6PD*)
  - Pyruvate kinase deficiency (*PKLR*)
  - Pyrimidine 5′-nucleotidase (*NT5C3A*)
- Associated findings:
  - Usually normocytic normochromic hemolytic anemia with no specific abnormalities of RBC morphology
  - Severity of hemolysis variable and may be a result of an external stressor (eg, infection, administration of drugs, or ingestion of some foods)
  - Nonhematological manifestations may include:
    - Myopathy
    - Neurological dysfunction
    - Intellectual disability

Hemoglobinopathies

Quantitative defect in biosynthesis of one type of hemoglobin (Hb) chain or a structurally abnormal Hb:

- Alpha or beta thalassemia results from a quantitative defect in the synthesis of either the alpha- or beta-globin chain.
  - The unpaired subunits precipitate, bind to the RBC membrane, and lead to hemolysis.
- Structural Hb variants result from a structurally abnormal Hb that may polymerize, precipitate, or crystalize within the RBC, leading to membrane changes and hemolysis.
- Next generation sequencing (NGS) analysis is complex in these disorders due to the high level of gene homology, copy number variations, and duplication within the alpha and beta globin operons. These disorders are not included as part of the current testing panel.

Prevalence

HHA disorders: 1/500-1,100

- HS: 1/2,000 northern Europeans
- HE/HPP: 1/2,000-4,000 worldwide
- G6PD deficiency: 400 million worldwide
  - Varies by ethnicity: 7/10 Kurdish Jewish males, 1/6-10 African American males, 1/7-9 Arabic males, 1/6-16 Southeast Asian males
- Pyruvate kinase deficiency: 1/20,000 Europeans

Inheritance

Dependent on gene: autosomal recessive, autosomal dominant, or X-linked

Test Description

Refer to Genes Tested table for genes included in the panel.

Clinical Sensitivity

Variable and dependent on phenotype/condition

Testing Strategy

Refer to ARUP Consult's Hemolytic Anemias Testing Algorithm.

- Molecular testing for hemolytic anemia is indicated when initial test results do not explain clinical presentation or mode of inheritance.

Limitations

- A negative result does not exclude a heritable form of hemolytic anemia.
- Diagnostic errors can occur due to rare sequence variations.
- Interpretation of this test result may be impacted if this individual has had an allogeneic stem cell transplantation or recently received a blood transfusion.
- The following will not be evaluated:
  - Variants outside the coding regions and intron-exon boundaries of the targeted genes
  - Regulatory region variants and deep intronic variants
  - Large deletions/duplications
  - Genes of the alpha- and beta-globin clusters
  - Noncoding transcripts
- The following may not be detected:
  - Deletions/duplications/insertions of any size by massively parallel sequencing
  - Some variants due to technical limitations in the presence of pseudogenes, repetitive, or homologous regions
  - Low-level somatic variants

Analytic Sensitivity

Variant Class	Analytic Sensitivity (PPA) Estimate[a] (%)	Analytic Sensitivity (PPA) 95% Credibility Region[a] (%)
SNVs	99.2	96.9-99.4
Deletions 1-10 bp	93.8	84.3-98.2
Deletions 11-44 bp	100	87.8-100
Insertions 1-10 bp	94.8	86.8-98.5
Insertions 11-23 bp	100	62.1-100

[a]Genes included on this test are a subset of a larger methods-based validation from which the PPA values are derived.

bp, base pairs; PPA, positive percent agreement; SNVs, single nucleotide variants

Genes Tested

Gene	MIM Number	Disorder	Inheritance
AK1	103000	Hemolytic anemia due to adenylate kinase deficiency	AR
ALDOA	103850	Glycogen storage disease XII	AR
ANK1	612641	Spherocytosis, type 1	AD
CDAN1	607465	Anemia, congenital dyserythropoietic, type IA	AR
CYB5R3	613213	Methemoglobinemia due to deficiency of methemoglobin reductase	AR
EPB41	130500	Elliptocytosis 1	AD
		Elliptocytosis 1	AR
EPB42	177070	Spherocytosis, type 5	AR
G6PD	305900	Nonspherocytic hemolytic anemia due to G6PD deficiency	XL
GCLC	606857	Hemolytic anemia due to gamma-glutamylcysteine synthetase deficiency	AR
GPI	172400	Nonspherocytic hemolytic anemia due to glucose phosphate isomerase deficiency	AR
GSR	138300	Hemolytic anemia due to glutathione reductase deficiency	AR
GSS	601002	Hemolytic anemia due to glutathione synthetase deficiency	AR
HK1	142600	Nonspherocytic Hemolytic Anemia due to Hexokinase Deficiency	AR
NT5C3A	606224	Hemolytic Anemia due to uridine 5-prime monophosphate hydrolase deficiency	AR
PFKM	610681	Glycogen storage disease VII	AR
PGK1	311800	Phosphoglycerate kinase 1 deficiency	XL
PIEZO1	611184	Dehydrated hereditary stomatocytosis with or without pseudohyperkalemia and/or perinatal edema	AD
PKLR	609712	Pyruvate kinase deficiency	AR
SEC23B	610512	Congenital dyserythropoietic anemia type II	AR
SLC4A1	109270	Spherocytosis, type 4 Ovalocytosis, southeast Asian type	AD

AD, autosomal dominant; AR, autosomal recessive; XL, X-linked

Gene	MIM Number	Disorder	Inheritance
		Cryohydrocytosis	
SLCO1B1	604843	Hyperbilirubinemia, rotor type	AR (digenic)
SLCO1B3	605495	Hyperbilirubinemia, rotor type	AR (digenic)
SPTA1	182860	Elliptocytosis 2	AD
		Pyropoikilocytosis Spherocytosis, type 3	AR
SPTB	182870	Spherocytosis, type 2 Elliptocytosis 3	AD
TPI1	190450	Hemolytic anemia due to triosephosphate isomerase deficiency	AR
UGT1A1	191740	Gilbert syndrome Crigler-Najjar syndrome, types I and II Hyperbilirubinemia, transient familial neonatal	AR
UGT1A6	606431		
UGT1A7	606432		

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Related Information

Hemolytic Anemias
Hemolytic Anemias Testing Algorithm

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