

## 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.

### 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.

### Tests to Consider

#### [Hereditary Hemolytic Anemia Panel Sequencing 2012052](#)

**Method:** Massively Parallel Sequencing

#### Indications for testing:

Determine etiology, elicit inheritance pattern, and assess recurrence risk in individuals with:

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

#### [Familial Mutation, Targeted Sequencing 2001961](#)

**Method:** Polymerase Chain Reaction/Sequencing

- Recommended test for a known familial sequence variant previously identified in a family member.
- A copy of the family member's test result documenting the familial variant is required.

See [Related Tests](#)

## 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 (PK)
  - 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 crystallize 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

- Hereditary spherocytosis – 1/2,000 northern Europeans
- Hereditary elliptocytosis/HPP – 1,2000-1,4000 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

See [Genes Tested](#) table for genes included in the panel.

# Clinical Sensitivity

Variable and dependent on phenotype/condition

## Testing Strategy

See 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

## Analytical Sensitivity

| Variant Class       | Analytical Sensitivity (PPA) Estimate <sup>a</sup> (%) | Analytical Sensitivity (PPA) 95% Credibility Region <sup>a</sup> (%) |
|---------------------|--|--|
| 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   |

<sup>a</sup>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 |
|------|------------|----------|-------------|
|------|------------|----------|-------------|

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

| Gene           | MIM Number | Disorder   | Inheritance     |
|----------------|------------|--|-----------------|
| <i>AK1</i>     | 103000     | Hemolytic anemia due to adenylate kinase deficiency  | AR              |
| <i>ALDOA</i>   | 103850     | Glycogen storage disease XII   | AR              |
| <i>ANK1</i>    | 612641     | Spherocytosis, type 1  | AD              |
| <i>CDAN1</i>   | 607465     | Anemia, congenital dyserythropoietic, type IA  | AR              |
| <i>CYB5R3</i>  | 613213     | Methemoglobinemia due to deficiency of methemoglobin reductase                                 | AR              |
| <i>EPB41</i>   | 130500     | Elliptocytosis 1   | AD              |
|                |            | Elliptocytosis 1   | AR              |
| <i>EPB42</i>   | 177070     | Spherocytosis, type 5  | AR              |
| <i>G6PD</i>    | 305900     | Nonspherocytic hemolytic anemia due to G6PD deficiency   | XL              |
| <i>GCLC</i>    | 606857     | Hemolytic anemia due to gamma-glutamylcysteine synthetase deficiency                           | AR              |
| <i>GPI</i>     | 172400     | Nonspherocytic hemolytic anemia due to glucose phosphate isomerase deficiency                  | AR              |
| <i>GSR</i>     | 138300     | Hemolytic anemia due to glutathione reductase deficiency                                       | AR              |
| <i>GSS</i>     | 601002     | Hemolytic anemia due to glutathione synthetase deficiency                                      | AR              |
| <i>HK1</i>     | 142600     | Nonspherocytic Hemolytic Anemia due to Hexokinase Deficiency                                   | AR              |
| <i>NT5C3A</i>  | 606224     | Hemolytic Anemia due to uridine 5-prime monophosphate hydrolase deficiency                     | AR              |
| <i>PFKM</i>    | 610681     | Glycogen storage disease VII   | AR              |
| <i>PGK1</i>    | 311800     | Phosphoglycerate kinase 1 deficiency   | XL              |
| <i>PIEZO1</i>  | 611184     | Dehydrated hereditary stomatocytosis with or without pseudohyperkalemia and/or perinatal edema | AD              |
| <i>PKLR</i>    | 609712     | Pyruvate kinase deficiency   | AR              |
| <i>SEC23B</i>  | 610512     | Congenital dyserythropoietic anemia type II  | AR              |
| <i>SLC4A1</i>  | 109270     | Spherocytosis, type 4<br>Ovalocytosis, southeast Asian type<br>Cryohydrocytosis                | AD              |
| <i>SLCO1B1</i> | 604843     | Hyperbilirubinemia, rotor type   | AR<br>(digenic) |

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

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

## References

1. Bolton-Maggs PHB, Langer JC, Iolascon A, et al. [Guidelines for the diagnosis and management of hereditary spherocytosis-2011 update](#) Br J Haematol. 2012; 156 (1): 37-49. PubMed
2. Gallagher PG. [Abnormalities of the erythrocyte membrane](#) Pediatr Clin North Am. 2013; 60 (6): 1349-62. PubMed
3. Koralkova P, van Solinge WW, van Wijk R. [Rare hereditary red blood cell enzymopathies associated with hemolytic anemia - pathophysiology, clinical aspects, and laboratory diagnosis.](#) Int J Lab Hematol. 2014; 36 (3): 388-97. PubMed

## Related Information

[Hemolytic Anemias](#)  
[Hemolytic Anemias Testing Algorithm](#)

## Related Tests

[Osmotic Fragility, Erythrocyte 2002257](#)

**Method:** Spectrophotometry

[RBC Band 3 Protein Reduction in Hereditary Spherocytosis 2008460](#)

**Method:** Qualitative Flow Cytometry

[Glucose-6-Phosphate Dehydrogenase 0080135](#)

**Method:** Quantitative Enzymatic

[Glucose-6-Phosphate Dehydrogenase Deficiency \(G6PD\) Sequencing 2007163](#)

**Method:** Polymerase Chain Reaction/Sequencing

## Glucose-6-Phosphate Dehydrogenase (G6PD) 2 Mutations 0051684

**Method:** Polymerase Chain Reaction/TaqMAN

## Pyruvate Kinase 0080290

**Method:** Quantitative Enzymatic

## Hemoglobin Evaluation Reflexive Cascade 2005792

**Method:** High Performance Liquid Chromatography/Electrophoresis/RBC Solubility/Polymerase Chain Reaction/Fluorescence Resonance Energy Transfer/Sequencing

## Alpha Globin (HBA1 and HBA2) Sequencing and Deletion/Duplication 2011708

**Method:** Polymerase Chain Reaction/Sequencing./Multiplex Ligation-dependent Probe Amplification.

## Beta Globin (HBB) Sequencing and Deletion/Duplication 2010117

**Method:** Polymerase Chain Reaction/Sequencing/Multiplex Ligation-dependent Probe Amplification

ARUP Laboratories is a nonprofit enterprise of the University of Utah and its Department of Pathology. 500 Chipeta Way, Salt Lake City, UT 84108 | (800) 522-2787 | (801) 583-2787 | aruplab.com | arupconsult.com  
Content Review January 2019 | Last Update January 2020

©2020 ARUP Laboratories. All Rights Reserved.



This site complies with the [HONcode standard for trustworthy health information](#). [Verify here.](#)