Hereditary Hemolytic Anemia Panel, Sequencing

**Indications for Ordering**

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

**Test Description**

- Targeted capture of all coding regions and intron/exon boundaries followed by massively parallel sequencing
- Sanger sequencing
  - Provides data for bases with insufficient coverage by massively parallel sequencing

**Tests to Consider**

**Typical testing strategy**

See ARUP Consult’s [Hemolytic Anemias Testing algorithm](#)

**Primary test**

[Hereditary Hemolytic Anemia Panel Sequencing 2012052](#)
- Confirm etiology of hemolytic anemia in individuals with hemolysis or a family history of hemolytic anemia

**Related tests**

- **Osmotic Fragility, Erythrocyte 2002257**
  - Functional testing of red blood cell (RBC) sensitivity to osmotic stress
  - Do not use to distinguish between spherocytes in hereditary spherocytosis and acquired autoimmune hemolytic anemia

- **RBC Band 3 Protein Reduction in Hereditary Spherocytosis 2008460**
  - Use to confirm diagnosis of hereditary spherocytosis when hemolytic anemia and spherocytes are present

- **Glucose-6-Phosphate Dehydrogenase (G6PD) 2 Mutations 0051684**
  - Preferred genetic test for individuals of African descent
  - Detects the single most common pathogenic G6PD variant (A- allele) in individuals of African descent

- **Pyruvate Kinase 0080290**
  - Preferred initial test to screen for pyruvate kinase deficiency

- **Hemoglobin Evaluation Reflexive Cascade 2005792**
  - Optimal test for the initial and confirmatory diagnosis of any suspected hemoglobinopathy

**Disease Overview**

**Prevalence**

Hereditary hemolytic anemia (HHA) disorders – 1/500-1,100

**Symptoms**

Highly variable clinical presentation
- Anemia
- Fatigue
- Gallstones
- Hyperbilirubinemia/jaundice
- Pallor
- Scleral icterus
- Splenomegaly

**Physiology**

HHA encompasses a diverse group of heterogeneous disorders
- Characterized by premature RBC destruction and anemia due to intrinsic RBC defects

**RBC membrane disorders**
- RBC membrane weakens, resulting in irregular shape, increased fragility, and hemolysis
- Most common types of RBC membrane disorders
  - Hereditary spherocytosis
  - Hereditary elliptocytosis or pyropoikilocytosis
  - Dehydrated hereditary stomatocytosis (xerocytosis)
Hereditary spherocytosis (HS)
• Inheritance
  o ~75% autosomal dominant
  o ~25% autosomal recessive or de novo
• Most common RBC membrane disorder
  o 1/2,000 northern Europeans
• Characterized by spherically shaped RBCs with decreased deformability
• RBC membrane proteins
  o Ankyrin
  o Band 3
  o α-spectrin
  o β-spectrin
  o Protein 4.2

Hereditary elliptocytosis or pyropoikilocytosis (HE/HPP)
• Inheritance
  o HE – autosomal dominant
  o HPP – autosomal recessive
• Prevalence of HE – 1/2,000-4,000 worldwide
  o Higher in individuals of African or Mediterranean descent
• Characterized by elliptically shaped RBCs on peripheral blood smear
• Defects in RBC membrane proteins α- and β-spectrin and less commonly protein 4.1
• Patients with HE are generally asymptomatic
• 10% have moderate to severe anemia

Dehydrated hereditary stomatocytosis (xerocytosis)
• Inheritance
  o Autosomal dominant
• Characterized by
  o Decreased intracellular potassium content
  o Loss of cell water
  o Increased cytoplasmic viscosity
  o Increased mean cell hemoglobin concentration
  o PIEZO1 is most commonly involved gene

RBC enzymopathies
• Inheritance
  o Most disorders are autosomal recessive
  o X-linked forms
    ▪ G6PD deficiency
    ▪ Phosphoglycerate kinase 1 (PGK1) deficiency
• Deficiencies of enzymes involved with
  o Glycolysis
  o Hexose monophosphate shunt
  o Glutathione metabolism
  o Nucleotide metabolism

• >20 disorders recognized
  o Common RBC enzymopathies
    ▪ G6PD deficiency
    ▪ Pyruvate kinase deficiency
  o Usually associated with normocytic normochromic hemolytic anemia with no specific abnormalities of RBC morphology
  o Severity of hemolysis is variable
    ▪ May be a result of an external stressor (eg, infection, administration of drugs, or ingestion of some foods)
  o Nonhematological manifestations possible (eg, myopathy, neurological dysfunction, intellectual disability)
  o Diagnosis based on detection of reduced specific enzyme activity and/or molecular testing to identify causative variant(s)

Hemoglobinopathies
• Quantitative defect in biosynthesis of one type of hemoglobin (Hb) chain or a structurally abnormal Hb
  o Alpha or beta thalassemia
    ▪ Quantitative defect in the synthesis of either the alpha- or beta-globin chain
    ▪ Unpaired subunits precipitate, bind to the RBC membrane, and lead to hemolysis
  o Structural Hb variants
    ▪ Result from a structurally abnormal Hb that may polymerize, precipitate, or crystalize within the RBC
    ▪ Leads to membrane changes and hemolysis
    ▪ Sickle cell diseases
    ▪ Unstable Hb variants

Diagnostic issues
Molecular testing for hemolytic anemia is indicated when initial test results do not explain
• Clinical outcome
• Mode of inheritance

Treatment issues
Splenectomy should be avoided in patients with some forms of hereditary stomatocytosis
• May predispose patient to life-threatening thrombotic events

Genetics

Genes tested – see table

Test Interpretation

Clinical sensitivity – unknown
Results

- Positive
  - One or more pathogenic variants detected
    - Autosomal dominant genes
    - One pathogenic variant predicts an HHA disorder
    - Autosomal recessive genes
    - One pathogenic variant predicts carrier status for an HHA disorder
    - Two pathogenic variants on opposite chromosomes predict an HHA disorder
    - X-linked genes
      - In females, one pathogenic variant predicts carrier status
      - In males, one pathogenic variant predicts an HHA disorder
- Negative
  - No pathogenic variants associated with an HHA disorder identified
    - Does not exclude a diagnosis of HHA
- Inconclusive
  - Variants of unknown clinical significance identified

Limitations

- Not detected
  - Variants in genes not tested
    - Alpha- and beta-globin genes are not analyzed due to high level of gene homology and frequency of large deletions
  - Large exonic deletions/duplications
  - Deep intronic or regulatory region variants
  - Small deletions or insertions may not be detected
  - Diagnostic errors can occur due to rare sequence variation
  - Presence of a highly homologous pseudogene may interfere with variant detection in PGK1

References


<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Description</th>
<th>NM #</th>
<th>OMIM #</th>
<th>Inh.</th>
<th>Associated Disorders</th>
<th>Other Associated Symptoms</th>
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AD, autosomal dominant; AR, autosomal recessive; Inh, inheritance; XL, X-linked