RhD Antigen (RHD) Genotyping

Indications for Ordering

- Determine paternal RHD gene copy number (heterozygous or homozygous) in phenotypically positive individual when reproductive partner has clinically significant alloantibody
  - Include father’s ethnicity and RhD phenotyping result with order
  - If father of pregnancy is homozygous for RHD allele, all of his offspring can be assumed to be RhD positive, negating the need for fetal RHD testing
- Determine fetal RHD gene copy number when mother has clinically significant alloantibody AND father is either heterozygous for RHD or unavailable for testing
  - Include father’s ethnicity and RhD phenotyping result with order

Contraindication for Ordering

This test does not identify the presence of weak or partial RHD genotypes

Test Description

Polymerase chain reaction followed by fluorescence monitoring

- RHD gene exons 5, 7, and a 37-base-pair insertion in the intron 3/exon 4 boundary
- Allelic height ratios to determine the number of copies of the RHD gene as compared to the RHCE gene

Tests to Consider

Primary test
RhD Gene (RHD) Copy Number 0051368
  - Determine the number of copies of the RHD gene

Related tests
  - Antigen Testing, Rh Phenotype 0013019
  - Antigen testing for D, C, E, c, e to assess maternal, paternal, or newborn Rh phenotype status

Assess risk for alloimmune hemolytic disease of the newborn (HDN) due to RHCE gene-related alloimmunization

- RhCc Antigen (RHCE) Genotyping 0050421
- RhEe Antigen (RHCE) Genotyping 0050423

Disease Overview

- HDN
- Caused by maternal/fetal RhD antigen incompatibility
- Despite routine screening and treatment, anti-D alloimmunization may still occur in some RhD-negative women due to
  - Blood transfusion
  - Unrecognized miscarriages
  - Failure to receive prophylactic anti-D immunoglobulin during and following pregnancy

Incidence

- 6-7/1,000 live births with maternal Rh alloimmunization in the U.S. (Martin, 2002)
  - Prevalence of Rh-negative phenotype differs greatly among ethnic groups (Daniels, 2005)
    - Caucasians – 15%
    - African Americans – 5%
    - Asians – <1%
- 13% of hydrops fetalis caused by antigen/antibody-mediated red blood cell (RBC) hemolysis
- RhD antigen causes ~50% of clinically significant maternal alloimmunization cases (Advent, 2000)

Symptoms of HDN

- Fetal hemolytic anemia
- Jaundice
- Hepatosplenomegaly
- Erythroblastosis
- Hydrops fetalis
- Stillbirth

Physiology

- Transplacentally transferred maternal IgG antibodies attack fetal RBCs in response to foreign, paternally inherited fetal antigens
- >50 different RBC antigens in addition to RhD are known to be associated with maternal alloimmunization and HDN

Genetics

Gene – RHD

Inheritance – autosomal recessive
Variants

- RhD-negative Caucasians
  - Most have complete deletions of both copies of RHD gene
    - Rarely, a nonfunctional RHD gene is due to sequence variant, insertions (eg, pseudogene at border of intron 3/exon 4), or a nonfunctional RHD-CE-D fusion gene
- RhD-negative African Americans
  - 25% have a 37-base-pair insertion inactivating the gene
  - Most others have a nonfunctional fusion gene or complete gene deletion (Singleton, 2000)
- RhD-negative Asians
  - 72% have partial or complete gene deletion (Okuda, 1997)
  - Remainder have sequence variant(s) or a nonfunctional fusion gene

Test Interpretation

Sensitivity/specificity

- Clinical sensitivity – >98% (Daniels, 2005; Okuda 1997; Singleton, 2000)
- Analytical sensitivity/specificity – >99%

Results

- Homozygous RHD
  - Two copies of RHD gene present – predicts RhD-positive phenotype
- Heterozygous RHD
  - One copy of RHD gene present – predicts RhD-positive phenotype
- RHD negative
  - No copies of RHD gene present – predicts RhD-negative phenotype
- Inconclusive due to
  - Presence of RHD exon 5 but absence of exon 7, or vice versa
  - Presence of the 37-base-pair insertion seen in African Americans
  - If fetal genotyping results are uncertain, testing of parental samples may be helpful to clarify whether fetus is RhD positive

Limitations

- Does not identify RHD gene variants resulting in weak or partial RhD
- Rare variants in RHD gene (eg, missense, nonsense, insertions, gene fusion, or small deletions) will not be detected
  - In these cases, specimen may be misinterpreted as RhD positive (false-positive)
- Diagnostic errors can result from rare sequence variations
- Bloody amniotic fluid specimens may give false-negative results due to maternal-cell contamination

References

- Singleton BK, Green CA. The presence of an RHD pseudogene containing a 37 base pair duplication and a nonsense mutation in Africans with the Rh D-negative blood group phenotype. Blood. 2000;95:12-18