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 Antigen (*RHD*) Genotyping 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 RhD 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

- Advent ND, Reid ME. The Rh blood group system: a review. Blood. 2000;95(2):375-387
- Daniels G. The molecular genetics of blood group polymorphism. Transpl Immunol. 2005;14(3-4):143-153
- Martin JA, Hamilton BE, et al. Births: final data for 2001. Natl Vital Stat Rep. 2002;51(2):1–102
- Okuda H, Kawano M, et al. The *RHD* gene is highly detectable in RhD-negative Japanese donors. J Clin Invest. 1997;100(2):373-379
- 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