RhD Antigen (RHD) Genotyping

Indications for Ordering

- Determine paternal RHD genotype (heterozygous or homozygous) in phenotypically positive individual when reproductive partner has clinically significant alloantibody
  - o Include father’s ethnicity and RhD phenotyping result with order
  - o 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 genotype when mother has clinically significant alloantibody AND father is either heterozygous for RHD or unavailable for testing
  - o Include father’s ethnicity and RhD phenotyping result with order

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

- Assess risk for alloimmune hemolytic disease of the newborn (HDN) in fetus or father of pregnancy

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 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
  - o Blood transfusion
  - o Unrecognized miscarriages
  - o 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)
  - o Prevalence of Rh-negative phenotype differs greatly among ethnic groups (Daniels, 2005)
    - Caucasi ans – 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
  o Most have complete deletions of both copies of \textit{RHD} gene
    ▪ Rarely, a nonfunctional \textit{RHD} gene is due to point mutations, insertions (eg, pseudogene at border of intron 3/exon 4), or a nonfunctional \textit{RHD-CE-D} fusion gene

• RhD-negative African Americans
  o 25% have a 37-base-pair insertion inactivating the gene
  o Most others have nonfunctional fusion gene or complete gene deletion (Singleton, 2000)

• RhD-negative Asians
  o 72% have partial or complete gene deletion (Okuda, 1997)
  o Remainder have point mutation(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 \textit{RHD}
  o Two copies of \textit{RHD} gene present
    ▪ Predicts RhD-positive phenotype

• Heterozygous \textit{RHD}
  o One copy of \textit{RHD} gene present
    ▪ Predicts RhD-positive phenotype

• \textit{RHD} negative
  o No copies of \textit{RHD} gene present
    ▪ Predicts RhD-negative phenotype

• Inconclusive due to
  o Presence of \textit{RHD} exon 5 but absence of exon 7, or vice versa
  o Presence of the 37-base-pair insertion seen in African Americans
  o If fetal genotyping results are uncertain, testing of parental samples may be helpful to clarify whether fetus is RhD positive

Limitations

• Rare variants in \textit{RHD} gene (eg, missense, nonsense, insertions, gene fusion, or small deletions) will not be detected
  o 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
• 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