Non-Invasive Prenatal Testing for RHD Genotyping, Fetal

**Indications for Ordering**

- Determine fetal RHD genotype to assess risk for alloimmune hemolytic disease of the newborn (HDN)
- RHD genotyping is appropriate only when the mother is Rh negative and the father is heterozygous for RHD or is unavailable for testing
- Useful after 10 weeks of gestation

**Test Description**

- Circulating cell-free DNA (cfDNA) is isolated from maternal plasma
- MALDI time-of-flight mass spectrometry analyzes
  - RHD exons 4, 5, 7
  - RHD psi-pseudogene 37-base-pair insertion at border of intron 3/exon 4
  - Y chromosome sequences (SRY, DBY, TTTY2) to determine fetal sex

**Tests to Consider**

**Primary test**

Non-Invasive Prenatal Testing for RhD Genotyping, Fetal 2009077

- Determines fetal RHD genotype using a maternal whole blood specimen to assess risk for alloimmune HDN
- Also determines fetal sex

**Related tests**

RhD Antigen (RhD) Genotyping 0051368

- Assess risk for alloimmune HDN in fetus
  - Specimen – amniocytes/chorionic villi
- Assess paternal contribution for risk of alloimmune HDN
  - Specimen – paternal whole blood

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**

- 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
- Identifying fetal genotype can
  - Avoid unnecessary immune prophylaxis
  - Guide management of sensitized pregnancies (eg, serial antibody testing and middle cerebral artery surveillance)

**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 is caused by antigen/antibody-mediated red blood cell (RBC) hemolysis
- RhD antigen causes ~50% of clinically significant maternal alloimmunization cases (Advent, 2000)

**Symptoms**

- 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 point mutations, 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 a complete gene deletion (Singleton, 2000)
- RhD-negative Asians
  - 72% have a partial or complete gene deletion (Okuda, 1997)
  - Remainder have point mutation(s) or a nonfunctional fusion gene

Test Interpretation

Sensitivity/specificity
- Clinical sensitivity – >98% (Bombard, 2011)
- Analytical sensitivity/specificity – >99%/98.3%

Results
- RHD-negative genotype
  - No copies of RHD gene present
    - Predicts RhD-negative phenotype
- RHD-positive genotype
  - At least one copy of RHD gene present
    - Predicts RhD-positive phenotype
- Psi(+) RHD variant
  - At least one copy of Psi(+) RHD gene present
  - Test cannot determine if variant is present in maternal DNA, fetal DNA, or both
  - Test cannot differentiate presence of one copy (RhD positive) from two copies (RhD negative)
    - Presence of variant is conservatively interpreted as a potential RhD-positive phenotype
- Inconclusive
  - Targeted analysis results are ambiguous
  - Fetal sex
    - Reported as female, male, or inconclusive

Limitations
- Rare variants (eg, missense, nonsense, insertions, gene fusion, or small deletions) in RHD gene will not be detected
  - In these cases, specimen may test as RhD positive and be misinterpreted as RhD positive (false-positive)
- Test is specific for RHD gene
- Other causes of alloimmune HDN will not be detected

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