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

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