

RhD Antigen (*RHD*) Genotyping

TO DETERMINE THE NUMBER OF COPIES OF THE RHD ALLELE AND RISK FOR FETAL ERYTHROBLASTOSIS DUE TO ISOIMMUNIZATION

Disease Overview

- Maternal-fetal RhD antigen incompatibility can cause alloimmune hemolytic disease in the fetus or newborn.
- Since the routine use of prophylactic anti-D immunoglobulin in the 1960s, hemolytic disease of the newborn has decreased dramatically, yet some RhD-negative women continue to be immunized through blood transfusion, unrecognized miscarriage, or failure to receive anti-D immunoglobulin during and following pregnancy.
- The RhD antigen causes approximately 50 percent of clinically significant maternal alloimmunization cases.

Epidemiology

Fifteen percent of Caucasians, 5 percent of African-Americans, and <1 percent of Asians are RhD negative.

Genetics

- Most RhD-negative Caucasians have complete deletions of both copies of the *RHD* gene. Rarely, a RhD-negative phenotype results from a nonfunctional *RHD* gene due to a nonsense mutation, missense mutation, small deletion, insertion, or hybrid *RHD-CE* gene.
- Twenty-five percent of RhD-negative African-Americans have a 37 base pair insertion inactivating the gene, while most of the remainder have a nonfunctional fusion gene or complete gene deletion.
- Seventy-two percent of RhD-negative Asians have a partial or complete gene deletion, and 28 percent have a missense mutation or fusion gene.

Indications for Ordering

- Paternal testing to determine *RHD* heterozygosity or homozygosity in a phenotypically positive individual when his reproductive partner has clinically significant alloantibody; if the father of the pregnancy is determined to be homozygous for the *RHD* allele, all of his offspring can be assumed to be RhD positive, negating the need for fetal *RHD* testing.
- Fetal testing when the mother has clinically significant alloantibody and the father of the pregnancy is either heterozygous for *RHD* or not available for testing.

Additional Ordering Notes

- Fetal testing: please provide parent's ethnicity and RhD phenotyping result.
- Paternal testing: please provide the father's ethnicity and RhD phenotyping result.

Interpretation

- Homozygous *RHD*: two copies of the *RHD* gene were present, predicting an RhD-positive phenotype.
- Heterozygous *RHD*: only one copy of the *RHD* gene was present, predicting an RhD-positive phenotype.
- *RHD* negative: no copies of the *RHD* gene were present, predicting an RhD-negative phenotype.
- Uncertain:
 - Presence of *RHD* exon 5 but absence of exon 7 or vice versa.
 - Presence of the 37 bp insertion seen in African-Americans.
 - If fetal genotyping results are uncertain, testing of parental samples may be helpful to clarify whether the fetus is RhD positive.

Limitations

- Most rare mutations in the *RHD* gene (i.e., missense, nonsense, insertions, gene fusion, or small deletions) will not be detected by this assay. In these cases, the sample may be misinterpreted as being RhD positive.
- Rare diagnostic errors may result from primer-site mutations.
- Bloody amniotic fluid specimens may give false-negative results due to maternal-cell contamination.

Methodology

- To determine the presence of the *RHD* exons 5, 7, and a 37 base pair insertion in the intron 3/exon 4 boundary by PCR and fluorescent monitoring.
- Allelic height ratios are used to determine the number of copies of the *RHD* as compared to the *RHCE* gene.
- Clinical sensitivity is greater than 98 percent.
- Analytic sensitivity and specificity are greater than 99 percent.

Related Tests

- RhCc Antigen (*RHCE*) Genotyping (0050421)
- RhEe Antigen (*RHCE*) Genotyping (0050423)
- Antigen Testing, Rh Phenotype (0013019)

References

1. Avent N, et al. The Rh blood group system: a review. *Blood* 2000; 95:375–87.
2. Singleton B, et al. The presence of an RhD pseudogene containing a 37 base pair duplication and a nonsense mutation in Africans with the RhD-negative blood group phenotype. *Blood* 2000; 95:12–8.
3. Suto Y, et al. Gene organization and rearrangements at the human Rhesus blood group locus revealed by fiber-Fish analysis. *Hum Genet* 2000; 106:164–71.

Test Information

0051368 **RhD Antigen (*RHD*) Genotyping**

For specific collection, transport, and testing information, refer to the ARUP Web site at www.aruplab.com.

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.