

RhEe and RhCc Antigen (*RHCE*) Genotyping

FOR DETERMINING FETAL RHESUS TYPE AT THE *RhCE* LOCI

Clinical Background

- The Rh blood group is composed of two genes, *RhD* and *RhCE*, which form integral red cell membrane proteins with at least 45 independent antigens.
- Although the D antigen causes 50 percent of maternal alloimmunization, incompatibility to Kell, C/c, G, E, Duffy, MNS, and Diego blood group systems is responsible for the remainder.
- If a woman is serologically negative for a specific Rh antigen and her reproductive partner is positive for the corresponding antigen, she is at risk for becoming sensitized. If blood from an Rh-positive fetus passes into the maternal circulatory system, she will form Rh antibodies.
- These antibodies can cross the placenta and cause destruction of the fetal red blood cells, resulting in severe fetal anemia, hydrops, demise, or delivery of an infant with jaundice and kernicterus.
- Prophylactic anti-D immunoglobulin is given to all RhD-negative women late in pregnancy and following delivery; therefore, sensitization to RhD is rare.
- The C/c and E/e antigens are less immunogenic than the D antigen but occasionally cause hemolytic reactions. Currently, anti-C/c or E is not prophylactically used to prevent maternal alloimmunization.
- Molecular analysis of the fetal Rh locus is routinely performed when a pregnant woman has a clinically significant alloantibody and the father of the pregnancy is positive for the corresponding antigen.
- Since fetal serologic Rh typing is difficult to perform, Rh genotyping of amniocytes is typically used to predict the fetal Rh phenotype. The optical density of the amniotic fluid and functional assays may also be used to determine if *in utero* treatment is necessary.

Epidemiology

Varies depending on ethnicity.

Genetics

- The C/c and E/e antigens are located on a single polypeptide encoded by the *RhCE* gene.
- The *RhD* and *RhCE* genes, located only 30 kilobase pairs apart on chromosome 1, are each composed of 10 exons, with 92 percent sequence homology.
- The basis of the C/c specificity results from six nucleotide substitutions in *RhCE*, causing four amino-acid substitutions: c.48C>T (p.C16W) in exon 1, and c.150C>T (p.I60L), c.178C>A (p.S68N), and c.307C>T (p.S103P) in exon 2. Only S103P is strictly correlated with C/c expression.
- Genotyping of *RhC* in the presence of *RhD* requires analysis of both exons 1 and 2, as *RhD* exon 1 is identical to *Rhc*, and *RhD* exon 2 is identical to *RhC*. A variant of *Rhc* [Rhc(cyt48)], exon 1 (which does not carry the S103P causative substitution) has the *RhC* sequence

- The E/e polymorphism results from a single nucleotide substitution in *RhCE* exon 5, c.676C>G (p.P226A). The proline at position 226 is necessary, but not sufficient, for full E antigen expression. Amino-acid substitutions at other positions and gene rearrangements may alter the expression of these antigens.

Indications for Ordering

- When a pregnant woman has a clinically significant alloantibody level and the father of the pregnancy is phenotypically positive for the corresponding Rh antigen.
- Please provide maternal and paternal ethnicities and blood group genotypes and phenotypes, as this may aid prediction of fetal Rh phenotype from genotype.

Interpretation

- Samples are reported as having one (heterozygous) or two (homozygous) copies of *RhC* or *c*, or *RhE* or *e*. These results need to be considered in conjunction with antigens and corresponding alloantibodies in the parents.
- High-risk pregnancies, predicted to be at low risk for maternal-fetal Rh incompatibility following fetal genotyping, should continue to be followed using non-invasive means (such as sonography and monitoring of maternal antibody titer) for the development of erythroblastosis and hydrops.

Methodology

- For *RhCc* genotyping: polymerase chain reaction (PCR) of exons 1 and 2 of the *RhCE* gene, followed by melting-curve analysis of allele-specific hybridization probes. To reduce the *RhC* false-positive rate, an additional 109 base-pair insertion specific to *RhC* is tested.
- For *RhEe* genotyping: PPCR of exon 5 of the *RhCE* gene, followed by melting-peak analysis.
- Analytic specificity and sensitivity are 99 percent.
- Clinical sensitivity is predicted to be greater than 99 percent.

Limitations

- Bloody amniotic fluid samples may give false-negative results due to maternal-cell contamination.
- Individuals with weak or no expression of the C/c and E/e antigens may result from *RhCE* gene alterations, such as *RhCE-D-CE* gene hybrids. Other hybrids allow for expression of the C, c, or e antigens on the RhD allele. Genotyping may result in false-negative *RhC*, *Rhc*, or *Rhe* predictions due to *RhCE-D-CE* fusion genes.
- Due to extreme variation in the Rh locus from multiple recombination events, nucleotide substitutions, small and large insertions/deletions, Rh genotyping is occasionally limited in predicting Rh genotype.
- Rare diagnostic errors may occur due to primer-site mutations.

Related Tests

- Rh Genotyping, D Antigen (0051368)
- Antigen Testing, Rh Phenotype (0013019)

References

1. Avent N, et al. The Rh blood group system: a review. *Blood* 2000; 95:375–87.
2. Hundhausen T, et al. *RhCE-D-CD* hybrid genes can cause false-negative DNA typing for the Rh e antigen. *Vox Sang* 2002; 83:268–72.
3. Noizat-Pirenne F, et al. Heterogeneity of blood group *RhE* variants revealed by serological analysis and molecular alteration of the *RhCE* gene and transcript. *Br J Haematol* 1998; 103:429–36.
4. Tax M, et al. *RhC* and *Rhc* genotyping in different ethnic groups. *Transfusion* 2002; 42:634–44.

Test Information

0050421 **RhCc Antigen (*RHCE*) Genotyping**
0050423 **RhEe Antigen (*RHCE*) 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.