Alloimmune Hemolytic Disease of the Fetus and Newborn (RhCc, RhEe, RhD, or Kell Antigen Genotyping)

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The Rh blood group is a complex human blood group. Rh antigens are encoded by two genes, *RHD* and *RHCE,* and are highly immunogenic. Antibodies against Rh antigens are the major cause of alloimmune hemolytic disease of the fetus and newborn (HDFN).[1]

The Kell blood group is complex with many antigens encoded by the *KEL* gene. The two major codominant alleles are *KEL*01* and *KEL*02* with corresponding K and k antigens. The K antigen is one of the most clinically significant antigens as anti-K causes severe fetal anemia by suppressing fetal red blood cell (RBC) synthesis.[2] Kell isoimmunization is the third most common cause of HDFN.[2]

In obstetrics, RBC antigen genotyping is useful when the mother has a clinically significant alloantibody level and the father is phenotypically positive for the corresponding antigen. Paternal genotyping can be used to assess risk for HDFN in offspring by determining copy number for the antigen of interest. Fetal genotyping is useful when the father of the fetus is either heterozygous for the corresponding antigen or unavailable for testing.

Disease Overview

Incidence of HDFN

- 13% of hydrops fetalis is caused by antigen/antibody-mediated RBC hemolysis.
- 6–7/1,000 live births with maternal RhD alloimmunization in the U.S.[3]
- RhD antigen causes ~50% of clinically significant maternal alloimmunization cases.[4]
  - Anti-c is one of the most common causes of severe HDFN, after anti-D.
  - Anti-C, anti-E, and anti-e are less common causes of HDFN.
    - When symptoms occur, they are usually mild to moderate.[5]
- ~4% of K negative (k/k) mothers will deliver a K positive baby with potential for HDFN.[6]

Symptoms of HDFN

- Fetal hemolytic anemia
- Jaundice
- Hepatosplenomegaly
- Erythroblastosis
- Hydrops fetalis
- Stillbirth

Physiology

- Transplacentally transferred maternal IgG antibodies attack fetal RBCs in response to foreign, paternally inherited antigens in the fetus.
- >50 different RBC antigens are known to be associated with maternal alloimmunization and HDFN.
- 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

Genetics

Featured ARUP Testing

RhC/c (RHCE) Antigen Genotyping 3002002

Method: Polymerase Chain Reaction (PCR)/Fluorescence Monitoring

- Genotyping to assess Rh blood group *RHCE*2* (C) and *RHCE*4* (c)
- Use to determine the paternal RhCc genotype when the reproductive partner has a clinically significant alloantibody.
- Immucor PreciseType HEA Molecular BeadChip which is FDA-approved for clinical testing
- For fetal testing, order RhC/c (RHCE) Antigen Genotyping, Fetal (3016679) using a fetal specimen.

RhD Gene (RHD) Copy Number 0051368

Method: Polymerase Chain Reaction (PCR)/Fluorescence Monitoring

- Determine the paternal *RHD* gene copy number (heterozygous or homozygous) in a phenotypically positive individual when the reproductive partner has a clinically significant alloantibody.
- This test does not assess for weak or partial *RHD* genotypes.
- For fetal testing, order RhD Gene (RHD) Copy Number, Fetal (3016640) using a fetal specimen.

RhE/e (RHCE) Antigen Genotyping 3002003

Method: Polymerase Chain Reaction (PCR)/Fluorescence Monitoring

- Genotyping to assess Rh blood group *RHCE*3* (E) and *RHCE*5* (e)
- Use to determine the paternal RhEe genotype when the reproductive partner has a clinically significant alloantibody.
- Immucor PreciseType HEA Molecular BeadChip which is FDA-approved for clinical testing
- For fetal testing, order RhE/e (RHCE) Antigen Genotyping, Fetal (3016682) using a fetal specimen.

Kell K/k (KEL) Antigen Genotyping 3002001

Method: Polymerase Chain Reaction (PCR)/Fluorescence Monitoring

- Genotyping to assess Kell blood group *KEL*01* (K), *KEL*02* (k)
- Use to determine the paternal K/k genotype when the reproductive partner has a clinically significant alloantibody.
- Immucor PreciseType HEA Molecular BeadChip which is FDA-approved for clinical testing

Variants Resulting in RhD-Negative Phenotypes

- RhD-negative White individuals
  - Most have complete deletions of both copies of *RHD* gene
    - Rarely, a nonfunctional *RHD* gene is due to sequence variants, 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 complete gene deletion[8]
- RhD-negative Asians
  - 72% have partial or complete gene deletion[9]
  - Remainder have sequence variant(s) or a nonfunctional fusion gene

Inheritance

Codominant

- For fetal testing, order Kell K/k (KEL) Antigen Genotyping, Fetal (3016676) using a fetal specimen.

Antigen Testing, Rh Phenotype 0013019

Method: Qualitative Hemagglutination (HA)

Antigen testing for D, C, E, c, e to assess maternal, paternal, or newborn Rh phenotype status

Genes and Variants

Blood Group	Gene	Allele	Antigen (ISBT #)[1]	ISBT Genotype	Variants Tested	Variants Used to Predict Allele	Phenotype Frequency[1,7]
Rh	*RHCE* (NM_020485.5)	C	RH2	*RHCE*2*	c.307C>T; p.Pro103Ser 109bp insertion	c.307T; p.Ser103 109bp insertion	C: 68% AA: 27% A: 93%
		c	RH4	*RHCE*4*		c.307C; p.Pro103	C: 80% AA: 98% A: 47%
		E	RH3	*RHCE*3*	c.676G>C; p.Ala226Pro	c.676C; p.Pro226	C: 29% AA: 22% A: 39%
		e	RH5	*RHCE*5*		c.676G; p.Ala226	C: 98% AA: 98% A: 96%
	RHD (NM_016124.4)	D	RH1	*RHD*01*	Presence of the *RHD* exons 5, 7, and a 37 base pair insertion in the intron 3/exon 4 boundary. Allelic height ratios are used to determine the number of copies of *RHD* as compared to *RHCE*.		C: 15% RhD negative AA: 8% RhD negative A: <1% RhD negative
Kell	*KEL* (NM_000420.2)	K	KEL1	*KEL*01*	c.578C>T; p.Thr193Met	c.578T; p.Met193	C: 9% AA: 2% A: Rare Iranian Jews: 12% Arabs: up to 25%
		k	KEL2	*KEL*02*		c.578C; p.Thr193	C: 99.8% AA: 100%

ISBT, International Society of Blood Transfusion

Test Interpretation

Clinical Sensitivity

- RhD: 98%[8,9,10]
- RhCc, RhEe, and Kell: 99%

Analytic Sensitivity/Specificity

- RhD: 99%
- RhCc, RhEe, and Kell: 99%

Results

- RhCc Genotyping
  - Homozygosity for C allele is predictive of RhC+c- phenotype.
  - Cc compound heterozygosity is predictive of RhC+c+ phenotype.
  - Homozygosity for c allele is predictive of RhC-c+ phenotype.
- RhD Copy Number
  - Presence of one or two copies of *RHD* gene predicts RhD positive phenotype.
  - No copies of the *RHD* gene predicts RhD negative phenotype.
  - Inconclusive results may occur due to:
    - Presence of *RHD* exon 5 but absence of exon 7, or vice versa
    - Presence of the 37-base-pair insertion seen in African Americans
- RhEe Genotyping
  - Homozygosity for E allele is predictive of RhE+e- phenotype.
  - Ee compound heterozygosity is predictive of RhE+e+ phenotype.
  - Homozygosity for e allele is predictive of RhE-e+ phenotype.
- Kell Genotyping
  - Homozygosity for K allele is predictive of K+k- phenotype.
  - Kk compound heterozygosity is predictive of K+k+ phenotype.
  - Homozygosity for k allele is predictive of K-k+ phenotype.
- Fetuses predicted to be unaffected following prenatal genotyping should continue to be monitored by noninvasive means for the development of erythroblastosis or hydrops.

Limitations

- Bloody amniotic fluid specimens may give false-negative results due to maternal cell contamination.
- A fetal sample is required when ordering fetal genotype testing.
- Rare nucleotide changes leading to altered or partial antigen expression may not be detected.
- Genotypes resulting in Rh null phenotypes will not be assessed.
- Weak or partial *RHD* genotypes are not assessed.
- This assay is occasionally limited in predicting genotype due to extreme variation in the Rh locus. False-negative RhC or Rhc predictions may result due to *RHCE-D-CE* fusion genes.
- Diagnostic errors can occur due to rare sequence variations.
- Abnormal signal intensities may result in indeterminate genotyping results.
- Patients who have had hematopoietic stem cell transplants may have inconclusive results.

References

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Related Information

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