Noninvasive Fetal RhD Genotyping Using Cell-Free Fetal DNA

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DESCRIPTION

The Rh blood group system includes a variety of surface markers or antigens, the most common of which is RhD. In general, the presence of the RhD antigen on red blood cells is referred to as “RhD positive” and the absence as “RhD negative.” The prevalence of RhD negative blood type generally varies by race and ethnicity. Approximately 15% of Caucasians, 5% to 8% of African Americans, and 1% to 2% of Asians and Native Americans are RhD negative, respectively.

Alloimmunization is a term that refers to the development of antibodies in an RhD negative mother if a sufficient number of red blood cells from an RhD positive fetus enter maternal circulation. Alloimmunization is often the result of fetomaternal hemorrhage during delivery, which has been reported in 15% to 50% of all births. Fetomaternal hemorrhage may be the result of miscarriage, pregnancy termination, trauma, or invasive

procedures, such as amniocentesis. Alloimmunization against the RhD antigen is the most common cause of hemolytic disease of the fetus and newborn (HDFN) in subsequent pregnancies, symptoms of which can vary from mild to severe. If left undiagnosed and untreated, alloimmunization can result in serious perinatal morbidity and mortality. However, the introduction of postpartum administration of anti-D prophylaxis immune globulin to RhD negative individuals has significantly reduced the morbidity and mortality of HDFN over the last 40 years

In cases of pregnant genotypic women without RhD hemolytic antibodies, knowledge that the fetus is RhD negative is useful in determining the need for prenatal and postnatal anti-D prophylaxis immune globulin. In the case of alloimmunized individuals with RhD antibodies, knowledge that the fetus is RhD negative may reduce need for intensive prenatal monitoring to predict and treat fetal anemia and continuously monitor high risk pregnancies. In addition, in alloimmunized individuals who require invasive prenatal testing for prenatal diagnosis of genetic abnormalities, knowledge of the fetal RhD genotype may be clinically useful when deciding whether to conduct first trimester chorionic villus sampling (CVS) or amniocentesis, both of which heighten the risk of worsening maternal sensitization and fetal HDFN. It should be noted that even with the presence of RhD incompatibility between mother and fetus, about 50% of alloimmunized pregnancies could be identified with fetal RhD genotyping as not being at increased risk, which would reduce or eliminate the need for unnecessary examinations and treatment with anti-D prophylaxis immune globulin, while also reducing parental anxiety.

The diagnosis of RhD alloimmunization is based upon detection of anti-RhD antibodies in maternal serum performed at the first prenatal visit. In RhD negative individuals with an initially negative result and uncomplicated pregnancy, this antibody screen should be repeated at 28 weeks of pregnancy, and again at delivery. The test most commonly used for diagnostic purposes is the indirect Coombs test, a technique used to determine antibody titers (antibodies in the plasma).

Alternatively, advances in fetal DNA testing have allowed the prediction of the fetal RhD phenotype. Conventional analysis of fetal DNA has relied upon invasive methods of sampling fetal tissues, such as amniocentesis and CVS, both of which are associated with a risk of fetal harm. Clinical guidelines published by the ACOG support the use of amniocentesis as the primary modality used to determine fetal blood type, while CVS is discouraged due to increased risk of fetomaternal hemorrhage.

Noninvasive alternatives to determine prenatal fetal RhD genotype are currently being investigated. One example is the SensiGene Genotyping test, a noninvasive prenatal blood test developed to determine the fetal RhD genotype in an RhD negative mother. The test is based upon current research demonstrating the presence of intact fetal cells and cell-free fetal nucleic acids (cffNA) that have crossed the placenta and enter maternal circulation. The SensiGene test detects circulating cffDNA extracted from a mother’s blood sample during the first or second trimester of pregnancy and uses a real-time polymerase chain reaction (PCR) to amplify the RhD genotype.

According to the manufacturer, the SensiGene Genotyping test (Sequenom, Inc., San Diego, CA) can be performed in the first trimester at 10 weeks of gestation. The test is intended for use in pregnant RhD negative alloimmunized individuals whose partners are either RhD positive or have an unknown RhD status. It is also intended to identify fetal RhD status when maternal antibody titers are unclear.

Testing Pregnant Women with Rhesus D-negative Blood Type

Clinical Content and Test Purpose
The purpose of genetic testing of individuals who are pregnant and have Rhesus D (RhD)-negative blood type is to determine the RhD status of the fetus to guide pregnancy management, including avoidance of invasive testing (chorionic villus sampling or amniocentesis) and administration of anti-D immunoglobulin.

Populations
The relevant population of interest includes individuals who are pregnant and have an RhD-negative blood type.

Interventions
The test being considered is noninvasive RhD genotyping of the fetus using cell-free DNA from maternal plasma.

Comparators
The following practices are currently being used: invasive methods to determine fetal Rhesus (Rh) status and management based on maternal RhD status.

Outcomes
The general outcomes of interest are test validity, morbid events, medication use, and treatment-related morbidity. The potential beneficial outcomes of primary interest are the avoidance of invasive testing (chorionic villus sampling or amniocentesis) and avoidance of unnecessary administration of RhD immunoglobulin.

Potentially harmful outcomes are those resulting from false-positive or false-negative test results. False-positive test results can lead to unnecessary administration of RhD immunoglobulins during pregnancy. False-negative test results can lead to lack of RhD immunoglobulin administration, development of maternal alloimmunization to RhD, and current and future pregnancy complications due to maternal alloantibodies to RhD.

Outcomes may be measured at various times. During a first pregnancy, testing may be conducted to detect the development of maternal alloimmunization to RhD and minimal-to-mild fetal or neonatal disease. In subsequent pregnancies, testing may be conducted to detect pregnancy complications due to maternal alloimmunization to RhD and potentially severe fetal or neonatal hemolytic anemia.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

The possible clinical utility of RhD genotyping using cffDNA includes the following scenarios. In the RhD-negative, nonalloimmunized pregnant patient:

- Avoidance of unnecessary anti-D immunoglobulin if the fetus is RhD-negative
- Avoidance of invasive procedures to obtain fetal tissue when the paternity is unknown, or the father is heterozygous for the D antigen.

In the RhD-negative, alloimmunized pregnant patient:

- Avoidance of invasive procedures to obtain fetal tissue if the RhD-negative pregnant individual is alloimmunized to determine fetal RhD status
- Avoidance of serial antibody testing in the mother and middle cerebral artery surveillance of the fetus if the fetus is determined to be RhD-negative.

This type of testing could lead to the avoidance of the use of anti-D immunoglobulin (e.g., RhoGAM) in RhD-negative mothers with RhD-negative fetuses. However, the false-negative test rate, while low, is not zero, and a certain percentage of RhD-negative women will develop alloimmunization to RhD-positive fetuses. Other issues that need to be defined include the optimal timing of testing during the pregnancy.

Review of Evidence
Sperling et. al. (2018) compared the guidelines from the American College of Obstetricians and Gynecologists as well as 3 international guidelines on the prevention of RhD alloimmunization. All 4 guidelines recommended that all women have an antibody screen with an indirect Coombs test at prenatal intake and at 24 to 28 weeks. None currently recommend screening with cell-free fetal DNA.

Moise et al (2016) analyzed blood samples collected in each trimester of pregnancy for 520 nonalloimmunized RhD-negative patients in a prospective, observational study using the Fetal RHD Genotyping test.[9,] Inconclusive results secondary to the presence of

the RhDy or an RhD variant were noted in 5.6%, 5.7%, and 6.1% of the first-, second-, and third-trimester samples, respectively. The false-positive rates for RhD (an RhD-negative fetus with an *RHD*-positive result) was 1.54% (95% CI, 0.42% to 5.44%), 1.53% (95% CI, 0.42% to 5.40%), and 0.82% (95% CI, 0.04% to 4.50%), respectively, across the 3 trimesters. There was only 1 (0.32%) false-negative diagnosis (an RhD-positive fetus with an *RHD*-negative result), which occurred in the first trimester (95% CI, 0.08% to 1.78%). Genotyping for mismatches across repeated samples revealed that this error was related to mislabeling of samples from 2 patients collected on the same day at a collection site. Overall test results were in agreement across all 3 trimesters (p>.99).

Vivanti et. al. (2016) in a retrospective observational study published promising results on the sensitivity, specificity, PPV and NPV of the SensiGene. Although the test continues to demonstrate a high degree of accuracy, both publications disclose conflicts of interest with the manufacturer of the test.

In 2014, Zhu et. al. published a meta-analysis of studies on the diagnostic accuracy of noninvasive fetal RhD genotyping using cell-free fetal DNA (cffDNA) which included 41 publications representing 11,129 samples from non-invasive Rh genotyping of cell-free fetal DNA (CffDNA) obtained from maternal blood. A total of 352 samples were excluded due to inconclusive results. The overall diagnostic accuracy from the remaining samples was 98.5%, and sensitivity and specificity were 99% and 98%, respectively. Diagnosis in the first trimester showed the highest accuracy at 99% and 30 studies reported 100% diagnostic accuracy of fetal RhD genotyping. It is not clear how many of the studies chosen for inclusion used the SensiGene test but based on this analysis, this non-invasive testing method warrants further investigation into its clinical utility and impact on perinatal outcomes.

In 2013, Moise et. al. in a prospective cohort study evaluated fetal RhD status in RhD negative pregnant women who were not previously alloimmunized (n=123). The relative accuracy of the test was based upon gestational age, and maternal blood samples were obtained during the first, second, and third trimesters of pregnancy. RhD status was initially confirmed by serological testing of neonatal cord blood. The overall accuracy of the SensiGene test to identify fetal RhD status was 99.1%, 99.1%, and 98.1% for the first, second, and third trimester, respectively. A total of 22 samples (6.3%) were determined to be inconclusive. The overall sensitivity of the SensiGene test was 99.6% (95% CI, 97.5%-100%). Of the 300 samples obtained, 3 false positives and 1 false negative were identified. Despite these preliminary promising results, the study was hampered by a few limitations, including lack of blinded assessors, participants and clinicians, and potential for conflicts of interest due to financial sponsorship by the manufacturer.

Section Summary
The clinical sensitivity of RhD genotyping is high. However, there is variability in the sensitivity based on the trimester when the test is performed. Clinical validation studies have found false-negative rates ranging from 0.5% to 2.0%. False-negative results in this clinical context would lead to lack of RhD immunoglobulin administration, development

of maternal alloimmunization to RhD, and current and future pregnancy complications due to maternal alloantibodies to RhD compared with standard management of RhD-negative pregnant women.

Direct evidence of the clinical utility of RhD genotyping using cffDNA is lacking. There is potential clinical utility in avoidance of unnecessary anti-D immunoglobulin administration, avoidance of invasive procedures to determine fetal RhD status, avoidance of serial antibody testing in alloimmunized pregnant patients, and avoidance of middle cerebral artery surveillance in an RhD-negative fetus. However, a certain percentage of RhD-negative individuals will develop alloimmunization to RhD-positive fetuses due to false-negative test results.

Summary of Evidence
Based on review of the peer reviewed medical literature, the results from studies consistently demonstrate a high diagnostic accuracy for the SensiGene test in identifying fetal RhD genotype in RhD negative pregnant individuals. The sensitivity, specificity, PPV, and NPV are comparable to those derived from invasive diagnostic assessments using amniocentesis. However, all studies to date are either small or of low quality. In particular, the available individual studies were characterized by several weaknesses, including the potential for conflicts of interest due to manufacturer sponsorship or involvement, and the occurrence of test failures due to invalid or insufficient sampling. There was no direct evidence to allow definitive conclusions with conventional diagnostic assessments. Additionally, there is no evidence assessing the clinical utility of the SensiGene test, and it is unclear whether its use will lead to improved health outcomes.

Questions persist regarding the clinical utility of the SensiGene test in helping pregnant individuals avoid unnecessary anti-D immune globulin treatment or avoid adverse events associated with invasive diagnostic procedures. Also, despite the relatively low false-negative rate associated with the SensiGene test, a certain percentage of women will still become alloimmunized to an RhD positive fetus. Additional research is needed to ascertain the test's relevance and usefulness in clinical practice. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

Practice Guidelines and Position Statements

American College of Obstetricians and Gynecologists (ACOG)
In 2018, the American College of Obstetricians and Gynecologists (ACOG) reaffirmed its 2006 position that detection of fetal Rhesus D (RhD) using molecular analysis of maternal plasma or serum can be assessed in the second trimester with an accuracy greater than 99% but that this test is not a widely used clinical tool.

In its 2017 Practice Bulletin Number 181 on the prevention of RhD alloimmunization, the College stated that "Despite the improved accuracies noted with noninvasive fetal RHD

genotyping, cost comparisons with current routine prophylaxis of anti-D immunoglobulin at 28 weeks of gestation have not shown a consistent benefit and, thus, this test is not routinely recommended."

Regulatory Status
The SensiGene RhD Genotyping test (Sequenom, Inc., San Diego, CA) is not subject to federal regulation by the Food and Drug Administration (FDA). Genetic tests are regulated under the Clinical Laboratory Improvement Amendments (CLIA) Act of 1988. Premarket approval by the FDA is not required provided the test is performed in a laboratory facility that observes CLIA regulations.

Sequenom offers the SensiGene™ Fetal RHD Genotyping test, performed by proprietary SEQureDx™ technology. The assay targets exons 4, 5, and 7 of the RhD gene located on chromosome 1, psi pseudogene in exon 4, and assay controls, which are 3 targets on the Y chromosome (SRY, TTTY, DBY) using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry-based nucleic acid analysis. The company claims that uses of its test include:

- Clarifying fetal RhD status without testing the father, thereby avoiding the cost of paternity testing and paternal genotyping
- Clarifying fetal RhD status when maternal anti-D titers are unclear
- Identifying the RhD-negative fetus in mothers who are opposed to immunization(s) and vaccines
- Identifying RhD-negative sensitized patients
- Avoiding invasive testing by CVS or genetic amniocentesis.

PRIOR APPROVAL

Not required.

POLICY

See Related Medical Policies

- 02.04.38 Prenatal Screening; Cell-Free Fetal DNA Testing
- 02.04.50 Whole Exome and Whole Genome Sequencing for Diagnosis of Genetic Disorders

Noninvasive fetal RhD genotyping using cell-free fetal DNA using the SensiGene Fetal RhD genotyping testing is considered **investigational,** because the evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

PROCEDURE CODES AND BILLING GUIDELINES

To report provider services, use appropriate CPT* codes, Alpha Numeric (HCPCS level 2) codes, Revenue codes, and/or ICD diagnosis codes.

- 81403 Molecular pathology procedure Level 4 (e.g., analysis of single exon DNA sequence analysis, analysis of > 10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons) [when specified as the following]:
  - RhD (Rh blood group, D antigen) (e.g., hemolytic disease of the fetus and newbor4n, Rh maternal/fetal compatibility), deletion analysis (e.g., exons 4, 5 and 7, pseudogene), performed on cell free fetal DNA in maternal blood (SensiGene Fetal RhD Genotyping blood test)

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POLICY HISTORY

Date	Reason	Action
February 2022		New Medical Policy Created

New information or technology that would be relevant for Wellmark to consider when this policy is next reviewed may be submitted to:

Wellmark Blue Cross and Blue Shield
Medical Policy Analyst
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