

2.04.108	Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA		
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Section:	2.0 Medicine	Page:	Page 1 of 13

Policy Statement

Noninvasive fetal *RHD* genotyping using cell-free fetal DNA is considered **investigational**.

Policy Guidelines

Genetics Nomenclature Update

The Human Genome Variation Society (HGVS) nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG1). The Society's nomenclature is recommended by the Human Variome Project, the Human Genome Organization (HUGO), and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG2 shows the recommended standard terminology—"pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign"—to describe variants identified that cause Mendelian disorders.

Table PG1. Nomenclature to Report on Variants Found in DNA

Previous	Updated	Definition
Mutation	Disease-associated variant	Disease-associated change in the DNA sequence
	Variant	Change in the DNA sequence
	Familial variant	Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives

Table PG2. ACMG-AMP Standards and Guidelines for Variant Classification

Variant Classification	Definition
Pathogenic	Disease-causing change in the DNA sequence
Likely pathogenic	Likely disease-causing change in the DNA sequence
Variant of uncertain significance	Change in DNA sequence with uncertain effects on disease
Likely benign	Likely benign change in the DNA sequence
Benign	Benign change in the DNA sequence

ACMG: American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

Genetic Counseling

Experts recommend formal genetic counseling for patients who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some patients; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing; further, genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

Coding

This testing is included in CPT code **81403**:

- *RHD* (Rh blood group, *D* antigen) (e.g., hemolytic disease of the fetus and newborn, Rh maternal/fetal compatibility), deletion analysis (e.g., exons 4, 5, and 7, pseudogene), performed on cell-free fetal DNA in maternal blood

Description

Rhesus D (RhD)-negative women who are exposed to RhD-positive red blood cells can develop anti-RhD antibodies, which can cross the placenta and cause fetal anemia. If undiagnosed and untreated, alloimmunization can cause significant perinatal morbidity and mortality. Determining the RhD status of the fetus may guide subsequent management of the pregnancy. Hence, the use of cell-free fetal DNA in maternal blood has been proposed as a noninvasive method to determine fetal *RHD* genotype.

Related Policies

- N/A

Benefit Application

Benefit determinations should be based in all cases on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Some state or federal mandates (e.g., Federal Employee Program [FEP]) prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

Regulatory Status

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

Sequenom offers the SensiGene™ Fetal RHD Genotyping test, performed by proprietary SEQuireDx™ technology. The assay targets exons 4, 5, and 7 of the *RHD* gene located on chromosome 1, psi (ψ) 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.

Rationale

Background

Alloimmunization

Alloimmunization refers to the development of antibodies in a patient whose blood type is Rhesus D (RhD)-negative and who is exposed to RhD-positive red blood cells (RBCs). This most commonly occurs from fetal-placental hemorrhage and entry of fetal blood cells into the maternal circulation. The management of an RhD-negative pregnant patient who is not alloimmunized and is carrying a known RhD-positive fetus, or if fetal RhD status is unknown, involves administration of RhD immunoglobulin at standardized during pregnancy to prevent the formation of anti-RhD antibodies. If the patient is already alloimmunized, monitoring the levels of anti-RhD antibody titers for the development of fetal anemia is performed. Noninvasive and invasive tests to determine fetal RhD status exist.

Rh Blood Groups

The Rh (Rhesus) system includes more than 100 antigen varieties found on RBCs. RhD is the most common and the most immunogenic. When people have the RhD antigen on their RBCs, they are considered to be RhD-positive; if their RBCs lack the antigen, they are considered to be RhD-negative. The RhD antigen is inherited in an autosomally dominant fashion, and a person may be heterozygous (Dd; ~60% of RhD-positive people) or homozygous (DD; ~40% of RhD-positive people). Homozygotes always pass the RhD antigen to their offspring, whereas heterozygotes have a 50% chance of passing the antigen to their offspring. A person who is RhD-negative does not have the Rh antigen. Although nomenclature refers to RhD-negative as dd, there is no small d antigen (i.e., they lack the *RHD* gene and the corresponding RhD antigen).

RhD-negative status varies across ethnic groups and is 15% in whites, 5% to 8% in blacks, and 1% to 2% in Asians and Native Americans.

In the white population, almost all RhD-negative individuals are homozygous for a deletion of the *RHD* gene. However, in black populations, only 18% of RhD-negative individuals are homozygous for an *RHD* deletion, and 66% of RhD-negative blacks have an inactive *RHD* pseudogene (*RHDy*).¹ There are also numerous rare variants of the D antigen, which are recognized by weakness of expression of D and/or by the absence of some of the epitopes of D. Some individuals with variant D antigens if exposed to RhD-positive RBCs, can make antibodies to one or more epitopes of the D antigen.¹

RhD-negative women can have a fetus that is RhD-positive if the fetus inherits the RhD-positive antigen from the paternal father.

Causes of Alloimmunization

By 30 days of gestation, the RhD antigen is expressed on the RBC membrane, and alloimmunization can be caused when fetal RhD-positive RBCs enter maternal circulation and the RhD-negative mother develops anti-D antibodies.² Once anti-D antibodies are present in a pregnant woman's circulation, they can cross the placenta and destroy fetal RBCs.

The production of anti-D antibodies in RhD-negative women is highly variable and significantly affected by several factors, including the volume of fetomaternal hemorrhage, the degree of the maternal immune response, concurrent ABO incompatibility, and fetal homozygosity vs heterozygosity for the D antigen. Therefore, although about 10% of pregnancies are RhD-incompatible, less than 20% of RhD-incompatible pregnancies actually lead to maternal alloimmunization.

Small fetomaternal hemorrhages of RhD-positive fetal RBCs into the circulation of an RhD-negative woman occurs in nearly all pregnancies, and percentages of fetomaternal hemorrhage increase as the pregnancy progresses: 7% in the first trimester, 16% in the second trimester, and 29% in the third trimester, with the greatest risk of RhD alloimmunization occurring

at birth (15%-50%). Transplacental hemorrhage accounts for almost all cases of maternal RhD alloimmunization.

Fetomaternal hemorrhage can also be associated with miscarriage, pregnancy termination, ectopic pregnancy, invasive in utero procedures (e.g., amniocentesis), in utero fetal death, maternal abdominal trauma, antepartum maternal hemorrhage, and external cephalic version. Other causes of alloimmunization include inadvertent transfusion of RhD-positive blood and RhD-mismatched allogeneic hematopoietic cell transplantation.

Consequences of Alloimmunization

Immunoglobulin G antibody-mediated hemolysis of fetal RBCs, known as hemolytic disease of the fetus and newborn, varies in severity and manifestations. The anemia can range from mild to severe, with associated hyperbilirubinemia and jaundice. In severe cases, hemolysis may lead to extramedullary hematopoiesis and reticuloendothelial clearance of fetal RBCs, which may result in hepatosplenomegaly, decreased liver function, hypoproteinemia, ascites, and anasarca. When accompanied by high-output cardiac failure and pericardial effusion, this condition is known as hydrops fetalis, which without intervention, is often fatal. Intensive neonatal care, including emergent exchange transfusion, is required.

Cases of hemolysis in the newborn that do not result in fetal hydrops can still lead to kernicterus, a neurologic condition observed in infants with severe hyperbilirubinemia due to the deposition of unconjugated bilirubin in the brain. Symptoms that manifest several days after delivery can include poor feeding, inactivity, loss of the Moro reflex, bulging fontanelle, and seizures. The 10% of infants who survive may develop spastic choreoathetosis, deafness, and/or mental retardation.

Hemolytic disease in the fetus or newborn was once a major contributor to perinatal morbidity and mortality. However, the widespread adoption of antenatal and postpartum use of RhD immunoglobulin in developed countries resulted in a major decrease in the frequency of this disease. In developing countries without prophylaxis programs, stillbirth occurs in 14% of affected pregnancies, and 50% of pregnancy survivors either die in the neonatal period or develop a cerebral injury.³

Prevention of Alloimmunization

There are four RhD immunoglobulin products available in the U. S., all of which undergo micropore filtration to eliminate viral transmission.³ To date, no reported cases of viral infection related to RhD immunoglobulin administration have been reported in the U. S.³ Theoretically, the Creutzfeldt-Jakob disease agent could be transmitted by the use of RhD immunoglobulin. Local adverse reactions may occur, including redness, swelling, and mild pain at the site of injection, and hypersensitivity reactions.

The American College of Obstetricians and Gynecologists and the American Association of Blood Banks have recommended the first dose of Rh_o(D) immunoglobulin (e.g., RhoGAM) be given at 28 weeks of gestation (or earlier if there's been an invasive event), followed by a postpartum dose given within 72 hours of delivery.

Diagnosis of Alloimmunization

The diagnosis of alloimmunization is based on detection of anti-RhD antibodies in the maternal serum. The most common test for determining antibodies in serum is the indirect Coombs test.³ The maternal serum is incubated with known RhD-positive RBCs. Any anti-RhD antibody present in the maternal serum will adhere to the RBCs. The RBCs are then washed and suspended in Coombs serum, which is antihuman globulin. RBCs coated with maternal anti-RhD will agglutinate, which is referred to as a positive indirect Coombs test. The indirect Coombs titer is the value used to direct management of pregnant alloimmunized women.

Management of Alloimmunization During Pregnancy

A patient's first alloimmunized pregnancy involves minimal fetal or neonatal disease. Subsequent pregnancies are associated with more severe degrees of fetal anemia. Treatment of an alloimmunized pregnancy requires monitoring maternal anti-D antibody titers and serial ultrasound assessment of middle cerebral artery peak systolic velocity of the fetus.

If severe fetal anemia is present near term, delivery is performed. If severe anemia is detected remote from term, intrauterine fetal blood transfusions may be performed.

Determining Fetal RhD Status

The American College of Obstetrician and Gynecologists has recommended that all pregnant women be tested during their first prenatal visit for ABO blood group typing and RhD type, and be screened for the presence of anti-RBC antibodies. These laboratory tests should be repeated for each subsequent pregnancy. The American Association of Blood Banks has also recommended that antibody screening be repeated before administration of anti-D immunoglobulin at 28 weeks of gestation, postpartum, and at the time of any event during pregnancy.

If the mother is determined to be RhD-negative, the paternal RhD status should also be determined at the initial management of a pregnancy. If paternity is certain and the father is RhD-negative, the fetus will be RhD-negative, and further assessment and intervention are unnecessary. If the father is RhD-positive, he can be either homozygous or heterozygous for the D allele. If homozygous for the D allele (i.e., D/D), then the fetus is RhD-positive. If the paternal genotype is heterozygous for Rh status or is unknown, determination of the RhD status of the fetus is the next step to assess the RhD compatibility of the pregnancy (first or any subsequent pregnancy).

Invasive and noninvasive testing methods to determine the RhD status of a fetus are available. These procedures use polymerase chain reaction assays to assess the fetal cellular elements in amniotic fluid by amniocentesis or chorionic villus sampling (CVS). Although CVS can be performed earlier in a pregnancy, amniocentesis is preferred because CVS is associated with disruption of the villi and the potential for larger fetomaternal hemorrhage and worsening alloimmunization if the fetus is RhD-positive. The sensitivity and specificity of fetal *RHD* genotyping by polymerase chain reaction are reported as 98.7% and 100%, respectively, with positive and negative predictive values of 100% and 96.9%, respectively.⁴

Noninvasive testing involves molecular analysis of cell-free fetal DNA (cffDNA) in the maternal plasma or serum. Lo et al (1998) showed that about 3% of cffDNA in the plasma of first-trimester pregnant women is of fetal origin, with this percentage rising to 6% in the third trimester.⁵ Fetal DNA cannot be separated from maternal DNA, but if the pregnant woman is RhD-negative, the presence of specific exons of the *RHD* gene, which are not normally present in the circulation of an RhD-negative patient, predicts an RhD-positive fetus. The cffDNA has been proposed as a noninvasive alternative to obtaining fetal tissue by invasive methods, which are associated with a risk of miscarriage.¹

The large quantity of maternal DNA compared with fetal DNA in the maternal circulation complicates the inclusion of satisfactory internal controls to test for successful amplification of fetal DNA. Therefore, reactions to detect Y chromosome-linked gene(s) can be included in the test, which will be positive when the fetus is a male.¹ When Y chromosome-linked genes are not detected, tests for variants may be performed to determine whether the result is derived from fetal not maternal DNA.

The cffDNA testing to determine the fetal *RHD* genotype is the standard of care in many European countries.³

Literature Review

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

Testing Pregnant Women with RhesusD-negative Blood Type**Clinical Context and Test Purpose**

The purpose of genetic testing of individuals who are pregnant and have 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.

The questions addressed in this evidence review include:

1. Does Rhesus D (*RHD*) genotyping reduce the need for invasive testing by chorionic villus sampling or amniocentesis?
2. Does *RHD* genotyping guide the administration of anti-D immunoglobulin during pregnancy?
3. Does *RHD* genotyping lead to improved pregnancy outcomes?

The following PICOs were used to select literature to inform this review.

Patients

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.

The primary setting would be in the obstetrics population where maternal blood type and RhD status are determined during the prenatal period and RhD-negative patients are monitored and/or treated to prevent alloimmunization to RhD.

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 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.

Technically Reliable

Assessment of technical reliability focuses on specific tests and operators and requires a review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

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).

Zhu et al (2014) published a meta-analysis of studies on the diagnostic accuracy of noninvasive fetal *RHD* genotyping using cell-free fetal DNA (cffDNA).⁶ Reviewers identified 37 studies conducted in RhD-negative pregnant women that had been published by the end of 2013. The studies included 11129 samples, and 352 inconclusive samples were excluded. When all data were pooled, the sensitivity of fetal *RHD* genotyping was 99% and the specificity was 98%. Diagnostic accuracy was higher in samples collected in the first trimester (99.0%) than in those collected in the second (98.3%) or third (96.4%) trimesters.

Chitty et al (2014)⁷ published a prospective study from the U.K. that was not included in the Zhu et al (2014) meta-analysis. Samples from 2288 RhD-negative women who initiated prenatal care before 24 weeks of gestation were analyzed using *RHD* genotyping. Overall, the sensitivity of the test was 99.34% and the specificity was 94.91%. The likelihood of correctly detecting RhD status in the fetus increased with gestational age, with high levels of accuracy after 11 weeks. In samples taken before 11 completed weeks of gestation, the sensitivity was 96.85% and the specificity was 94.40%; at 14 to 17 weeks of gestation, the sensitivity was 99.67% and specificity was 95.34%. These findings of increased diagnostic accuracy as pregnancies advanced differ from those of the Zhu et al (2014) meta-analysis, which found the highest diagnostic accuracy in the first trimester.

Two key studies reporting on the clinical validity of fetal *RHD* genotyping with the Sequenom assay, which is commercially available in the U. S., are detailed next, and findings are summarized in Table 1.

Table 1. Sequenom SensiGene Clinical Validation Studies

Author	Accuracy for RhD Status Determination, %	False-Negative Rate RhD Determination, %
Moise et al (2012) ³ , Bombard et al (2011) ⁸	98.1%-99.1%, depending on trimester when test performed	0.45
Cohort 1	97.1	1.9
Cohort 2	99.5	0

RhD: Rhesus D.

Moise et al (2012) analyzed samples from 120 patients enrolled prospectively from multiple centers.³ All were RhD-negative pregnant patients with no evidence of alloimmunization. The samples were analyzed using the SensiGene Fetal RHD test using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry to detect control and fetal-specific DNA signals. The determination of fetal sex was defined as follows: three Y chromosome markers is a male fetus, two markers are inconclusive, and one or no marker is a female fetus. The algorithm for *RHD* determination was defined as follows: pseudogene present is inconclusive, three *RHD* markers present is an *RHD*-positive fetus, two markers present is inconclusive, one or no marker is

an *RHD*-negative fetus. If the results were *RHD*-positive and male, the fetus was determined to be *RHD*-positive and male, and if *RHD*-negative and male results were noted, the fetus was determined to be *RHD*-negative and male. If the results were *RHD*-positive and female, the fetus was determined to be *RHD*-positive and female. If an *RHD*-negative and female result was noted, reflex testing was performed with a panel of 92 single nucleotide variants. If a minimum of six informative paternal alleles (uniquely and unambiguously fetal in nature) were detected, the result was an *RHD*-negative, female fetus. If fewer than six alleles were detected, the sample was reported as inconclusive. Cord blood was obtained at delivery and RhD typing was determined using standard serologic methods, and phenotype assessment of the newborns was used to assign sex. The pregnant patients underwent planned venipunctures during 3 time periods in gestation: 11 to 13,^{6/7} 16 to 19,^{6/7} and 28 to 29^{6/7} weeks. At the second blood draw, two patients were not evaluated because they did not return during the prescribed gestational age window; and at the time of the third-trimester blood draw, seven patients did not have a sample obtained.

Median gestational ages of the first-, second-, and third-trimester samplings were 12.4 weeks (range, 10.6-13.9 weeks), 17.6 weeks (range, 16-20.9 weeks), and 28.7 weeks (range, 27.9-33.9 weeks), respectively. Three samples in the first trimester and two in the second trimester were insufficient in quantity to perform the DNA assay (1.4% of the total samples). Twenty-two samples (6.3% of the total samples; 2.5% of the patients) were deemed inconclusive. In 23% of these inclusive cases, there was an *RHD*-negative, female result, but an insufficient number of paternal single nucleotide variants detected to confirm the presence of fetal DNA. In the remaining 77% of the inconclusive results (4.8% of the total samples), the *RHD* pseudogene (*RHDy*) was detected, and the sample was deemed inconclusive. Erroneous results were observed for 6 (1.7%) of the samples, and included discrepancies in 4 (1.1%) *RHD* genotyping tests and 2 (0.6%) fetal sex determinations following data unblinding. Three cases of RhD typing were false-positives (cffDNA was *RHD*-positive but neonatal serology RhD-negative) and one case was a false-negative (cffDNA: *RHD*-negative but neonatal serology RhD-positive). Accuracy for determination of the *RHD* status of the fetus was 99.1%, 99.1%, and 98.1%, respectively for each of the 3 consecutive trimesters of pregnancy, and accuracy of fetal sex determination was 99.1%, 99.1%, and 100%, respectively.

Bombard et al (2011) analyzed the performance of the SensiGene Fetal RHD Genotyping test in 2 cohorts.⁸ Cohort 1 used as a reference point the clinical RhD serotype obtained from cord blood at delivery. Samples from cohort 2 were originally genotyped at one Sequenom location and results were used for clinical validation of genotyping performed at another Sequenom facility.

In cohort 1, *RHD* genotyping was performed on 236 maternal plasma samples from singleton, nonsensitized pregnancies with documented fetal RhD serology. The samples were obtained at 11 to 13 weeks of gestation. The ethnic origin of the pregnant women was white (77.1%), African (19.1%), mixed-race (3.4%), and South Asian (0.4%). Neonatal RhD phenotype, determined by serology at the time of birth, was positive in 69.1% of samples and negative in 30.9% of samples. In 2 (0.9%) of the 236 samples, the results were classified as invalid. In the 234 (99.1%) samples with sufficient DNA, the result was conclusive in 207 (88.5%) samples, inconclusive in 16 (6.8%) samples; and y-positive/*RHD* variant in 11 (4.7%) samples. In the 207 samples with a conclusive result, the neonatal RhD phenotype was positive in 142 (68.6%) samples and negative in 65 (31.4%) samples. The Fetal RHD Genotyping test correctly predicted the neonatal RhD phenotype in 201 (97.1%) of 207 samples (95% confidence interval [CI], 93.5% to 98.8%). In the 142 samples with RhD-positive fetuses, the test predicted that the fetus was positive in 138 and was negative in 4, for an RhD-positive sensitivity of 97.2% (95% CI, 93.0% to 98.9%). In 63 of the 65 samples with RhD-negative fetuses, the Fetal RHD Genotyping test predicted that the fetus was negative and, in the remaining 2, that it was positive, for an RhD-positive specificity of 96.9% (95% CI, 89.5% to 99.1%). The test predicted that the fetus was RhD-positive in 140 samples, of which 138 were predicted correctly, for a positive predictive value of 98.6% (95% CI, 94.9% to 99.6%). The test predicted that the fetus was RhD-negative in 67 samples, of which 63 were predicted

correctly, for a negative predictive value for RhD-positive fetuses of 94.0% (95% CI, 85.6% to 97.6%).

Cohort 2 consisted of 205 samples from 6 to 30 weeks of gestation. Testing sought to detect the presence of *RHD* exon sequences 4, 5, 7, the *RHDy*, and three, Y chromosome sequences (SRY, DBY, TTTY2), using Matrix-Assisted Laser Desorption Ionization-Time Of Flight mass spectrometry-based nucleic acid analysis (the Fetal RHD Genotyping laboratory-developed test). The laboratory performing the assays for both cohorts was blinded to the sex and fetal *RHD* genotype. In cohort 2, the test correctly classified 198 of 199 patients, for a test accuracy of 99.5%, with a sensitivity and specificity for prediction of *RHD* genotype of 100.0% and 98.3%, respectively.

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 two patients collected on the same day at a collection site. Overall test results were in agreement across all 3 trimesters ($p > 0.99$).

Section Summary: Clinically Valid

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 the 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.

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

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.

No published data were identified showing that fetal *RHD* genotyping leads to improved health outcomes.

Chain of Evidence

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 cfDNA 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 RhD-negative pregnant woman 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, which is 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.

Section Summary: Clinically Useful

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 patient, and avoidance of middle cerebral artery surveillance in an RhD-negative fetus. However, a certain percentage of RhD-negative women will develop alloimmunization to RhD-positive fetuses due to false-negative test results.

Summary of Evidence

For individuals who are pregnant and have RhD-negative blood type who receive noninvasive *RHD* genotyping of the fetus using cell-free DNA from maternal plasma, the evidence includes a meta-analysis and additional prospective studies (for clinical validity) and no direct evidence for clinical utility. The relevant outcomes are test validity, morbid events, medication use, and treatment-related morbidity. Clinical validity studies have demonstrated that the sensitivity and specificity of the test are high; however, the false-negative test rate, which is low, is not zero, potentially leading to alloimmunization of the RhD-negative mothers in these cases. It is uncertain whether *RHD* genotyping using cffDNA will lead to improved health outcomes. The evidence is insufficient to determine the effects of the technology on health outcomes.

Supplemental Information

Practice Guidelines and Position Statements

American Association of Blood Banks

The American Association of Blood Banks has not issued specific practice guidelines or recommendations on the use of fetal Rhesus D (*RHD*) genotyping.

American College of Obstetricians and Gynecologists

The American College of Obstetricians and Gynecologists (2018) reaffirmed its 2006 position that detection of fetal 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.^{10,11}

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."¹²

Sperling et al (2018) compared the guidelines from the American College of Obstetricians and Gynecologists as well as 3 international on the prevention of RhD alloimmunization.¹³ 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.

U.S. Preventive Services Task Force Recommendations

No U.S. Preventive Services Task Force recommendations addressing fetal *RHD* genotyping were identified.

Medicare National Coverage

There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

Ongoing and Unpublished Clinical Trials

A search of ClinicalTrials.gov in June 2019 did not identify any ongoing or unpublished phase 3 trials that would likely influence this review.

References

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Documentation for Clinical Review

- No records required

Coding

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy. Inclusion or exclusion of codes does not constitute or imply member coverage or provider reimbursement.

IE

The following services may be considered investigational.

Type	Code	Description
CPT®	81403	Molecular Pathology Procedure Level 4
HCPCS	None	
ICD-10 Procedure	None	

Policy History

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

Effective Date	Action	Reason
06/01/2016	BCBSA Medical Policy adoption	Medical Policy Committee
07/01/2017	Policy revision without position change	Medical Policy Committee
07/01/2018	Policy revision without position change	Medical Policy Committee
10/01/2019	Policy revision without position change	Medical Policy Committee

Definitions of Decision Determinations

Medically Necessary: A treatment, procedure, or drug is medically necessary only when it has been established as safe and effective for the particular symptoms or diagnosis, is not investigational or experimental, is not being provided primarily for the convenience of the patient or the provider, and is provided at the most appropriate level to treat the condition.

Investigational/Experimental: A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

Split Evaluation: Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment, procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

Prior Authorization Requirements (as applicable to your plan)

Within five days before the actual date of service, the provider must confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department. Please call (800) 541-6652 or visit the provider portal at www.blueshieldca.com/provider.

Disclaimer: This medical policy is a guide in evaluating the medical necessity of a particular service or treatment. Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.