# Medical Policy

Blue Cross Blue Shield Blue Care Network of Michigan

Nonprofit corporations and independent licensees of the Blue Cross and Blue Shield Association



***Current Policy Effective Date: 5/1/22**
**(See policy history boxes for previous effective dates)**

# Title: Genetic Testing-Fetal RHD Genotyping Using Maternal Plasma

## Description/Background

### ALLOIMMUNIZATION

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

### Rh Blood Groups

The (Rhesus) Rh 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 Rh-positive people) or homozygous (DD) (≈40% of Rh-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 among ethnic group and is 15% in white population, 5% to 8% in black population, 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 the black population, only 18% of RhD-negative individuals are homozygous for an RHD

deletion, and 66% of RhD-negative blacks have an inactive RHDψ. There are also numerous rare variants of the D antigen, which are recognized by weakness of expression of D and/or by 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 Rh-positive RBCs enter maternal circulation, and the Rh-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 cause destruction of 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 maternal immune response, concurrent ABO incompatibility, and fetal homozygosity versus heterozygosity for the D antigen. Therefore, although about 10% of pregnancies are Rh-incompatible, less than 20% of Rh-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 stem-cell transplantation.

**Consequences of Alloimmunization**

IgG antibody–mediated hemolysis of fetal RBCs, known as hemolytic disease of the fetus and newborn, varies in severity and can have a variety of 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, with the widespread adoption of antenatal and postpartum use of Rh immune globulin in developed countries, the result has been a major decrease in 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 cerebral injury.[3]

**Prevention of Alloimmunization**

There are 4 currently in use Rh immune globulin products available in the United States, all of which undergo micropore filtration to eliminate viral transmission.[3] To date, no reported cases of viral infection related to Rh immune globulin administration have been reported in the United States. Theoretically, the Creutzfeldt-Jakob disease agent could be transmitted by use of Rh immune globulin. Local adverse reactions may occur, including redness, swelling, and mild pain at the site of injection, and hypersensitivity reactions have been reported.

The American College of Obstetricians and Gynecologists (ACOG) and the American Association of Blood Banks (AABB) recommend the first dose of Rho(D) immune globulin (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] 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 of 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**

ACOG recommends that all pregnant women should be tested at the time of their first prenatal visit for ABO blood group typing and Rh-D type and be screened for the presence of anti-RBC antibodies. These laboratory tests should be repeated for each subsequent pregnancy. AABB also recommends that antibody screening be repeated before administration of anti-D immune globulin at 28 weeks of gestation, postpartum, and at the time of any event during pregnancy.

If the mother is determined to be Rh-negative, the paternal Rh status should also be determined at the initial management of a pregnancy. If paternity is certain and the father is Rh-negative, the fetus will be Rh-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 he is 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 Rh-status of the fetus is the next step.

Invasive and noninvasive testing methods to determine the Rh status of a fetus are available. These procedures use polymerase chain reaction (PCR) assays to assess the fetal cellular elements in amniotic fluid by amniocentesis or by chorionic villus sampling (CVS). Although CVS can be performed earlier in a pregnancy, amniocentesis is the preferred method because CVS is associated with disruption of the villi and the potential for larger fetomaternal hemorrhage and worsening alloimmunization if the fetus if RhD-positive. The sensitivity and specificity of fetal RHD typing by PCR 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. In 1998, Lo et al 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. 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 polymorphisms may be performed to determine whether the result is derived from fetal but not maternal DNA.

CffDNA testing to determine the fetal *RHD* genotype is standard of practice in many European countries.[3]

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## Regulatory Status

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). No genotyping tests were found. Laboratories that offer LDTs must be licensed by CLIA 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 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 (ψ) pseudogene in exon 4, and assay controls which are 3 targets on the Y chromosome (SRY, TTTY, DBY).

The company claims that the uses of its test include:

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

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## Medical Policy Statement

Fetal RHD genotyping using maternal plasma is experimental/investigational. It has not been scientifically demonstrated to improve patient clinical outcomes.

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## Inclusionary and Exclusionary Guidelines (Clinically based guidelines that may support individual consideration and pre-authorization decisions)

N/A

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## CPT/HCPCS Level II Codes *(Note: The inclusion of a code in this list is not a guarantee of coverage. Please refer to the medical policy statement to determine the status of a given procedure.)*

### *Established codes:*

N/A

### *Other codes (investigational, not medically necessary, etc.):*

81403

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## Rationale

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 FEMALES WITH RHD-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 [CVS] or amniocentesis) and administration of anti-D immunoglobulin.

The questions addressed in this evidence review include:
1. Does *RHD* genotyping reduce the need for invasive testing by CVS 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.

### Populations

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

### Interventions

The relevant intervention of interest is noninvasive *RHD* genotyping of the fetus using cell-free DNA from maternal plasma.

### Comparators

The relevant comparators of interest are 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 avoidance of invasive testing (CVS or amniocentesis) and avoidance of unnecessary administration of RhD immunoglobulin.

Potential harmful outcomes are those resulting from a 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).

**Review of Evidence**
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.[6] The investigators identified 37 studies conducted in RhD-negative pregnant women that were published by the end of 2013. The studies included a total of 11,129 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 those collected in the second (98.3%) or third (96.4%) trimesters.

Also in 2014, Chitty et al published a prospective study from the U.K. that was not included in the Zhu meta-analysis.[7] Samples from 2288 Rh-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. For example, for samples taken before 11 completed weeks of gestation, the sensitivity was 96.85% and the specificity was 94.40%, and at 14 to 17 weeks' gestation, sensitivity was 99.67% and specificity was 95.34%. The finding in the Chitty study of increased accuracy as pregnancies advanced differs from that of the Zhu meta-analysis, which found highest diagnostic accuracy in the first trimester.

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

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: 3 Y chromosome markers is a male fetus, 2 markers are inconclusive, and 1 or no marker is a female fetus. The algorithm for *RHD* determination was defined as follows: pseudogene present is inconclusive, 3 *RHD* markers present is an *RHD*-positive fetus, 2 markers present is inconclusive, 1 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 6 informative paternal alleles (uniquely and unambiguously fetal in nature) were detected, the result was an *RHD*-negative, female fetus. If fewer than 6 alleles were detected, the sample was reported as inconclusive. Cord blood was obtained at delivery and RhD typing was determined using standard serologic methods. 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, 16 to 19, and 28 to 29 weeks. At the second blood draw, 2 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, 7 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 to 13.9 weeks), 17.6 weeks (range, 16 to 20.9 weeks), and 28.7 weeks (range,

27.9 to 33.9 weeks), respectively. Three samples in the first trimester and 2 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 was *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 a single 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, and 7, the *RHDy*, and 3 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.

**Table 1. Sequenom SensiGene Clinical Validation Studies**

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

RhD: Rhesus D.

In 2016, Moise et al 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 *RHD*ψ 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>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 to 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 cell-free fetal DNA includes the following scenarios. In the RhD-negative, nonalloimmunized pregnant patient:

- Avoidance of unnecessary anti-D immune globulin if the fetus is Rh-negative.
- Avoidance of invasive procedure 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 procedure to obtain fetal tissue if Rh-negative pregnant woman is alloimmunized to determine fetal Rh status.
- Avoidance of serial antibody testing in the mother and middle cerebral artery surveillance of the fetus if the fetus is determined to be Rh-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 Rhesus D (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 analytic validity). Clinical validity studies have demonstrated that the sensitivity and specificity of the test are high; however, the false negative rate of the test, which is low, is not zero, potentially leading to alloimmunization of the Rh-negative mothers in these cases. It is uncertain whether *RHD* genotyping using cell free fetal DNA will lead to improved health outcomes. The evidence is insufficient to determine the effects of the technology on health outcomes.

## ONGOING AND UNPUBLISHED CLINICAL TRIALS

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

## SUPPLEMENTAL INFORMATION

### PRACTICE GUIDELINES AND POSITION STATEMENTS

**American College of Obstetricians and Gynecologists**

In 2018, the American College of Obstetricians and Gynecologists 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 it 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.

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## Government Regulations

### National:

There is no national coverage determination (NCD)

### Local:

There is no local coverage determination (LCD)

*(The above Medicare information is current as of the review date for this policy. However, the coverage issues and policies maintained by the Centers for Medicare & Medicare Services [CMS, formerly HCFA] are updated and/or revised periodically. Therefore, the most current CMS information may not be contained in this document. For the most current information, the reader should contact an official Medicare source.)*

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## Related Policies

N/A

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*The articles reviewed in this research include those obtained in an Internet based literature search for relevant medical references through January 2022, the date the research was completed.*

## Joint BCBSM/BCN Medical Policy History

| Policy Effective Date | BCBSM Signature Date | BCN Signature Date | Comments |
|---|---|---|---|
| 5/1/16 | 2/16/16 | 2/16/16 | Joint policy established |
| 5/1/17 | 2/21/17 | 2/21/17 | Routine policy maintenance, no change in policy status. |
| 5/1/18 | 2/20/18 | 2/20/18 | Updated rationale, added reference #9. No change in policy status. |
| 5/1/19 | 2/19/19 | | Routine policy maintenance, added references 11-13. No change in policy status. |
| 5/1/20 | 2/18/20 | | Routine policy maintenance. No added references. No change in policy status. |
| 5/1/21 | 2/16/21 | | Routine policy maintenance. No change in policy status. |
| 5/1/22 | 2/15/22 | | Routine policy maintenance. No change in policy status. |

Next Review Date: 1st Qtr. 2023

# BLUE CARE NETWORK BENEFIT COVERAGE
# POLICY: GENETIC TESTING-FETAL RHD GENOTYPING USING MATERNAL PLASMA

## I. Coverage Determination:

| | |
|---|---|
| **Commercial HMO (includes Self-Funded groups unless otherwise specified)** | Not covered |
| **BCNA (Medicare Advantage)** | See government section |
| **BCN65 (Medicare Complementary)** | Coinsurance covered if primary Medicare covers the service. |

## II. Administrative Guidelines:

- The member's contract must be active at the time the service is rendered.
- Coverage is based on each member's certificate and is not guaranteed. Please consult the individual member's certificate for details. Additional information regarding coverage or benefits may also be obtained through customer or provider inquiry services at BCN.
- The service must be authorized by the member's PCP except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Services must be performed by a BCN-contracted provider, if available, except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Payment is based on BCN payment rules, individual certificate and certificate riders.
- Appropriate copayments will apply. Refer to certificate and applicable riders for detailed information.
- CPT - HCPCS codes are used for descriptive purposes only and are not a guarantee of coverage.