Medical Policy

Blue Cross Blue Shield Blue Care Network of Michigan

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Title: Genetic Testing-Noninvasive Prenatal Testing for Fetal RBC Antigen Status

Description/Background

ALLOIMMUNIZATION

Alloimmunization, also called isoimmunization, refers to the development of antibodies against antigens from a different person of the same species which during pregnancy can result in hemolytic disease of the newborn. An example of this can be found in a pregnant individual whose blood type is Rh D-negative and who is exposed to Rh D-positive red blood cells (RBCs). This most commonly occurs from fetal-placental hemorrhage and entry of fetal blood cells into maternal circulation.

Rh Blood Groups

The (Rhesus) Rh system includes more than 50 antigen varieties found on RBCs. The most common antigens that induce antibodies are D, C, c, E, and e. There is no d antigen, but C and c, E and e are alternate alleles with codominant expression. Rh D is the most common and the most immunogenic. When people have the Rh D antigen on their RBCs, they are considered to be Rh D-positive; if their RBCs lack the antigen, they are considered to be Rh D-negative. The Rh D-antigen is inherited in an autosomally dominant fashion, and a person may be heterozygous (D-) (≈60% of Rh-positive people) or homozygous (DD) (≈40% of Rh-positive people). Homozygotes always pass the Rh D antigen to their offspring, whereas heterozygotes have a 50% chance of passing the antigen to their offspring. A person who is Rh D-negative does not have the Rh D antigen. Although nomenclature refers to Rh D-negative as dd, there is no small d antigen (i.e., they lack the *Rh D* gene and the corresponding Rh D antigen).

Rh D-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 Rh D-negative individuals are homozygous for a deletion of the *Rh D* gene. However, in the black population, only 18% of Rh D-negative individuals are homozygous for an Rh D deletion, and 66% of Rh D-negative blacks have an inactive RH Dψ. 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 Rh D-positive RBCs, can make antibodies to one or more epitopes of the D antigen.[1]

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

Causes of Alloimmunization

In addition to the Rh blood group antigens mentioned above, antigens from other blood group systems such as Colton, Diego, Duffy, Gerbich, Globoside, H, Kell, Mittenberger, and MNSs can cause alloimmunization that can lead to moderate to severe hemolytic disease of the fetus and newborn (HDFN).

By 30 days of gestation, the Rh D antigen is expressed on the RBC membrane, and alloimmunization can be caused when fetal Rh-positive RBCs enter maternal circulation, and the Rh D-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 Rh D-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 Rh D-positive fetal RBCs into the circulation of an Rh D-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 Rh D alloimmunization occurring at birth (15%-50%). Transplacental hemorrhage accounts for almost all cases of maternal Rh D 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 Rh D-positive blood and Rh D-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 Rh D Alloimmunization in the Setting of Rh Immune Globulin Shortage
The management of an Rh D-negative pregnant individual who is not alloimmunized and is carrying a known Rh D-positive fetus (or if fetal Rh status is unknown) would involve administration of Rh D immune globulin at standardized times during the pregnancy to prevent formation of anti-Rh D antibodies. If the individual is already alloimmunized, monitoring the levels of anti-Rh D antibody titers for the development of fetal anemia is performed. Both noninvasive and invasive tests to determine fetal Rh status exist.

There are currently 4 Rh immune globulin (RhIg) 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.

Although current American College of Obstetricians and Gynecologists (ACOG) guidance does not recommend routine use of noninvasive prenatal testing (NIPT) to determine fetal Rh(D) status based on cost-effectiveness analyses[18] the use of NIPT to prioritize use of RhIg and conserve RhIg supply is a reasonable consideration in the practice setting that is experiencing RhIg shortages. Noninvasive fetal red blood cell antigen genotyping utilizing cell-free DNA (cfDNA) isolated from maternal plasma has demonstrated high sensitivity and specificity for detection of fetal Rh(D) antigen status.[4-8] If cfDNA testing results confirm an Rh(D)-negative fetus, RhIg would not need to be routinely administered in the antepartum period (for bleeding, abortion, pregnancy loss, or at 28 weeks of gestation).

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 has 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 antibodies to red blood cell antigens in the maternal serum. The most common test for determining antibodies in serum is the indirect Coombs test.[3] Maternal serum is incubated with selected RBCs. Antibody present in the maternal serum will adhere to the selected RBCs. The RBCs are then washed and

suspended in Coombs serum, which is antihuman globulin. RBCs coated with maternal antibodies 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

An individual'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 RBC Antigen 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 or have a positive antibody screen that can be seen with HDFN, the paternal antigen status should then be determined. If paternity is certain and the father is negative for the antigen in question, the fetus will be antigen negative, and further assessment and intervention are unnecessary. If the father is positive for the antigen, he can be either homozygous or heterozygous. If he is homozygous for the implicated antigen and paternity is assured, then the fetus is assumed antigen positive and testing of the fetal antigen status is not necessary. If the paternal genotype is heterozygous for the antigen in question or is unknown, determination of the antigen status of the fetus is the next step.

Invasive and noninvasive testing methods to determine the antigen status of a fetus are available. Invasive 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 antigen positive. The sensitivity and specificity of fetal Rh D typing by PCR are reported as 98.7% and 100%, respectively, with positive and negative predictive values of 100% and 96.9%, respectively.[9] Invasive testing, addition to the risk of worsening of alloimmunization as stated above, includes risks of rupture of the membranes and loss of the pregnancy by miscarriage.

Noninvasive fetal red blood cell antigen genotyping using cell-free DNA (cfDNA) from maternal plasma has emerged as a reliable alternative to invasive genetic testing in the setting of maternal alloimmunization. In a recent meta-analysis involving a large cohort of 16 studies, pooled sensitivity and specificity for RH D detection were 99.3% (95% CI, 98.7–99.7%) and 98.4% (95% CI, 97.4–99.0%), respectively.[5]

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

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). Currently unavailable in the United States.

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

Another noninvasive Rh D test is the Unity Screen™ test from BillionToOne. In addition to testing for Rh D, the test evaluates the C, c, E, Fy^a, and K antigens, aneuploidy, and recessive conditions including cystic fibrosis, spinal muscular atrophy, sickle cell disease, alpha and beta thalassemia, and fragile X syndrome.

Natera, Inc., announced on May 1, 2024 the launch of a new cfDNA based fetal Rh D test. Natera's test can be performed as early as nine weeks gestation and determines fetal Rh D status from the blood of a pregnant patient, including complex pseudogene and Rh D-CE-D hybrid variants.

Medical Policy Statement

Non-invasive prenatal testing for fetal Rh D antigen in non-alloimmunized pregnancies is established when criteria are met.

Non-invasive prenatal testing for fetal RBC antigen in alloimmunized pregnancies is established when criteria are met.

Inclusionary and Exclusionary Guidelines

Inclusions:

Non-invasive prenatal testing for Rh D antigen in non-alloimmunized pregnant individuals when **ALL** of the following are met:

- The pregnant individual is confirmed Rh D-negative, **and**
- The father of the baby is Rh D-positive, unavailable for testing, or paternity is uncertain.

Non-invasive prenatal testing for fetal C, c, D, E, Fy^a (Duffy), and/or K (Kell) antigens in alloimmunized pregnant individuals when **ALL** of the following are met:

- Matenal alloantibody is detected for one or more of the following: D, C,c, E, Kell or Duffy, **and**
- The father of the baby's antigen status is positive heterozygote, unavailable for testing or paternity is uncertain, **and**
- Pregnant individual declines amniocentesis.

Testing is only covered for the specific antigen(s) the pregnant individual is positive for.

Exclusions:
Individuals not meeting the above criteria.

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:

81403 0488U 0494U

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

N/A

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 Non-ALLOIMMUNIZED PREGNANT INDIVIDUALS WITH RH D-NEGATIVE BLOOD TYPE AND TESTING OF PREGNANT INDIVIDUALS WHO ARE ALLOIMMUNIZED

Clinical Context and Test Purpose
The purpose of genetic testing of individuals who are pregnant and non alloimmunized and have Rh D-negative blood type and those that are alloimmunized is to determine the Rh D

antigen status of the fetus to guide pregnancy management including avoidance of administration of anti-D immunoglobulin and invasive testing (chorionic villus sampling [CVS] or amniocentesis).

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 Rh D-negative blood type who are non-alloimmunized and those that are pregnant and alloimmunized.

Interventions
The relevant intervention of interest is noninvasive *Rh D* genotyping and fetal antigen status of in alloimmunized pregnancies from maternal plasma

Comparators
Rh D The relevant comparators of interest are invasive methods to determine 1. fetal Rhesus (Rh) status in non-alloimmunized pregnancies and 2. fetal antigen status in alloimmunized pregnancies and management based on these results.

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 Rh D 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 Rh D immunoglobulins during pregnancy and unnecessary prenatal testing, both invasive and non-invasive. For non alloimmunized Rh D pregnancies, False-negative test results can lead to lack of Rh D immunoglobulin administration, development of maternal alloimmunization to Rh D, and current and future pregnancy complications due to maternal alloantibodies to Rh D. For alloimmunized pregnancies, false negative test results can lead to the development of hemolytic disease of the fetus and newborn which could result in death of the fetus or newborn.

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

Review of Evidence
In 2014, Zhu et al published a meta-analysis of studies on the diagnostic accuracy of noninvasive fetal Rh D genotyping using cell-free fetal DNA.[10] The investigators identified 37 studies conducted in Rh D-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 Rh D 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.[11] Samples from 2288 Rh-negative women who initiated prenatal care before 24 weeks of gestation were analyzed using Rh D genotyping. Overall, the sensitivity of the test was 99.34% and the specificity was 94.91%. The likelihood of correctly detecting Rh D 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.

In 2021, Alshehri et al published a systematic review and meta-analysis on NIPT's beneficial application, in conjunction with quantitative maternal alloantibody analysis, for early diagnosis of pregnancies at risk.[17] Nineteen eligible studies were critically appraised. Almost all NIPT studies were conducted prospectively through experimental estimation of fetal blood status in cross-sectional Rh D-negative pregnant women at specified time intervals. Only 2 studies had enrolled Rh D-negative pregnant participants retrospectively. NIPT was estimated highly sensitive/specific for fetal Rh D genotyping beyond 11-week gestation. The diagnostic performance of NIPT, for fetal Rh D -genotyping, was clearly illustrated through pooled sensitivity and specificity of the 16 included studies. Forest plots show high pooled diagnostic sensitivity and specificity; 95% confidence intervals (99.3% at 98.7-99.7% and 98.4% at 97.4-99%, respectively) being statistically significant at P -value < .00. They concluded that NIPT allows evidence-based provision of routine anti-D immunoprophylaxis and estimates potential fetal risks for guiding further interventions.

Moise et al (2012) analyzed samples from 120 patients enrolled prospectively from multiple centers.[3,] All were Rh D-negative pregnant patients with no evidence of alloimmunization. The samples were analyzed using the SensiGene Fetal Rh D 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 *Rh D* determination was defined as follows: pseudogene present is inconclusive, 3 *Rh D* markers present is an *Rh D*-positive fetus, 2 markers present is inconclusive, 1 or no marker is an *Rh D*-negative fetus. If the results were *Rh D*-positive and male, the fetus was determined to be *Rh D*-positive and male, and if *Rh D*-negative and male results were noted, the fetus was determined to be *Rh D*-negative and male. If the results were *Rh D*-positive and female, the fetus was determined to be *Rh D*-positive and female. If an *Rh D*-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 *Rh D*-negative, female fetus. If fewer than 6 alleles were detected, the sample was reported as inconclusive. Cord blood was obtained at delivery and Rh D 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 *Rh D*-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 *Rh D* pseudogene (*Rh D*) 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%) *Rh D* genotyping tests and 2 (0.6%) fetal sex determinations following data unblinding. Three cases of Rh D typing were false-positives (cffDNA was *Rh D*-positive but neonatal serology Rh D-negative) and one case was a false-negative (cffDNA was *Rh D*-negative but neonatal serology Rh D-positive). Accuracy for determination of the *Rh D* 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 Rh D Genotyping test in 2 cohorts.[12,] Cohort 1 used as a reference point the clinical Rh D 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, *Rh D* genotyping was performed on 236 maternal plasma samples from singleton, nonsensitized pregnancies with documented fetal Rh D 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 Rh D 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/*Rh D* variant in 11 (4.7%) samples. In the 207 samples with a conclusive result, the neonatal Rh D phenotype was positive in 142 (68.6%) samples and negative in 65 (31.4%) samples. The Fetal Rh D Genotyping test correctly predicted the neonatal Rh D phenotype in 201 (97.1%) of 207 samples (95% confidence interval [CI], 93.5% to 98.8%). In the 142 samples with Rh D-positive fetuses, the test predicted that the fetus was positive in 138 and was negative in 4, for an Rh D-positive sensitivity of 97.2% (95% CI, 93.0% to 98.9%). In 63 of the 65 samples with Rh D-negative fetuses, the Fetal Rh D Genotyping test predicted that the fetus was negative and, in the remaining 2, that it was positive, for an Rh D-positive specificity of 96.9% (95% CI, 89.5% to 99.1%). The test predicted that the fetus was Rh D-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 Rh D-negative in 67 samples, of which 63 were predicted correctly, for a negative predictive value for Rh D-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 *Rh D* exon sequences 4, 5, and 7, the *Rh Dy*, 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 Rh D Genotyping laboratory-developed test). The laboratory performing the assays for both cohorts was blinded to the sex and fetal *Rh D* 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 *Rh D* genotype of 100.0% and 98.3%, respectively.

Table 1. Sequenom SensiGene Clinical Validation Studies

Author	Accuracy for Rh D Status Determination	False-Negative Rate Rh D 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%

Rh D: Rhesus D.

In 2016, Moise et al analyzed blood samples collected in each trimester of pregnancy for 520 nonalloimmunized Rh D-negative patients in a prospective, observational study using the Fetal Rh D Genotyping test.[13] Inconclusive results secondary to the presence of the *RH Dψ* or an *Rh D* 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 Rh D (an Rh D-negative fetus with an *RH D*-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 Rh D-positive fetus with an *Rh D* 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).

Londero et al (2023) noted that fetal Rh D genotyping of cfDNA from Rh D-negative pregnant women can be used to guide anti-D prophylaxis: the knowledge of fetal Rh D type can direct and restrict the use of prenatal anti-D immunoglobulin exclusively to Rh D-negative women carrying a Rh D-positive fetus.[14] Since November 2019 in the region of Friuli Venezia Giulia (Italy) a pre-natal screening service has been offered to Rh D-negative women at 22 to 24 weeks of gestation. The cfDNA was extracted from a simple peripheral maternal blood sample to analyze the fetal Rh D gene: the results were interpreted as Rh D-positive fetus, Rh D-negative fetus, or Inconclusive. The service was shared with all regional hospitals and tests were provided free-of-charge by the National Health System. A total of 142 Rh D-negative pregnant women were recruited in nearly 2 years. Fetal Rh D genotyping was negative in 53 pregnancies, and positive in 89 pregnancies. The authors found the sensitivity of fetal Rh D genotyping using cfDNA to be 100% and specificity was 98%. Therefore, unnecessary treatment of pregnant women and exposure to a scarce plasma-derived medicinal product was avoided, by the use of a single blood sample, in 37.8 % of cases, representing 100 % of the Rh D-negative women carrying a Rh D-negative fetus in this cohort. The authors concluded

that the 1st Italian region-wide screening service for fetal Rh D genotyping has been implemented for 2 years, despite the COVID-19 pandemic, in order to obtain the predicted fetal Rh D phenotype before the 28th week of gestation, during which pre-natal prophylaxis is usually administered. Giving pre-natal anti-D immunoglobulin exclusively to Rh D-negative women carrying a Rh D-positive fetus reduced the overall use of anti-D immunoglobulin, which is becoming an ever more limited resource. The high sensitivity of the procedure provided evidence that the implementation of a diagnostic test in a reference laboratory guaranteed the quality of the results, the concordance of reports and the sustainability of costs, representing an excellent guide to targeted use of prophylactic use of RHIG in pregnancy, and customer satisfaction has been excellent.

Earlier based NIPT assays employed qualitative polymerase chain reaction technology with the assumption that an Rh D-negative fetus will be homozygous for the Rh D gene deletion. However, up to 30% of Rh D-negative Black individuals in the United States (U.S.), and 60% of Rh D-negative Africans, have non-deletion Rh D gene variants, which would not be detected by this tesing.[7,8] Due to this, Alford et al (2023) developed and validated a next generation sequencing-(NGS) based NIPT assay using quantitative counting template (QCT) technology to detect Rh D, C, c, E, K (Kell), and Fy^a (Duffy) fetal antigen genotypes from maternal blood samples in the ethnically diverse U.S. population. Quantitative counting template technology is employed to enable quantification and detection of paternally derived fetal antigen alleles in cfDNA with high sensitivity and specificity.[4] In an analytical validation, fetal antigen status was determined for 1,061 pre-clinical samples with a sensitivity of 100 % (95 % CI: 99 % to 100 %) and specificity of 100 % (95 % CI: 99 % to 100 %). Independent analysis of 2 duplicate plasma samples was carried out for 1,683 clinical samples, showing precision of 99.9 %. More importantly, in clinical practice the no-results rate was 0 % for 711 Rh D-negative non-allo-immunized pregnant people; and 0.1 % for 769 allo-immunized pregnancies. In a clinical validation, NIPT results were 100 % concordant with corresponding neonatal antigen genotype/serology for 23 Rh D-negative pregnant individuals, and 93 antigen evaluations in 30 allo-immunized pregnancies. Overall, this NGS-based fetal antigen NIPT assay had high performance that was comparable to invasive diagnostic assays in a validation study of a diverse U.S. population as early as 10 weeks of gestation, without the need for a sample from the biological partner. The authors concluded that these findings suggested that NGS-based fetal antigen NIPT may identify more fetuses at risk for hemolytic disease than current clinical practice, which relies on paternal genotyping and invasive diagnostics and thus, is limited by adherence rates and incorrect results due to non-paternity. Clinical adoption of NIPT for the detection of fetal antigens for both allo-immunized and Rh D-negative non-allo-immunized pregnant individuals may streamline care and reduce unnecessary treatment, monitoring, and patient anxiety.

Rego et al (2024) evaluated the accuracy of next-generation sequencing-based quantitative cell free DNA analysis for fetal antigen genotyping in individuals with alloimmunized pregnancies undergoing clinical testing in practices across the United States as early as 10 weeks of gestation, with the objective of identifying individuals with pregnancies at risk for hemolytic disease of the fetus and newborn and guiding management.[15] This prospective cohort study included patients with alloimmunized pregnancies undergoing clinical fetal antigen cell-free DNA analysis between 10 0/7 and 37 0/7 weeks of gestation at 120 clinical sites. Both the pregnant person with the alloimmunized pregnancy and the neonates resulting from the pregnancies were included. The laboratory issued the cell-free DNA results prospectively as a part of clinical care. After delivery, neonatal buccal swabs collected between 0 and 270 days of

life were sent to an outside independent laboratory for antigen geno-typing. The outside laboratory was blinded to the fetal cell-free DNA results, and the results were compared. Concordance was reported for the fetal antigen cell-free DNA analysis for antigens to which the pregnant person was alloimmunized and for all antigens for which the pregnant person was genotype negative. A total of 156 pregnant people who received clinically ordered cell-free DNA fetal antigen testing provided neonatal buccal swabs for genotyping after delivery. Overall, 15.4% of participants were Hispanic, 9.0% were non-Hispanic Black, 65.4% were non-Hispanic White, 4.5% were Asian, 1.3% were more than one race or ethnicity, and 4.5% were unknown. The median gestational age at the time of testing was 16.4 weeks with a median fetal fraction of 11.1 %. Concordance between cell-free DNA analysis results and neonatal genotype was determined for 465 antigen calls for the following antigens: K1 (n = 143), E (124), C (60), Fy^a (50), c (47), and D(Rh D) (41). These 465 calls included 145 in which the fetus was antigen positive and 320 in which the fetus was antigen negative. The authors observed complete concordance between prenatal fetal antigen cell-free DNA analysis results and neonatal genotypes for the 465 calls, resulting in 100% sensitivity, specificity, and accuracy.

Nino et al (2024) evaluated the performance of a next generation sequencing (NGS) with quantitative counting template (QCT) technology prenatal cell free DNA (cf DNA) assay in detecting the fetal Rh D genotype in a diverse Rh D-negative pregnant population in the United States (US).[16] A total of 401 non-alloimunized Rh D-negative pregnancies were included in the analysis. Fetal Rh D was detected in 261 cases (65%), whereas it was negative in 140 (35%). The D antigen cfDNA result was 100% concordant with the neonatal serology, resulting in 100% sensitivity and positive predictive value and (both 95% CI: 98.6%-100%) 100% specificity and negative predictive value (both 95% CI: 97.4%-100%). There were 10 pregnancies where the cfDNA analysis identified a non-RHO gene deletion, including Rh Dψ (n=5) and RH D-CE-D hybrid variants (n=5). A total of 616 doses of RhIG were administered. Despite the fact that the study occurred prior to the current RhIG shortage and the recent American College (ACOG) advisory change, there was a marked decrease in the use of antenatal RhIG based on cfDNA results. This decrease was greater at certain sites and at later study periods. If the cfDNA results were fully utilized during the entire study period, up to 147 RhIG doses (24% of administered doses) could have been avoided, indicating the importance of guideline changes to support the use of cfDNA for fetal Rh D detection to conserve this resource.

SUMMARY OF EVIDENCE

For individuals who are pregnant and have Rhesus D (Rh D)-negative blood type who receive noninvasive Rh D genotyping of the fetus using cell-free DNA from maternal plasma, early studies showed a high degree of sensitivity, 99-100%, and specificity, 95-100%. With the newer next generation sequencing based NIPT assay using quantitative counting template (QCT) technology, the sensitivity and specificity of this testing, in addition to testing for Rh C, c, E, K (Kell), and Fy^a (Duffy) fetal antigen genotypes, has been shown to have 100% sensitivity and specificity. In addition, two studies have evaluated the accuracy of this newer testing technology and have found complete concordance between prenatal fetal antigen cell-free DNA analysis results and neonatal genotypes for individuals with alloimmunized pregnancies and for the detection of fetal Rh D genotype in non-alloimmunized pregnancies. The evidence is sufficient to determine that the technology results in an improvement in the health outcome.

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

ACOG issued a clinical practice update, Paternal and Fetal Genotyping in the Management of Alloimmunization in Pregnancy,[19] (August 2024) in which they state:

- Determination of paternal genotype in pregnant patients with Rh-D alloimmunization: "Paternal Rh D zygosity testing using genotypic analysis is recommended for Rh-D alloimmunization risk assessment. It may be reasonable to defer or discontinue fetal surveillance for anemia in the setting of paternal genotyping that is Rh D homozygous negative"
- Determination of fetal genotype in pregnant patients with Rh-D alloimmunization: "Because cfDNA testing possesses performance characteristics that appear comparable with those of molecular testing, while avoiding the rare complications and costs associated with diagnostic genetic testing, it is reasonable to use it as an alternative tool for fetal RH D testing among alloimmunized patients with potentially at-risk pregnancies who decline amniocentesis"
- Determination of paternal and fetal genotyping in pregnant patients with non-Rh-D alloimmunization: "Cell-free DNA for the assessment of selected non-Rh-D red blood cell antigens may be considered for pregnant patients declining amniocentesis, after weighing cost, access, and the encouraging-yet-limited date supporting its use"

ACOG issued a Practice Advisory in March 2024, Rho(D) Immune Globulin Shortages, in which they state the following:[20]

- Although current ACOG guidance does not recommend routine use of noninvasive prenatal testing (NIPT) to determine fetal Rh(D) status based on cost-effectiveness analyses, the use of NIPT to prioritize use of RhIg and conserve RhIg supply is a reasonable consideration in the practice setting that is experiencing RhIg shortages.

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.)

Related Policies

- Genetic Testing-Noninvasive Prenatal Screening For Fetal Aneuploidies, Microdeletions, Single-Gene Disorders, and Twin Zygosity Using Cell-Free Fetal DNA

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The articles reviewed in this research include those obtained in an Internet based literature search for relevant medical references through September 2024, 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.
5/1/23	2/21/23		Routine policy maintenance, no change in policy status. (ds)
5/1/24	2/20/24		Routine policy maintenance, no change in status. Vendor managed: N/A (ds)
1/1/25	10/15/24		Status changed from E/I to established with criteria. Title change to “Genetic Testing-Non-Invasive Prenatal Testing for Fetal antigen Status. Added codes 0494U, 0488U as established, also code 81403 is now established. Vendor managed: N/A (ds)

Next Review Date: 4th Qtr. 2025

BLUE CARE NETWORK BENEFIT COVERAGE
POLICY: GENETIC TESTING-NONINVASIVE PRENATAL TESTING FOR FETAL RBC ANTIGEN STATUS

I. Coverage Determination:

Commercial HMO (includes Self-Funded groups unless otherwise specified)	Covered per policy
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.