BlueCross BlueShield of Alabama

Name of Policy:
Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA

Policy #: 554
Category: Laboratory
Latest Review Date: May 2018
Policy Grade: B

Background/Definitions:

As a general rule, benefits are payable under Blue Cross and Blue Shield of Alabama health plans only in cases of medical necessity and only if services or supplies are not investigational, provided the customer group contracts have such coverage.

The following Association Technology Evaluation Criteria must be met for a service/supply to be considered for coverage:

1. *The technology must have final approval from the appropriate government regulatory bodies;*
2. *The scientific evidence must permit conclusions concerning the effect of the technology on health outcomes;*
3. *The technology must improve the net health outcome;*
4. *The technology must be as beneficial as any established alternatives;*
5. *The improvement must be attainable outside the investigational setting.*

Medical Necessity means that health care services (e.g., procedures, treatments, supplies, devices, equipment, facilities or drugs) that a physician, exercising prudent clinical judgment, would provide to a patient for the purpose of preventing, evaluating, diagnosing or treating an illness, injury or disease or its symptoms, and that are:

1. *In accordance with generally accepted standards of medical practice; and*
2. *Clinically appropriate in terms of type, frequency, extent, site and duration and considered effective for the patient's illness, injury or disease; and*
3. *Not primarily for the convenience of the patient, physician or other health care provider; and*
4. *Not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of that patient's illness, injury or disease.*

Description of Procedure or Service:

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 Rh status of the fetus may guide subsequent management of the pregnancy. The use of cell-free fetal DNA in maternal blood has been proposed as a noninvasive method to determine fetal *RhD* genotype.

Alloimmunization

Alloimmunization refers to the development of antibodies in a patient whose blood type is 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 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 immune globulin at standardized times during the pregnancy to prevent 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. Both 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 Rh-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 among ethnic group and is 15% in whites, 5 to 8% in African Americans, 5% to 8%, 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 African-American population, only 18% of RhD-negative individuals are homozygous for an RHD deletion, and 66% of RhD-negative African Americans have an inactive *RHD* pseudogene (RHDy). 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.

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 red blood cell (RBC) membrane, and alloimmunization can be caused when fetal RhD-positive RBCs enter maternal circulation, and the RhD-negative mother develops anti-D antibodies. 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 ~10% of pregnancies are RhD-incompatible, <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 feto-maternal 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% to 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

Immunoglobulin (Ig) G 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 RhD 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.

Prevention of Alloimmunization

There are four RhD immune globulin products available in the U.S., all of which undergo micropore filtration to eliminate viral transmission. To date, no reported cases of viral infection related to RhD immune globulin administration have been reported in the U.S. Theoretically, the Creutzfeldt-Jakob disease (CJD) agent could be transmitted by use of RhD immune globulin. 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 (ACOG) and the American Association of Blood Banks (AABB) recommend the first dose of Rh_o(D) immune globulin (e.g., RhoGAM®) be given at 28 weeks' 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. 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 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 immune globulin at 28 weeks' 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 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 RhD-status of the fetus is the next step to determine the RhD compatibility 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 (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.

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 cell-free DNA in the plasma of first trimester pregnant women is of fetal origin, with this percentage rising to 6% in the third trimester. 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. ccfDNA has been proposed as a noninvasive alternative to obtaining fetal tissue by invasive methods, which are associated with a risk of miscarriage.

The large quantity of maternal DNA compared to 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. 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.

Cell-free fetal DNA testing to determine the fetal *RHD* genotype is standard of practice in many European countries.

Policy:

Noninvasive fetal *RHD* genotyping using cell-free fetal DNA does not meet Blue Cross and Blue Shield of Alabama's medical criteria for coverage and is considered **investigational**.

Blue Cross and Blue Shield of Alabama does not approve or deny procedures, services, testing, or equipment for our members. Our decisions concern coverage only. The decision of whether or not to have a certain test, treatment or procedure is one made between the physician and his/her patient. Blue Cross and Blue Shield of Alabama administers benefits based on the member's contract and corporate medical policies. Physicians should always exercise their best medical judgment in providing the care they feel is most appropriate for their patients. Needed care should not be delayed or refused because of a coverage determination.

Key Points:

This policy was created in 2013, with the most recent update using MEDLINE database. The most recent literature review was performed through March 5, 2018.

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.

Literature was sought on fetal *RHD* genotyping using cell-free fetal DNA in the following areas: clinical validity (ability to detect a variant in cell-free fetal DNA to determine RhD-negative or RhD-positive genotype status); and clinical utility (the impact of a variant on the management of patients and on relevant health outcomes).

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 RhesusD (RhD)-negative blood type is to determine the RhD status of the fetus to guide pregnancy management including avoidance of invasive testing (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 chorionic villus sampling (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 PICOTS were used to select literature to inform this review.

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

Interventions
Noninvasive *RHD* genotyping of the fetus using cell-free DNA from maternal plasma.

Comparators
Invasive methods to determine fetal Rh status and management based on maternal RhD status.

Outcomes
The potential beneficial outcomes of primary interest are avoidance of invasive testing (CVS or amniocentesis) and avoidance on 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.

Timing
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.

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

Simplifying Test Terms
There are three core characteristics for assessing a medical test. Whether imaging, laboratory, or other, all medical tests must be:

- Technically reliable
- Clinically valid
- Clinically useful.

Because different specialties may use different terms for the same concept, we are highlighting the core characteristics. The core characteristics also apply to different uses of tests, such as diagnosis, prognosis, and monitoring treatment.

Diagnostic tests detect presence or absence of a condition. Surveillance and treatment monitoring are essentially diagnostic tests over a time frame. Surveillance to see whether a condition develops or progresses is a type of detection. Treatment monitoring is also a type of detection because the purpose is to see if treatment is associated with the disappearance, regression, or progression of the condition.

Prognostic tests predict the risk of developing a condition in the future. Tests to predict response to therapy are also prognostic. Response to therapy is a type of condition and can be either a beneficial response or adverse response. The term predictive test is often used to refer to response to therapy. To simplify terms, we use prognostic to refer both to predicting a future condition or to predicting a response to therapy

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires 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).

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. Reviewers 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. Samples from 2288 RhD-negative women who initiated prenatal care before 24 weeks' 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%, and at 14 to 17 weeks' gestation, sensitivity was 99.67% and specificity was 95.34%. The findings of increased diagnostic accuracy as pregnancies advanced differ 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 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 2012	98.1-99.1, depending on trimester during which it was performed	.45
Bombard 2011		
Cohort 1	97.1	1.9
Cohort 2	99.5	0

RhD: Rhesus D.

In 2012, Moise and colleagues analyzed samples from 120 patients who were enrolled prospectively from multiple centers. All patients were RhD-negative pregnant patients with no evidence of alloimmunization. The samples were analyzed using the SensiGENE 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: three Y-chromosome markers=male fetus, two markers=inconclusive, and one or no markers=female fetus. The algorithm for RhD determination was: pseudogene present=inconclusive, three RhD markers present=RhD-positive fetus, two markers present=inconclusive, one or no markers= 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 (SNVs). 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 less 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 gender. The pregnant patients underwent planned venipunctures during three time periods in gestation: $11–13^{6/7}$, $16–19^{6/7}$, and $28–29^{6/7}$ 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 age of the first, second and third trimester samplings was 12.4 (range, 10.6–13.9) weeks, 17.6 (16–20.9) weeks and 28.7 (27.9–33.9) weeks, respectively. There were three samples in the first trimester and two samples in the second trimester insufficient in the quantity of samples 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 there were an insufficient number of paternal SNVs detected to confirm the presence of fetal DNA. In the remaining 77% of the inconclusive results (4.8% of the total samples), the *RHD* psi (y)-pseudogene was detected, and the sample was deemed inconclusive. Erroneous results were observed for six of the samples (1.7%), and included discrepancies in four RhD typings (1.1%) and two fetal sex determinations (0.6%) following data un-blinding. Three cases of RhD typing were false positives (ccffDNA was RhD-positive but neonatal serology RhD-negative) and one case was a

false negative (ccffDNA: 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 three consecutive trimesters of pregnancy, and accuracy of fetal sex determination was 99.1%, 99.1%, and 100%, respectively.

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

In cohort one, *RhD* genotyping was performed on 236 maternal plasma samples from singleton, non-sensitized pregnancies with documented fetal RhD serology. The samples were obtained at 11 to 13 weeks' gestation. 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 two (0.9%) of the 236 samples, there the results were classified as invalid. In the 234 (99.1%) samples with sufficient DNA extraction, the result was conclusive in 207 samples (88.5%); inconclusive in 16 samples (6.8%); and psi (+)/*RhD* variant in 11 samples (4.7%). In the 207 samples with a conclusive result, the neonatal RhD phenotype was positive in 142 samples (68.6%) and negative in 65 samples (31.4%). The RHD Genotyping test correctly predicted the neonatal RhD phenotype in 201 of 207 samples for an accuracy of 97.1% (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 in four that it was negative, for a sensitivity of prediction of RhD positivity of 97.2% (95% CI, 93.0 to 98.9). In 63 of the 65 samples with RhD-negative fetuses, the RHD Genotyping test predicted that the fetus was negative and, in the remaining two, that it was positive, for a specificity for the prediction of RhD positivity of 96.9% (95% CI, 89.5 to 99.1). The test predicted that the fetus was RhD-positive in 140 samples, of which, in 138 of these the prediction was correct, 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, in 63 of these the prediction was correct, for a negative predictive value for RhD-positive fetuses of 94.0% (95% CI, 85.6 to 97.6).

Cohort two consisted of 205 samples from 6 to 30 weeks' gestation. Testing was for the presence of *RhD* exon sequences 4, 5, 7, the psi-pseudogene, and three Y-chromosome sequences (SRY, DBY and TTTY2), using MALDI-TOF MS-(the RHD Genotyping laboratory developed test). The laboratory performing the assays for both cohorts was blinded to the sex and fetal *RhD* genotype. In cohort two, 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.

In 2016, Moise et al analyzed blood samples collected in each trimester of pregnancy in 520 non-alloimmunized RhD-negative patients in a prospective, observational study using the SensiGene RHD test. Inconclusive results secondary to the presence of the *RhD* pseudogene or an *RhD* variant were noted in 5.6%, 5.7%, and 6.1% of the first-, second-, and third-trimester samples, respectively. The incidence of 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. There was only one false-negative diagnosis (an RhD-positive fetus with an RhD-negative result), which occurred in the first trimester (0.32%; 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 1 of the collection sites. Overall test results were in agreement across all three trimesters ($p>0.99$).

Section Summary: Clinical Validity

The clinical sensitivity of *RhD* genotyping is high. However, there is variability in the sensitivity based on the trimester during which the test is performed. Clinical validation studies have found the false-negative rate 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, non-alloimmunized pregnant patient:

- Avoidance of unnecessary anti-D immune globulin if the fetus is RhD-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 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 immune globulin (e.g., RhoGAM) in RhD-negative mothers with Rh-negative fetuses. However, the false negative rate of the test, which is low, is not zero, and a certain percentage of RhD-negative women will develop alloimmunization to RhD-positive fetuses. Other issues that still need to be defined include the optimal timing of testing during the pregnancy.

Section Summary: Clinical Utility
Direct evidence of the clinical utility of *RhD* genotyping using cell-free fetal DNA is lacking. There is potential clinical utility in avoidance of unnecessary anti-D immune globulin 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 a 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 women 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 clinical utility). 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 rate of the test, 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 cell free fetal DNA will lead to improved health outcomes. The evidence is insufficient to determine the effects of the technology on health outcomes.

Practice Guidelines and Position Statements
American Association of Blood Banks
The American Association of Blood Banks (AABB) has not issued specific practice guidelines or recommendations on the use of fetal *RhD* genotyping.

American College of Obstetricians and Gynecologists (ACOG)
In 2016, the American College of Obstetricians and Gynecologists reaffirmed its 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.

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."

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. 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.

Key Words:
RHD genotyping, Rh genetic testing, SensiGene™, Fetal, RHD

Approved by Governing Bodies:
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). 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 (y) pseudogene in exon 4, and assay controls which are three targets on the Y chromosome (SRY, TTTY, DBY) using matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry-based nucleic acid analysis.

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
- Identify RhD (-) sensitized patients
- Avoid invasive testing by CVS or genetic amniocentesis

Benefit Application:
Coverage is subject to member's specific benefits. Group specific policy will supersede this policy when applicable.
ITS: Home Policy provisions apply.
FEP: Special benefit consideration may apply. Refer to member's benefit plan. FEP does not consider investigational if FDA approved and will be reviewed for medical necessity.

Current Coding:

CPT Codes:

81403	Molecular pathology procedure, Level 4 (e.g., analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons) including—*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
81479	Unlisted molecular pathology procedure

References:

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3. Chitty LS, Finning K, Wade A, et al. Diagnostic accuracy of routine antenatal determination of fetal RHD status across gestation: population based cohort study. BMJ. 2014; 349:g5243.
4. Committee on Practice B-O. Practice Bulletin No. 181: Prevention of Rh D Alloimmunization. Obstet Gynecol. Aug 2017; 130(2):e57-e70.
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11. Sperling JD, Dahlke JD, Sutton D, et al. Prevention of RhD alloimmunization: a comparison of four national guidelines. Am J Perinatol. Jan 2018; 35(2):110-119.
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13. Wikman AT, Tiblad E, Karlsson A et al. Noninvasive single-exon fetal RHD determination in a routine screening program in early pregnancy. Obstet Gynecol 2012; 120(2 Pt 1):227-34.

14. Zhu YJ, Zheng YR, Li L, et al. Diagnostic accuracy of non-invasive fetal RhD genotyping using cell-free fetal DNA: a meta analysis. J Matern Fetal Neonatal Med. Feb 10 2014.

Policy History:

Medical Policy Panel, November 2013
Medical Policy Group, September 2014 **(1):** New policy, previously only listed on Investigational Listing; remains investigational
Medical Policy Administration Committee, September 2014
Available for comment September 16 through October 31, 2014
Medical Policy Panel, November 2014
Medical Policy Group, November 2014 **(1):** Updates to Key Points, References, and Current Coding. No policy change.
Medical Policy Panel, November 2015
Medical Policy Group, January 2016 **(3):** 2015 Updates to Title, Key Points & Key Words; clarification to policy statement with no change in policy intent.
Medical Policy Panel, May 2017
Medical Policy Group, June 2017 **(3)**: 2017 Updates to Description, Key Points, Approved by Governing Bodies & References. No change in policy statement.
Medical Policy Panel, May 2018
Medical Policy Group, May 2018 **(4):** Updates to Key Points, and References. No change to policy statement.