2.04.108	Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA		
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Section:	2.0 Medicine	**Page:**	Page 1 of 14

Policy Statement

I. Measurement of cell-free DNA for fetal genotyping for RhD antigen may be **medically necessary** when **all** of the following criteria are met:
   A. Pregnancy may be at risk for alloimmunization due to maternal RhD negative status or the presence of maternal red cell antigen antibodies
   B. Paternal antigen typing is unavailable or heterozygous
   C. Amniocentesis is declined or contraindicated.

NOTE: Refer to Appendix A to see the policy statement changes (if any) from the previous version.

Policy Guidelines

Genetics Nomenclature Update

Plans may need to alter local coverage medical policy to conform to state law regarding coverage of biomarker testing.

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

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

Table PG1. Nomenclature to Report on Variants Found in DNA

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

Table PG2. American College of Medical Genetics and Genomics-Association for Molecular Pathology Standards and Guidelines for Variant Classification

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

Variant Classification	Definition
Benign	Benign change in the DNA sequence

Coding
See the Codes table for details.

Description

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

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 for clinical validity includes 2 meta-analyses and additional prospective studies and one retrospective cohort for clinical utility. Relevant outcomes are test validity, morbid events, medication use, and treatment-related morbidity. Clinical validity studies have demonstrated that the sensitivity of both currently available tests are 100% and the specificity of both tests is very high (99.3% to 100%). Prospective studies comparing outcomes in patients managed with and without the test are lacking. The American College of Obstetricians and Gynecologists endorses cell-free fetal DNA testing in pregnancies with when paternity status is unknown or heterozygous and in clinical circumstances when an alternative to amniocentesis is recommended or desired. The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

Related Policies

- N/A

Benefit Application

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

Some state or federal law may prohibit health plans from denying FDA-approved Healthcare Services as investigational or experimental. In these instances, Blue Shield of California may be obligated to determine if these FDA-approved Healthcare Services are Medically Necessary.

Regulatory Status

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). Laboratories that offer laboratory-developed tests 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.

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

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

At the time of the 2025 policy update, the availability of the SensiGene™ Fetal RHD Genotyping test could not be confirmed. The evidence review no longer considers this test.

Another noninvasive RhD test is the Unity Screen™ test from BillionToOne. In addition to testing for RhD, the test evaluates the C, c, D, 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. The Unity Screen test uses a proprietary technology (Quantitative Counting Templates) to quantify fetal DNA with as little as a single base pair alteration. The quantitative counting templates are traceable synthetic DNA fragments that are added to the patient's sample. After amplification, the number of fragments is added to a calculation that determines the number of DNA fragments of interest in the patient sample.

Natera offers an add-on fetal RhD test to its noninvasive prenatal Panorama™ test, which uses next-generation sequencing technology. The manufacturer states that Panorama is the only single nucleotide polymorphism-based noninvasive prenatal test. More than 13,000 single nucleotide polymorphisms are included in the screening test. The assay involves DNA primers to regions that specifically identify the RHD psi (ψ) pseudogene.

Rationale

Background

Alloimmunization

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

Rhesus Blood Groups

The Rhesus (Rh) system includes more than 100 antigen varieties found on RBCs. Rhesus D 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; approximately 60% of RhD-positive people) or homozygous (DD; approximately 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).

Rhesus D-negative status varies across ethnic groups and is 15% in White populations, 5% to 8% in Black populations, and 1% to 2% in Asians and Native Americans.

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

Rhesus D-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 occur when fetal RhD-positive RBCs enter maternal circulation and the RhD-negative mother develops anti-D antibodies.[2,] Once anti-D antibodies are present in a pregnant person's circulation, they can cross the placenta and destroy fetal RBCs.

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

Small fetomaternal hemorrhages of RhD-positive fetal RBCs into the circulation of an RhD-negative individual occur in nearly all pregnancies, and incidence of fetomaternal hemorrhage increases 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 cell transplantation.

Consequences of Alloimmunization

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

Cases of hemolysis in the newborn that do not result in fetal hydrops can still lead to kernicterus, a neurologic condition observed in infants with severe hyperbilirubinemia due to the deposition of

unconjugated bilirubin in the brain. Symptoms that manifest several days after delivery can include poor feeding, inactivity, loss of the Moro reflex, bulging fontanelle, and seizures. The 10% of infants who survive may develop spastic choreoathetosis, deafness, and/or mental retardation.

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

Prevention of Alloimmunization

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

The American College of Obstetricians and Gynecologists and the American Association of Blood Banks have recommended that the first dose of Rh_o(D) immunoglobulin (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 anti-RhD antibodies in the maternal serum. The most common test for determining antibodies in serum is the indirect Coombs test.[3,] The maternal serum is incubated with known RhD-positive RBCs. Any anti-RhD antibody present in the maternal serum will adhere to the RBCs. The RBCs are then washed and suspended in Coombs serum, which is antihuman globulin. Red blood cells 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

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 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 Rhesus D Status

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

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

or is unknown, determination of the RhD status of the fetus is the next step to assess the RhD compatibility of the pregnancy (first or any subsequent pregnancy).

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

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

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

Use of cffDNA testing to determine the fetal *RHD* genotype is the standard of care in many European countries.[3,]

Literature Review

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

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

Testing Pregnant Women with Rhesus D-negative Blood Type

Clinical Context and Test Purpose

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

The questions addressed in this evidence review include:

- Does *RHD* genotyping reduce the need for invasive testing by chorionic villus sampling or amniocentesis?
- Does *RHD* genotyping guide the administration of anti-D immunoglobulin during pregnancy?

- Does *RHD* genotyping lead to improved pregnancy outcomes?

The following PICO was used to select literature to inform this review.

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

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

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

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

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

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

Study Selection Criteria
For the evaluation of clinical validity, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology.
- Included a suitable reference standard.
- Patient/sample clinical characteristics were described.
- Patient/sample selection criteria were described.

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
Systematic Reviews
Mustafa et al (2025) published a systematic review and meta-analysis of 84 cohort studies (77 prospective, 7 retrospective) on the diagnostic accuracy of cell-free fetal DNA (cffDNA) for fetal *RHD* genotyping.[6,] Alloimmunized pregnancies were included in 10 studies, nonalloimmunized pregnancies were included in 66 studies, and a combination of alloimmunized and nonalloimmunized pregnancies were included in 8 studies. A total of 77,187 fetal antigen samples were evaluated in the included studies. Several laboratory methods were used including polymerase chain reaction, next-generation sequencing, and matrix-assisted laser desorption/ionization-time of flight. Overall, the

sensitivity and specificity of cffDNA were 99% and 99%, respectively. Sensitivity and specificity for each of the studied antigens (RhD, RhC, Rhc, RhE, Kell, and Duffy (Fy^a) were similar to the overall cohort.

Zhu et al (2014) published a meta-analysis of studies on the diagnostic accuracy of noninvasive fetal *RHD* genotyping using cffDNA.[7,] Reviewers identified 37 studies conducted in RhD-negative pregnant women that had been published by the end of 2013. The studies included 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 in those collected in the second (98.3%) or third (96.4%) trimesters.

Observational Studies

Chitty et al (2014) published a prospective study from the U.K. that was not included in the Zhu et al (2014) meta-analysis.[8,] Samples from 2288 RhD-negative women who initiated prenatal care before 24 weeks of gestation were analyzed using *RHD* genotyping. Overall, the sensitivity of the test was 99.34% and the specificity was 94.91%. The likelihood of correctly detecting RhD status in the fetus increased with gestational age, with high levels of accuracy after 11 weeks. In samples taken before 11 completed weeks of gestation, the sensitivity was 96.85% and the specificity was 94.40%; at 14 to 17 weeks of gestation, the sensitivity was 99.67% and specificity was 95.34%. These findings of increased diagnostic accuracy as pregnancies advanced differ from those of the Zhu et al (2014) meta-analysis, which found the highest diagnostic accuracy in the first trimester.

The Unity screen, which assesses RhD, K1, Fy^a, C, c, and E antigens, demonstrated 100% sensitivity and specificity in a validation study in 1683 clinical samples.[9,] Rego et al (2024) conducted a prospective cohort study of the Unity test in 156 individuals with an alloimmunized pregnancy who underwent clinical testing to determine their risk of hemolytic disease of the fetus/newborn.[10,] The pregnancies were alloimmunized to 191 antigens, most commonly E (34%), K1 (29.5%), RhD (26.3%), C (17.3%), c (12.8%), and Fy^a (2.6%). Both maternal cffDNA and neonatal DNA were analyzed. Concordance between cffDNA and neonatal genotype was 100% for the 190 antigens to which the patient was alloimmunized and for 465 antigens to with the patient had a negative genotype. Among these concordant results, the fetus was antigen positive in 145 of case and antigen negative in 320 cases. The test sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were all 100% with confidence intervals [CI] ranging from 96.0% to 100%.

Gilstrop Thompson et al (2025) published a clinical validation study of the add-on RHD genotype cffDNA assay as part of the Panorama prenatal screening test.[11,] The prospective cohort included 655 patients in the U.S. (74.0% White, 13.7% Hispanic, 7.0% Black, 2.1% Asian). Sensitivity and specificity of the assay were 100% (95% CI, 98.9% to 100%) and 99.3% (95% CI, 97.6% to 99.8%), respectively.

Section Summary: Clinically Valid

The clinical sensitivity of both currently available *RHD* genotyping tests is high. cfDNA testing possesses performance characteristics that appear comparable with those of molecular testing. False-negative results with cfDNA for RHD determination are rare (less than 1%) and attributable to either low levels of fetal DNA or genes
responsible for partial D or weak D phenotypes. Low levels of fetal DNA can be limited by restricting cfDNA testing to no earlier than the minimum gestational duration required by the specific test used. Partial D and weak D phenotypes are often, but not always, weakly immunogenic, further limiting the subset of pregnant patients with false negative results who are truly at risk of missing RhD immunoglobulin administration, development of maternal alloimmunization and fetal anemia.

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.

Review of Evidence

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.

Mateus-Nino et al (2025) conducted a retrospective cohort study of 410 pregnant individuals who underwent cffDNA genotyping with the Panorama test.[12,] The investigators found that sensitivity, specificity, positive predictive value, and negative predictive value were all 100%. Among patients with an RhD-positive fetus (n=261), Rh immune globulin was given in 93.1% of cases; among patients with an RhD-negative fetus (n=140), Rh immune globulin was given in 75.0% of cases (p<.001). Application of these results is limited due to lack of a control group (i.e., patients who did not undergo *RHD* genotyping). The authors also noted that rates of Rh immune globulin administration declined over time in patients with negative cffDNA genotype tests, which likely confounded the results.

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 cffDNA includes the following scenarios. In the RhD-negative, nonalloimmunized pregnant patient:

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

In the RhD-negative, alloimmunized pregnant patient:

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

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

Section Summary: Clinically Useful

Prospective and controlled evidence of the clinical utility of *RHD* genotyping using cffDNA is lacking. Clinical utility can be inferred from the avoidance of unnecessary anti-D immunoglobulin administration, avoidance of invasive procedures to determine fetal RhD status, avoidance of serial antibody testing in alloimmunized pregnant patients, and avoidance of middle cerebral artery surveillance in an RhD-negative fetus.

Supplemental Information

The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

Practice Guidelines and Position Statements

Guidelines or position statements will be considered for inclusion in 'Supplemental Information' if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

American College of Obstetricians and Gynecologists

In 2018, the American College of Obstetricians and Gynecologists reaffirmed its 2006 position that detection of fetal Rhesus D (RhD) using molecular analysis of maternal plasma or serum can be assessed in the second trimester with an accuracy greater than 99% but that this test is not a widely used clinical tool.[13,14,] This statement was last reaffirmed in 2024.

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."[15,] This statement was last reaffirmed in 2024.

Sperling et al (2018) compared the guidelines from the American College of Obstetricians and Gynecologists as well as 3 international guidelines on the prevention of RhD alloimmunization.[16,] 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.

In 2024, ACOG published updated guidance for management of alloimmunization in pregnancy.[17,] The update recommends fetal RhD antigen testing when the paternal genotype is heterozygous or unknown. Cell-free fetal DNA is described as an "alternative tool" for patients who are unwilling to undergo amniocentesis.

A 2024 Practice Advisory statement on RhD immune globulin shortages endorses using noninvasive prenatal testing with cell-free fetal DNA in the setting of a shortage to help with supply conservation efforts.[18,] Postpartum administration should be prioritized first, followed by 28 weeks of antepartum prophylaxis if there is sufficient supply.

U.S. Preventive Services Task Force Recommendations

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

Medicare National Coverage

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

Ongoing and Unpublished Clinical Trials

A search of ClinicalTrials.gov in July 2025 did not identify any ongoing or unpublished phase 3 trials that would likely influence this review.

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Documentation for Clinical Review

Please provide the following documentation:

- History and physical and/or consultation notes including:
  - Clinical findings (i.e., pertinent symptoms and duration)
  - Reason for procedure/test/device, when applicable
  - Past and present diagnostic testing and results

Post Service (in addition to the above, please include the following):

- Results/reports of tests performed
- Procedure report(s)

Coding

The list of codes in this Medical Policy is intended as a general reference and may not cover all codes. Inclusion or exclusion of a code(s) does not constitute or imply member coverage or provider reimbursement policy.

Type	Code	Description
CPT®	0198U	Red cell antigen (RH blood group) genotyping (RHD and RHCE), gene analysis Sanger/chain termination/conventional sequencing, RHD (Rh blood group D antigen) exons 1-10 and RHCE (Rh blood group CcEe antigens) exon 5 Includes Navigator RHD/CE Sequencing, Grifols Immunohematology Center, Grifols Immunohematology Center
	0222U	Red cell antigen (RH blood group) genotyping (RHD and RHCE), gene analysis, next-generation sequencing, RH proximal promoter, exons 1-10, portions of introns 2-3 Includes Navigator Rh Blood Group NGS, Grifols Immunohematology Center, Grifols Immunohematology Center
	0488U	Obstetrics (fetal antigen noninvasive prenatal test), cell-free DNA sequence analysis for detection of fetal presence or absence of 1 or more of the Rh, C, c, D, E, Duffy (Fya), or Kell (K) antigen in alloimmunized pregnancies, reported as selected antigen(s) detected or not detected Includes UNITY Fetal Antigen™ NIPT, BillionToOne Laboratory, BillionToOne, Inc
	0494U	Red blood cell antigen (fetal RhD gene analysis), next-generation sequencing of circulating cell-free DNA (cfDNA) of blood in pregnant individuals known to be RhD negative, reported as positive or negative Includes Rh Test, Natera™
	0536U	Red blood cell antigen (fetal RhD), PCR analysis of exon 4 of RHD gene and housekeeping control gene GAPDH from whole blood in pregnant individuals at 10+ weeks gestation known to be RhD negative, reported as fetal RhD status Includes Prenatal Detect RhD, Devyser Genomic Laboratories, Devyser AB
	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)
HCPCS	None	

Policy History

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

Effective Date	Action
06/01/2016	BCBSA Medical Policy adoption
07/01/2017	Policy revision without position change
07/01/2018	Policy revision without position change
10/01/2019	Policy revision without position change
04/01/2026	Policy reactivated. Previously archived from 04/01/2020 to 03/31/2026.

Feedback

Blue Shield of California is interested in receiving feedback relative to developing, adopting, and reviewing criteria for medical policy. Any licensed practitioner who is contracted with Blue Shield of California or Blue Shield of California Promise Health Plan is welcome to provide comments, suggestions, or concerns. Our internal policy committees will receive and take your comments into consideration. Our medical policies are available to view or download at www.blueshieldca.com/provider.

For medical policy feedback, please send comments to: MedPolicy@blueshieldca.com

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department at (800) 541-6652, or the Transplant Case Management Department at (800) 637-2066 ext. 3507708 or visit the provider portal at www.blueshieldca.com/provider.

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

Appendix A

POLICY STATEMENT	
BEFORE	**AFTER** **Blue font: Verbiage Changes/Additions**
Reactivated Policy **Policy Statement:** N/A	**Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA 2.04.108** **Policy Statement:** I. Measurement of cell-free DNA for fetal genotyping for RhD antigen may be **medically necessary** when **all** of the following criteria are met: A. Pregnancy may be at risk for alloimmunization due to maternal RhD negative status or the presence of maternal red cell antigen antibodies B. Paternal antigen typing is unavailable or heterozygous C. Amniocentesis is declined or contraindicated.