Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA

Policy # 00400
Original Effective Date: 02/19/2014
Current Effective Date: 03/08/2021

Applies to all products administered or underwritten by Blue Cross and Blue Shield of Louisiana and its subsidiary, HMO Louisiana, Inc.(collectively referred to as the "Company"), unless otherwise provided in the applicable contract. Medical technology is constantly evolving, and we reserve the right to review and update Medical Policy periodically.

Services Are Considered Investigational

Coverage is not available for investigational medical treatments or procedures, drugs, devices or biological products.

Based on review of available data, the Company considers noninvasive fetal RHD genotyping using cell-free fetal DNA to be **investigational.***

Policy Guidelines

Genetics Nomenclature Update

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. ACMG-AMP Standards and Guidelines for Variant Classification

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

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

Background/Overview

Alloimmunization

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

Rh Blood Groups

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

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

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

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

Causes of Alloimmunization

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

The production of anti-D antibodies in RhD-negative women is highly variable and significantly affected by several factors, including the volume of fetomaternal hemorrhage, the degree of the maternal immune response, concurrent ABO incompatibility, and fetal homozygosity vs

heterozygosity for the D antigen. Therefore, although about 10% of pregnancies are RhD-incompatible, less than 20% of RhD-incompatible pregnancies actually lead to maternal alloimmunization.

Small fetomaternal hemorrhages of RhD-positive fetal RBCs into the circulation of an RhD-negative woman occurs in nearly all pregnancies, and percentages of fetomaternal hemorrhage increase as the pregnancy progresses: 7% in the first trimester, 16% in the second trimester, and 29% in the third trimester, with the greatest risk of RhD alloimmunization occurring at birth (15%-50%). Transplacental hemorrhage accounts for almost all cases of maternal RhD alloimmunization.

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

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

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

Hemolytic disease in the fetus or newborn was once a major contributor to perinatal morbidity and mortality. However, the widespread adoption of antenatal and postpartum use of RhD

immunoglobulin in developed countries resulted in a major decrease in the frequency of this disease. In developing countries without prophylaxis programs, stillbirth occurs in 14% of affected pregnancies, and 50% of pregnancy survivors either die in the neonatal period or develop a cerebral injury.

Prevention of Alloimmunization
There are 4 RhD immunoglobulin 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 immunoglobulin administration have been reported in the U. S. Theoretically, the Creutzfeldt-Jakob disease agent could be transmitted by the use of RhD immunoglobulin. Local adverse reactions may occur, including redness, swelling, and mild pain at the site of injection, and hypersensitivity reactions.

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

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

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

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

Determining Fetal RhD Status

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

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

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. Fetal DNA cannot be separated from maternal DNA, but if the pregnant woman is RhD-negative, the presence of specific exons of the *RHD* gene, which are not normally present in the circulation of an RhD-negative patient, predicts an RhD-positive fetus. The cffDNA has been proposed as a noninvasive

alternative to obtaining fetal tissue by invasive methods, which are associated with a risk of miscarriage.

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. When Y chromosome-linked genes are not detected, tests for variants may be performed to determine whether the result is derived from fetal not maternal DNA.

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

FDA or Other Governmental Regulatory Approval

U.S. Food and Drug Administration (FDA)

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

Sequenom offers the 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) using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry-based nucleic acid analysis. The company claims that uses of its test include:

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

Rationale/Source

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

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

Supplemental Information

Practice Guidelines and Position Statements

American Association of Blood Banks

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

American College of Obstetricians and Gynecologists

In 2018, the American College of Obstetricians and Gynecologists reaffirmed its 2006 position that detection of fetal RhD using molecular analysis of maternal plasma or serum can be assessed in the second trimester with an accuracy greater than 99% but that 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.

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 May 2020 did not identify any ongoing or unpublished phase 3 trials that would likely influence this review.

References

1. Blue Cross and Blue Shield Association, Medical Policy Reference Manual, "Fetal RHD Genotyping Using Maternal Plasma", 2.04.108, 09:2020.
2. Daniels G, Finning K, Martin P, et al. Fetal RhD genotyping: a more efficient use of anti-D immunoglobulin. Transfus Clin Biol. Dec 2007; 14(6): 568-71. PMID 18436463
3. Moise K. Overview of Rhesus (Rh) alloimmunization in pregnancy. In: Lockwood CJ, ed. UpToDate. Waltham, MA: UpToDate; 2013.
4. Moise KJ, Argoti PS. Management and prevention of red cell alloimmunization in pregnancy: a systematic review. Obstet Gynecol. Nov 2012; 120(5): 1132-9. PMID 23090532
5. Van den Veyver IB, Moise KJ. Fetal RhD typing by polymerase chain reaction in pregnancies complicated by rhesus alloimmunization. Obstet Gynecol. Dec 1996; 88(6): 1061-7. PMID 8942854

6. Lo YM, Tein MS, Lau TK, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. Am J Hum Genet. Apr 1998; 62(4): 768-75. PMID 9529358
7. 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. Dec 2014; 27(18): 1839-44. PMID 24422551
8. 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. Sep 04 2014; 349: g5243. PMID 25190055
9. Bombard AT, Akolekar R, Farkas DH, et al. Fetal RHD genotype detection from circulating cell-free fetal DNA in maternal plasma in non-sensitized RhD negative women. Prenat Diagn. Aug 2011; 31(8): 802-8. PMID 21626507
10. Moise KJ, Gandhi M, Boring NH, et al. Circulating Cell-Free DNA to Determine the Fetal RHD Status in All Three Trimesters of Pregnancy. Obstet Gynecol. Dec 2016; 128(6): 1340-1346. PMID 27824757
11. American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No. 75: Management of alloimmunization during pregnancy. Obstet Gynecol. Aug 2006; 108(2): 457-64. PMID 16880320
12. ACOG Practice Bulletin No. 192 Summary: Management of Alloimmunization During Pregnancy. Obstet Gynecol. Mar 2018; 131(3): 611-612. PMID 29470338
13. Silver RM. Practice Bulletin No. 181: Prevention of Rh D Alloimmunization. Obstet Gynecol. Aug 2017; 130(2): e57-e70. PMID 28742673
14. 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. PMID 28910850

Policy History

Original Effective Date: 02/19/2014
Current Effective Date: 03/08/2021

02/06/2014	Medical Policy Committee review
02/19/2014	Medical Policy Implementation Committee approval. New policy
02/05/2015	Medical Policy Committee review
02/18/2015	Medical Policy Implementation Committee approval. No change to coverage.
08/03/2015	Coding update: ICD10 Diagnosis code section added; ICD9 Procedure code

	section removed.
02/04/2016	Medical Policy Committee review
02/17/2016	Medical Policy Implementation Committee approval. No change to coverage.
01/01/2017	Coding update: Removing ICD-9 Diagnosis Codes
02/02/2017	Medical Policy Committee review
02/15/2017	Medical Policy Implementation Committee approval. Title changed. "Maternal plasma" replaced with "cell-free fetal DNA" in the policy statement.
02/01/2018	Medical Policy Committee review
02/21/2018	Medical Policy Implementation Committee approval. No change to coverage.
02/07/2019	Medical Policy Committee review
02/20/2019	Medical Policy Implementation Committee approval. No change to coverage.
02/06/2020	Medical Policy Committee review
02/12/2020	Medical Policy Implementation Committee approval. No change to coverage.
02/04/2021	Medical Policy Committee review
02/10/2021	Medical Policy Implementation Committee approval. No change to coverage.

Next Scheduled Review Date: 02/2022

Coding

The five character codes included in the Blue Cross Blue Shield of Louisiana Medical Policy Coverage Guidelines are obtained from Current Procedural Terminology (CPT®)‡, copyright 2020 by the American Medical Association (AMA). CPT is developed by the AMA as a listing of descriptive terms and five character identifying codes and modifiers for reporting medical services and procedures performed by physician.

The responsibility for the content of Blue Cross Blue Shield of Louisiana Medical Policy Coverage Guidelines is with Blue Cross and Blue Shield of Louisiana and no endorsement by the AMA is intended or should be implied. The AMA disclaims responsibility for any consequences or liability attributable or related to any use, nonuse or interpretation of information contained in Blue Cross Blue Shield of Louisiana Medical Policy Coverage Guidelines. Fee schedules, relative value units, conversion factors and/or related components are not assigned by the AMA, are not part of CPT, and the AMA is not recommending their use. The AMA does not directly or indirectly practice medicine or dispense medical services. The AMA assumes no liability for data contained or not contained herein. Any use of CPT outside of Blue Cross Blue Shield of Louisiana Medical Policy

Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA

Policy # 00400
Original Effective Date: 02/19/2014
Current Effective Date: 03/08/2021

Coverage Guidelines should refer to the most current Current Procedural Terminology which contains the complete and most current listing of CPT codes and descriptive terms. Applicable FARS/DFARS apply.

CPT is a registered trademark of the American Medical Association.

Codes used to identify services associated with this policy may include (but may not be limited to) the following:

Code Type	Code
CPT	81403, 81479
HCPCS	No codes
ICD-10 Diagnosis	O36.0110-O36.0119, O36.0120-O36.0129, O36.0130-O36.0139, O36.0190-O36.0199, O36.0910-O36.0919, O36.0920-O36.0929, O36.0930-O36.0939, O36.0990-O36.0999, Z31.82, Z36.0-Z36.9

*Investigational – A medical treatment, procedure, drug, device, or biological product is Investigational if the effectiveness has not been clearly tested and it has not been incorporated into standard medical practice. Any determination we make that a medical treatment, procedure, drug, device, or biological product is Investigational will be based on a consideration of the following:

A. Whether the medical treatment, procedure, drug, device, or biological product can be lawfully marketed without approval of the U.S. Food and Drug Administration (FDA) and whether such approval has been granted at the time the medical treatment, procedure, drug, device, or biological product is sought to be furnished; or
B. Whether the medical treatment, procedure, drug, device, or biological product requires further studies or clinical trials to determine its maximum tolerated dose, toxicity, safety, effectiveness, or effectiveness as compared with the standard means of treatment or diagnosis, must improve health outcomes, according to the consensus of opinion among experts as shown by reliable evidence, including:
    1. Consultation with the Blue Cross and Blue Shield Association technology assessment program (TEC) or other nonaffiliated technology evaluation center(s);
    2. Credible scientific evidence published in peer-reviewed medical literature generally recognized by the relevant medical community; or
    3. Reference to federal regulations.

‡ Indicated trademarks are the registered trademarks of their respective owners.

NOTICE: If the Patient's health insurance contract contains language that differs from the BCBSLA Medical Policy definition noted above, the definition in the health insurance contract will be relied upon for specific coverage determinations.

NOTICE: Medical Policies are scientific based opinions, provided solely for coverage and informational purposes. Medical Policies should not be construed to suggest that the Company recommends, advocates, requires, encourages, or discourages any particular treatment, procedure, or service, or any particular course of treatment, procedure, or service.