



Louisiana

Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA

Policy # 00400

Original Effective Date: 02/19/2014

Current Effective Date: 02/21/2018

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**.*

Background/Overview

ALLOIMMUNIZATION

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

Rh blood groups

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

RhD-negative status varies among ethnic group and is 15% in whites, 5 to 8% in African Americans, 5% to 8%, and 1 to 2% in Asians and Native Americans, respectively.

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

©2018 Blue Cross and Blue Shield of Louisiana

Blue Cross and Blue Shield of Louisiana is an independent licensee of the Blue Cross and Blue Shield Association and incorporated as Louisiana Health Service & Indemnity Company.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, or otherwise, without permission from Blue Cross and Blue Shield of Louisiana.



Louisiana

Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA

Policy # 00400

Original Effective Date: 02/19/2014

Current Effective Date: 02/21/2018

for an RHD deletion, and 66% of RhD-negative African Americans have an inactive 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 RBC membrane, and alloimmunization can be caused when fetal Rh-positive RBCs enter maternal circulation, and the Rh-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 Rh-incompatible, <20% of Rh-incompatible pregnancies actually lead to maternal alloimmunization.

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

©2018 Blue Cross and Blue Shield of Louisiana

Blue Cross and Blue Shield of Louisiana is an independent licensee of the Blue Cross and Blue Shield Association and incorporated as Louisiana Health Service & Indemnity Company.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, or otherwise, without permission from Blue Cross and Blue Shield of Louisiana.



Louisiana

Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA

Policy # 00400

Original Effective Date: 02/19/2014

Current Effective Date: 02/21/2018

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 cerebral injury.

Prevention of alloimmunization

There are four currently in use Rh 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 Rh immune globulin administration have been reported in the U.S. Theoretically, the Creutzfeldt-Jakob disease (CJD) agent could be transmitted by use of Rh immune globulin. Local adverse reactions may occur, including redness, swelling, and mild pain at the site of injection, and hypersensitivity reactions have been reported.

The American College of Obstetricians and Gynecologists (ACOG) and the American Association of Blood Banks (AABB) recommend the first dose of 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.

©2018 Blue Cross and Blue Shield of Louisiana

Blue Cross and Blue Shield of Louisiana is an independent licensee of the Blue Cross and Blue Shield Association and incorporated as Louisiana Health Service & Indemnity Company.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, or otherwise, without permission from Blue Cross and Blue Shield of Louisiana.



Louisiana

Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA

Policy # 00400

Original Effective Date: 02/19/2014

Current Effective Date: 02/21/2018

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

Determining fetal RhD status

ACOG recommends that all pregnant women should be tested at the time of their first prenatal visit for ABO blood group typing and Rh-D type and be screened for the presence of anti-RBC antibodies. These laboratory tests should be repeated for each subsequent pregnancy. The AABB also recommends 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 Rh-negative, the paternal Rh status should also be determined at the initial management of a pregnancy. If paternity is certain and the father is Rh-negative, the fetus will be Rh-negative, and further assessment and intervention are unnecessary. If the father is RhD-positive, he can be either homozygous or heterozygous for the D allele. If he is homozygous for the D allele (i.e., D/D), then the fetus is RhD-positive. If the paternal genotype is heterozygous for Rh status or is unknown, determination of the Rh-status of the fetus is the next step.

Invasive and noninvasive testing methods to determine the Rh status of a fetus are available.

Invasive procedures use polymerase chain reaction (PCR) assays to assess the fetal cellular elements in amniotic fluid by amniocentesis or by chorionic villus sampling (CVS). Although CVS can be performed earlier in a pregnancy, amniocentesis is the preferred method because CVS is associated with disruption of the villi and the potential for larger fetomaternal hemorrhage and worsening alloimmunization if the fetus is 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.

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

©2018 Blue Cross and Blue Shield of Louisiana

Blue Cross and Blue Shield of Louisiana is an independent licensee of the Blue Cross and Blue Shield Association and incorporated as Louisiana Health Service & Indemnity Company.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, or otherwise, without permission from Blue Cross and Blue Shield of Louisiana.



Louisiana

Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA

Policy # 00400

Original Effective Date: 02/19/2014

Current Effective Date: 02/21/2018

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 (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). No genotyping tests were found. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the FDA has chosen not to require any regulatory review of this test.

Sequenom offers SensiGene™[‡] Fetal RHD Genotyping test, performed™[‡] by proprietary SEQuireDx™[‡] 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 3 targets on the Y chromosome (SRY, TTTY, DBY).

The company claims that the uses of its test include:

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

Centers for Medicare and Medicaid Services (CMS)

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

Rationale/Source

Literature was sought on fetal Rhesus D (*RHD*) genotyping using cffDNA in the following areas: analytic validity (ability to detect a variant that is known to be present and the ability to rule out variants when they are absent); clinical validity (ability to detect a variant in cffDNA to determine RhD-negative or RhD-positive genotype status); and clinical utility (the impact of knowing 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 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 CVS or amniocentesis?

©2018 Blue Cross and Blue Shield of Louisiana

Blue Cross and Blue Shield of Louisiana is an independent licensee of the Blue Cross and Blue Shield Association and incorporated as Louisiana Health Service & Indemnity Company.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, or otherwise, without permission from Blue Cross and Blue Shield of Louisiana.



Louisiana

Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA

Policy # 00400

Original Effective Date: 02/19/2014

Current Effective Date: 02/21/2018

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

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

Comparators

The relevant comparators of interest are invasive methods to determine fetal 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 of unnecessary administration of RhD immunoglobulin.

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

Timing

The time frame for outcomes measures varies from short-term development of maternal alloimmunization to RhD during first pregnancy and minimal-to-mild fetal or neonatal disease to long-term pregnancy complications in future pregnancies 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 and/or treated to prevent alloimmunization to RhD.

Analytic Validity

No studies were identified that provide direct evidence of the analytic validity of *RHD* genotyping. The commercially available test uses matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF)

©2018 Blue Cross and Blue Shield of Louisiana

Blue Cross and Blue Shield of Louisiana is an independent licensee of the Blue Cross and Blue Shield Association and incorporated as Louisiana Health Service & Indemnity Company.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, or otherwise, without permission from Blue Cross and Blue Shield of Louisiana.



Louisiana

Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA

Policy # 00400

Original Effective Date: 02/19/2014

Current Effective Date: 02/21/2018

mass spectrometry-based nucleic acid analysis. Neither the routine quality control procedures used for the test, nor the analytic performance metrics has been published.

Clinical Validity

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 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.

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 of gestation were analyzed using *RHD* genotyping. Overall, the sensitivity of the test was 99.34% and the specificity was 94.91%. The likelihood of correctly detecting RhD status in the fetus increased with gestational age, with high levels of accuracy after 11 weeks. For example, for samples taken before 11 completed weeks of gestation, the sensitivity was 96.85% and the specificity was 94.40%; at 14 to 17 weeks of gestation, sensitivity was 99.67% and specificity was 95.34%. The finding in the Chitty study of increased diagnostic accuracy as pregnancies advanced differs from that of the Zhu meta-analysis, which found highest diagnostic accuracy in the first trimester.

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

Table 1. Sequenom SensiGene Clinical Validation Studies

Author	Accuracy for RhD Status Determination	False-Negative Rate RhD Determination
Moise et al (2012)	98.1%-99.1%, depending on trimester when test performed	.45%
Bombard et al (2011)		
Cohort 1	97.1%	1.9%
Cohort 2	99.5%	0%

RhD: Rhesus D.

In 2012, Moise et al analyzed samples from 120 patients enrolled prospectively from multiple centers. All were RhD-negative pregnant patients with no evidence of alloimmunization. The samples were analyzed using the SensiGene Fetal RHD test using MALDI-TOF mass spectrometry to detect control and fetal-specific DNA signals. The determination of fetal sex was: 3 Y-chromosome markers= male fetus, 2 markers=inconclusive, and 1 or no markers=female fetus. The algorithm for *RHD* determination was: pseudogene present=inconclusive, 3 *RHD* markers present=*RHD*-positive fetus, 2 markers present=inconclusive, 1 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

©2018 Blue Cross and Blue Shield of Louisiana

Blue Cross and Blue Shield of Louisiana is an independent licensee of the Blue Cross and Blue Shield Association and incorporated as Louisiana Health Service & Indemnity Company.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, or otherwise, without permission from Blue Cross and Blue Shield of Louisiana.



Louisiana

Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA

Policy # 00400

Original Effective Date: 02/19/2014

Current Effective Date: 02/21/2018

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 6 informative paternal alleles (uniquely and unambiguously fetal in nature) were detected, the result was an *RHD*-negative, female fetus. If fewer than 6 alleles were detected, the sample was reported as inconclusive. Cord blood was obtained at delivery and RhD typing was determined using standard serologic methods, and phenotype assessment of the newborns was used to assign sex. The pregnant patients underwent planned venipunctures during 3 time periods in gestation: 11 to 13^{6/7}, 16 to 19^{6/7}, and 28 to 29^{6/7} weeks. At the second blood draw, 2 patients were not evaluated because they did not return during the prescribed gestational age window; and at the time of the third trimester blood draw, 7 patients did not have a sample obtained.

Median gestational ages of the first-, second-, and third-trimester samplings were 12.4 weeks (range, 10.6-13.9 weeks), 17.6 weeks (range, 16-20.9 weeks), and 28.7 weeks (range, 27.9-33.9 weeks), respectively. Three samples in the first trimester and 2 in the second trimester were insufficient in quantity to perform the DNA assay (1.4% of the total samples). Twenty-two samples (6.3% of the total samples; 2.5% of the patients) were deemed inconclusive. In 23% of these inclusive cases, there was an *RHD*-negative, female result, but an insufficient number of paternal 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* pseudogene (*RHDψ*)-pseudogene was detected, and the sample was deemed inconclusive. Erroneous results were observed for 6 (1.7%) of the samples, and included discrepancies in 4 (1.1%) *RHD* genotyping tests and 2 (0.6%) fetal sex determinations following data unblinding. Three cases of RhD typing were false positives (cffDNA was *RHD*-positive but neonatal serology RhD-negative) and 1 case was a false negative (cffDNA: *RHD*-negative but neonatal serology RhD-positive). Accuracy for determination of the *RHD* status of the fetus was 99.1%, 99.1%, and 98.1%, respectively for each of the 3 consecutive trimesters of pregnancy, and accuracy of fetal sex determination was 99.1%, 99.1%, and 100%, respectively.

In 2011, Bombard et al analyzed the performance of the SensiGene Fetal RHD Genotyping test in 2 cohorts. Cohort 1 used as a reference point the clinical RhD serotype obtained from cord blood at delivery. Samples from cohort 2 were originally genotyped at 1 Sequenom location and results were used for clinical validation of genotyping performed at another Sequenom facility.

In cohort 1, *RHD* genotyping was performed on 236 maternal plasma samples from singleton, nonsensitized pregnancies with documented fetal RhD serology. The samples were obtained at 11 to 13 weeks of gestation. Ethnic origin of the pregnant women was white (77.1%), African (19.1%), mixed race (3.4%), and South Asian (0.4%). Neonatal RhD phenotype, determined by serology at the time of birth, was positive in 69.1% of samples and negative in 30.9% of samples. In 2 (0.9%) of the 236 samples, the results were classified as invalid. In the 234 (99.1%) samples with sufficient DNA, the result was conclusive in 207 (88.5%) samples, inconclusive in 16 (6.8%) samples; and ψ (+)/*RHD* variant in 11 (4.7%) samples. In the 207 samples with a conclusive result, the neonatal RhD phenotype was positive in 142 (68.6%) samples and negative in 65 (31.4%) samples. The Fetal RHD Genotyping test correctly predicted the neonatal RhD

©2018 Blue Cross and Blue Shield of Louisiana

Blue Cross and Blue Shield of Louisiana is an independent licensee of the Blue Cross and Blue Shield Association and incorporated as Louisiana Health Service & Indemnity Company.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, or otherwise, without permission from Blue Cross and Blue Shield of Louisiana.



Louisiana

Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA

Policy # 00400

Original Effective Date: 02/19/2014

Current Effective Date: 02/21/2018

phenotype in 201 (97.1%) of 207 samples (95% confidence interval [CI], 93.5% to 98.8%). In the 142 samples with RhD-positive fetuses, the test predicted that the fetus was positive in 138 and was negative in 4, for an RhD-positive sensitivity of 97.2% (95% CI, 93.0% to 98.9%). In 63 of the 65 samples with RhD-negative fetuses, the Fetal RHD Genotyping test predicted that the fetus was negative and, in the remaining 2, that it was positive, for an RhD-positive specificity of 96.9% (95% CI, 89.5% to 99.1%). The test predicted that the fetus was RhD-positive in 140 samples, of which 138 were predicted correctly, for a positive predictive value of 98.6% (95% CI, 94.9% to 99.6%). The test predicted that the fetus was RhD-negative in 67 samples, of which 63 were predicted correctly, for a negative predictive value for RhD-positive fetuses of 94.0% (95% CI, 85.6% to 97.6%).

Cohort 2 consisted of 205 samples from 6 to 30 weeks of gestation. Testing sought to detect the presence of *RHD* exon sequences 4, 5, 7, the *RHD* ψ , and 3 Y-chromosome sequences (SRY, DBY, TTTY2), using MALDI-TOF MS-based nucleic acid analysis (the Fetal RHD Genotyping laboratory developed test). The laboratory performing the assays for both cohorts was blinded to the sex and fetal *RHD* genotype. In cohort 2, the test correctly classified 198 of 199 patients, for a test accuracy of 99.5%, with a sensitivity and specificity for prediction of *RHD* genotype of 100.0% and 98.3%, respectively.

In 2016, Moise et al analyzed blood samples collected in each trimester of pregnancy for 520 nonalloimmunized RhD-negative patients in a prospective, observational study using the Fetal RHD Genotyping test. Inconclusive results secondary to the presence of the *RHD* ψ or an *RHD* variant were noted in 5.6%, 5.7%, and 6.1% of the first-, second-, and third-trimester samples, respectively. The false-positive rates for RhD (an RhD-negative fetus with an *RHD*-positive result) was 1.54% (95% CI, 0.42% to 5.44%), 1.53% (95% CI, 0.42% to 5.40%), and 0.82% (95% CI, 0.04% to 4.50%), respectively, across the 3 trimesters. There was only 1 (0.32%) false-negative diagnosis (an RhD-positive fetus with an *RHD*-negative result), which occurred in the first trimester (95% CI, 0.08% to 1.78%). Genotyping for mismatches across repeated samples revealed that this error was related to mislabeling of samples from 2 patients collected on the same day at a collection site. Overall test results were in agreement across all 3 trimesters ($p > 0.99$).

Section Summary: Clinical Validity

The clinical sensitivity of *RHD* genotyping is high. However, there is variability in the sensitivity based on the trimester when the test is performed. Clinical validation studies have found the false-negative rates ranging from 0.5% to 2.0%. False-negative results in this clinical context would lead to lack of RhD immunoglobulin administration, development of maternal alloimmunization to RhD, and current and future pregnancy complications due to maternal alloantibodies to RhD compared to standard management of RhD-negative pregnant women.

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.

©2018 Blue Cross and Blue Shield of Louisiana

Blue Cross and Blue Shield of Louisiana is an independent licensee of the Blue Cross and Blue Shield Association and incorporated as Louisiana Health Service & Indemnity Company.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, or otherwise, without permission from Blue Cross and Blue Shield of Louisiana.



Louisiana

Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA

Policy # 00400

Original Effective Date: 02/19/2014

Current Effective Date: 02/21/2018

- 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 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.

No published data were identified showing that fetal *RHD* genotyping leads to improved health outcomes. This type of testing could lead to the avoidance of the use of anti-D immunoglobulin (eg, RhoGAM) in RhD-negative mothers with RhD-negative fetuses. However, the false-negative test rate, which is low, is not zero, and a certain percentage of RhD-negative women will develop alloimmunization to RhD-positive fetuses. Other issues that need to be defined include the optimal timing of testing during the pregnancy.

Section Summary: Clinical Utility

Direct evidence of the clinical utility of *RHD* genotyping using cfDNA is lacking. There is potential clinical utility in avoidance of unnecessary anti-D immunoglobulin administration, avoidance of invasive procedures to determine fetal RhD status, avoidance of serial antibody testing in alloimmunized pregnant patient, and avoidance of middle cerebral artery surveillance in an RhD-negative fetus. However, a certain percentage of RhD-negative women will develop alloimmunization to RhD-positive fetuses due to false-negative test results.

SUMMARY OF EVIDENCE

For individuals who are pregnant and have RhD-negative blood type who receive noninvasive *RHD* genotyping of the fetus using cell-free DNA from maternal plasma, the evidence includes a meta-analysis and additional prospective studies (for clinical validity) and no direct evidence (for analytic validity). Relevant outcomes are test accuracy and 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.

References

1. Blue Cross and Blue Shield Association, Medical Policy Reference Manual, "Fetal *RHD* Genotyping Using Maternal Plasma", 2.04.108, 05:2017.
2. Daniels G, Finning K, Martin P et al. Fetal RhD genotyping: a more efficient use of anti-D immunoglobulin. *Transfus Clin Biol* 2007; 14(6):568-71.
3. Moise K. Overview of Rhesus (Rh) alloimmunization in pregnancy. In: UpToDate, Lockwood CJ, ed. *UpToDate*. Waltham, MA, 2013.

©2018 Blue Cross and Blue Shield of Louisiana

Blue Cross and Blue Shield of Louisiana is an independent licensee of the Blue Cross and Blue Shield Association and incorporated as Louisiana Health Service & Indemnity Company.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, or otherwise, without permission from Blue Cross and Blue Shield of Louisiana.



Louisiana

Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA

Policy # 00400

Original Effective Date: 02/19/2014

Current Effective Date: 02/21/2018

4. Moise KJ, Jr., Argoti PS. Management and prevention of red cell alloimmunization in pregnancy: a systematic review. *Obstet Gynecol* 2012; 120(5):1132-9.
5. Van den Veyver IB, Moise KJ, Jr. Fetal RhD typing by polymerase chain reaction in pregnancies complicated by rhesus alloimmunization. *Obstet Gynecol* 1996; 88(6):1061-7.
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* 1998; 62(4):768-75.
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.* Feb 10 2014. 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.* 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* 2011; 31(8):802-8.
10. American College of O, Gynecologists. ACOG Practice Bulletin No. 75: Management of alloimmunization during pregnancy. *Obstet Gynecol* 2006; 108(2):457-64.
11. Moise KJ, Jr., 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

Policy History

Original Effective Date: 02/19/2014

Current Effective Date: 02/21/2018

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.
Next Scheduled Review Date:	02/2019

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 2017 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,

©2018 Blue Cross and Blue Shield of Louisiana

Blue Cross and Blue Shield of Louisiana is an independent licensee of the Blue Cross and Blue Shield Association and incorporated as Louisiana Health Service & Indemnity Company.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, or otherwise, without permission from Blue Cross and Blue Shield of Louisiana.



Louisiana

Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA

Policy # 00400

Original Effective Date: 02/19/2014

Current Effective Date: 02/21/2018

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 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. 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: 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.

©2018 Blue Cross and Blue Shield of Louisiana

Blue Cross and Blue Shield of Louisiana is an independent licensee of the Blue Cross and Blue Shield Association and incorporated as Louisiana Health Service & Indemnity Company.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, or otherwise, without permission from Blue Cross and Blue Shield of Louisiana.