MEDICAL POLICY

POLICY TITLE	NONINVASIVE FETAL *RHD* GENOTYPING USING CELL-FREE FETAL DNA
POLICY NUMBER	MP 2.261

CLINICAL BENEFIT	☐ MINIMIZE SAFETY RISK OR CONCERN. ☒ MINIMIZE HARMFUL OR INEFFECTIVE INTERVENTIONS. ☐ ASSURE APPROPRIATE LEVEL OF CARE. ☐ ASSURE APPROPRIATE DURATION OF SERVICE FOR INTERVENTIONS. ☐ ASSURE THAT RECOMMENDED MEDICAL PREREQUISITES HAVE BEEN MET. ☐ ASSURE APPROPRIATE SITE OF TREATMENT OR SERVICE.
Effective Date:	**4/1/2025**

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I. POLICY

Noninvasive fetal Rhesus D (RHD*)* genotyping using cell-free fetal DNA is considered **investigational**. There is insufficient evidence to support a general conclusion concerning the health outcomes or benefits associated with this procedure.

II. PRODUCT VARIATIONS

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This policy is only applicable to certain programs and products administered by Capital Blue Cross please see additional information below, and subject to benefit variations as discussed in Section VI below.

FEP PPO- Refer to FEP Medical Policy Manual. The FEP Medical Policy Manual can be found at: https://www.fepblue.org/benefit-plans/medical-policies-and-utilization-management-guidelines/medical-policies

III. DESCRIPTION/BACKGROUND

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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. The use of cell-free fetal DNA in maternal blood has been proposed as a noninvasive method to determine fetal *RHD* genotype.

ALLOIMMUNIZATION

Alloimmunization refers to the development of antibodies in a patient whose blood type is 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 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 formation of anti-RhD antibodies. If the patient is already alloimmunized, monitoring the levels of anti-RhD antibody titers and 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 (i.e., they lack the *RHD* gene and the corresponding RhD antigen).

RhD-negative status varies across ethnic group 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 absence of some of the epitopes of D. Some individuals with variant D antigens, if exposed to RhD-positive RBCs, can make antibodies to 1 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 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 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 (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 (Ig) 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 cerebral injury.

Prevention of Alloimmunization

There are 4 RhD immunoglobulin products available in the United States, 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 United States. Theoretically, the Creutzfeldt-Jakob disease agent could be transmitted by 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 (ACOG) and the American Association of Blood Banks (AABB) have recommended the first dose of Rh(D) immunoglobulin (e.g., 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. 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

ACOG 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. AABB 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 the pregnancy (first or any subsequent pregnancy).

Invasive and noninvasive testing methods to determine the RhD status of a fetus are available. These procedures use polymerase chain reaction (PCR) assays to assess the fetal cellular elements in amniotic fluid by amniocentesis or 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 if RhD-positive. The sensitivity and specificity of fetal *RHD* genotyping 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 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. 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 polymorphisms may be performed to determine whether the result is derived from fetal but not maternal DNA.

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

REGULATORY STATUS

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

Sequenom offers SensiGene™ Fetal RHD Genotyping test, performed by proprietary SEQureDx™ technology. The assay targets exons 4, 5, and 7 of the Rhesus D (*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 (-) fetus in mothers who are opposed to immunization(s) and vaccines
- Identifying RhD (-) sensitized patients
- Avoiding invasive testing by chorionic villus sampling or genetic amniocentesis.

IV. RATIONALE

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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 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.

V. DEFINITIONS

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NA

VI. BENEFIT VARIATIONS

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The existence of this medical policy does not mean that this service is a covered benefit under the member's health benefit plan. Benefit determinations are based on the applicable health benefit plan language. Medical policies do not constitute a description of benefits. Members and providers should consult the member's health benefit plan for information or contact Capital Blue Cross for benefit information.

VII. DISCLAIMER

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Capital Blue Cross' medical policies are developed to assist in administering a member's benefits. These medical policies do not constitute medical advice and are subject to change. Treating providers are solely responsible for medical advice and treatment of members. Members should discuss any medical policy related to their coverage or condition with their provider and consult their benefit information to determine if the service is covered. If there is a discrepancy between this medical policy and a member's benefit information, the benefit information will govern. If a provider or a member has a question concerning the application of this medical policy to a specific member's plan of benefits, please contact Capital Blue Cross' Provider Services or Member Services. Capital Blue Cross considers the information contained in this medical policy to be proprietary and it may only be disseminated as permitted by law.

VIII. CODING INFORMATION

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Note: This list of codes may not be all-inclusive, and codes are subject to change at any time. The identification of a code in this section does not denote coverage as coverage is determined by the terms of member benefit information. In addition, not all covered services are eligible for separate reimbursement.

Investigational for all indications:

Procedure Codes								
0198U	0222U	0494U	0536U	81403				

IX. REFERENCES

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15. *Blue Cross Blue Shield Association Medical Policy Reference Manual. 2.04.108,*

POLICY TITLE	NONINVASIVE FETAL *RHD* GENOTYPING USING CELL-FREE FETAL DNA
POLICY NUMBER	MP 2.261

Noninvasive Fetal RHD Genotyping Using Cell-Free Fetal DNA. September, 2023

X. POLICY HISTORY

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MP 2.261	**01/29/2020 Consensus Review.** No changes to policy statements. References reviewed; no updates needed.
	09/08/2020 Administrative Update. New code 0222U added to policy.
	01/12/2021 Consensus Review. No changes to policy statements. References updated. Added CPT 0198U as investigational.
	11/15/2022 Consensus Review. No changes to policy statements. References updated.
	12/21/2023 Consensus Review. No changes to policy statement. Updated references. Coding reviewed, no changes.
	09/23/2024 Administrative Update. New code 0494U added effective 10/01/2024.
	12/13/2024 Consensus Review. No changes to policy statement. Reviewed references. Coding reviewed, no changes.
	03/12/2025 Administrative Update. Added new code 0536U to code table.

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