ONTARIO HEALTH TECHNOLOGY ASSESSMENT SERIES

Noninvasive Fetal RhD Blood Group Genotyping: A Health Technology Assessment

KEY MESSAGES

What Is This Health Technology Assessment About?

RhD incompatibility occurs in pregnancy when the fetus's blood type is RhD positive (RhD+) and the mother's is RhD negative (RhD−). As a result, the mother's immune system creates antibodies to the RhD+ red blood cells. In the event of another RhD incompatible pregnancy, these antibodies can attack the fetus's blood cells. This can cause serious, sometimes fatal, health problems for the baby before or after birth.

As a precaution, all RhD− pregnant people receive a shot that prevents "anti-D" antibodies from developing. If antibodies do develop, any future pregnancies are closely monitored. But the injections or monitoring are unnecessary in pregnancies without RhD incompatibility. A blood test called noninvasive fetal RhD blood group genotyping (noninvasive fetal RhD genotyping) can look at fetal DNA in the mother's blood and see if the pregnancy is RhD incompatible.

This health technology assessment looked at how accurate, clinically useful, and cost-effective this genotyping test is for guiding the care of RhD− pregnancies. It also looked at the budget impact of publicly funding the test and at the experiences, preferences, and values of patients and health care providers related to care for RhD incompatible pregnancies.

What Did This Health Technology Assessment Find?

With current routine prenatal screening for blood group antibodies, noninvasive fetal RhD blood group genotyping is an accurate test to identify RhD incompatibility. In RhD− pregnancies without antibodies, testing can reduce the use of unnecessary preventive treatment and, in pregnancies with antibodies, it can reduce unnecessary monitoring where there is no RhD incompatibility.

For managing RhD− pregnancies without antibodies, noninvasive fetal RhD genotyping would generally not be viewed as cost-effective compared with usual care, unless the cost of testing is much lower than what is proposed now. For managing RhD− pregnancies with antibodies, noninvasive fetal RhD genotyping is cost saving (i.e., less costly and more effective than usual care). Publicly funding noninvasive fetal RhD genotyping in RhD− pregnancies without antibodies in Ontario would cost an additional $14.8 million in total over the next 5 years. At the same time, the test could result in savings of more than $50 million by avoiding unnecessary monitoring in pregnancies with antibodies.

Patients and health care providers generally felt positively about the potential use of noninvasive fetal RhD genotyping. Patients we spoke with said they would want assurances the test is safe. Patients and clinical experts also raised ethical questions about the risks and benefits of widespread use of this genetic test.

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A NOTE ABOUT TERMINOLOGY

As a government agency, Ontario Health can play an active role in ensuring that people of all identities and expressions recognize themselves in what they read and hear from us. We recognize that gender identities are individual and that many people who give birth do not identify as women, despite being assigned female sex at birth. Thus, in this health technology assessment, we use gender-inclusive pronouns and terms as much as possible. However, when citing published literature that uses the terms “woman,” “women,” “mother,” “pregnancy,” or “maternal,” we also use these terms for consistency with these cited studies.

ABSTRACT

Background

RhD blood group incompatibility during pregnancy can cause serious health problems for the fetus. Noninvasive fetal RhD blood group genotyping is a test for fetal RhD status that may help prevent unnecessary preventive treatment (Rh immunoglobulin [RhIG] injections) and intensive pregnancy monitoring. We conducted a health technology assessment of noninvasive fetal RhD blood group genotyping for RhD-negative (RhD−) pregnancies. Our assessment evaluated the test's diagnostic accuracy, clinical utility, and cost-effectiveness, the budget impact of publicly funding this test, and patients' and providers' preferences and values.

Methods

We performed a systematic literature search of the clinical and economic evidence to conduct an overview of reviews for test accuracy, a systematic review for clinical utility, and a review of the test's cost-effectiveness compared with usual care. We assessed the risk of bias of each included systematic review and study using the ROBIS and RoBANs tools, respectively. We assessed the quality of the body of clinical evidence according to the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) Working Group criteria. We developed probabilistic Markov microsimulation models to determine the cost-effectiveness and cost–utility of noninvasive fetal RhD genotyping compared with usual care from the Ontario Ministry of Health perspective. We also estimated the 5-year budget impact of publicly funding this test in Ontario. To examine patient and provider preferences related to noninvasive fetal RhD genotyping, we conducted a literature survey of quantitative studies on preference; the Canadian Agency for Drugs and Technologies in Health (CADTH) performed a review of qualitative literature about patient preferences; and we conducted interviews and an online survey with Ontario patients.

Results

We included six systematic reviews in the overview of reviews on diagnostic test accuracy and 11 studies in the clinical utility review. Across systematic reviews, test accuracy was high for noninvasive fetal RhD genotyping. The evidence suggests that implementation of noninvasive fetal RhD genotyping may lead to avoidance of unnecessary RhIG prophylaxis (GRADE: Low), good compliance with targeted RhIG prophylaxis (GRADE: Very low), and high uptake of genotyping (GRADE: Low). Alloimmunization may not increase when using noninvasive fetal RhD genotyping to target prenatal RhIG prophylaxis (GRADE: Very low), and may allow unnecessary monitoring and invasive procedures to be avoided in alloimmunized pregnancies (GRADE: Very low).

We included eight published economic studies that reported inconsistent results regarding the cost-effectiveness of noninvasive fetal RhD genotyping. In nonalloimmunized RhD− pregnancies, compared with usual care, the intervention identified more maternal alloimmunization cases (probability: 0.0022 vs. 0.0020) and was associated with a reduced number of RhIG injections per pregnancy (1.79 vs 1.43). It was more expensive ($154, 95% credible interval [CrI] $139 to $169) but had little impact on the QALYs of newborns followed over a 10-year time horizon (0.0007, 95% CrI −0.01 to 0.01). The cost of noninvasive fetal RhD genotyping and inclusion of paternal RhD typing were drivers of the cost-effectiveness results in this population. In alloimmunized RhD− pregnancies, noninvasive fetal RhD genotyping was associated with lower resource use during the pregnancy. Compared with usual care, it was less costly (−$6,280, 95% CrI −$6,325 to −$6,229) and more effective (0.19 QALYs, 95% CrI 0.17 to 0.20).

The annual budget impact of publicly funding noninvasive fetal RhD genotyping in nonalloimmunized RhD− pregnancies in Ontario ranges from $2.6 million in year 1 (uptake of 80%) to $3.4 million in year 5 (uptake of 100%), with a 5-year total of about $14.8 million. In alloimmunized pregnancies, we estimate cost savings, from about $9 million in year 1 to about $12 million in year 5, with 5-year total savings of about $51.5 million.

We included two studies in the survey of quantitative preferences literature. In the quantitative literature, RhD− pregnant people support routine offering of noninvasive fetal RhD genotyping as part of pregnancy care, with a preference to be adequately informed about the test process, attributes, timing, and risks in advance of the test, ideally in a dialogue with their health care provider. More than half of obstetric health care providers were supportive of offering the test. The qualitative review by CADTH and our own engagement with Ontario patients yielded similar results. Participants consistently expressed a desire for more information about the test and assurance about its safety. They also consistently mentioned the prevention of unnecessary monitoring and treatment as potential benefits.

Conclusions

Noninvasive fetal RhD blood group genotyping is an accurate test to determine RhD incompatibility and guide management of RhD− pregnancies. Compared with usual care, noninvasive fetal RhD genotyping is less costly and more effective for the management of alloimmunized pregnancies. For nonalloimmunized pregnancies, noninvasive fetal RhD genotyping would generally not be considered cost-effective, compared with usual care, unless the cost of testing is much lower than what is proposed now. Publicly funding noninvasive fetal RhD genotyping for guiding the management of RhD− pregnancies in Ontario over next 5 years is associated with a total budget impact of about $15 million in nonalloimmunized pregnancies and total cost savings of about $51 million in alloimmunized pregnancies. Patients and providers indicated support for the routine use of noninvasive fetal RhD genotyping in RhD− pregnancies.

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OBJECTIVE

This health technology assessment evaluates the diagnostic test accuracy, clinical utility, and cost-effectiveness of noninvasive fetal RhD blood group genotyping. It also evaluates the budget impact of publicly funding noninvasive fetal RhD blood group genotyping, and the experiences, preferences, and values of people and health care providers related to care for RhD-incompatible pregnancies.

BACKGROUND

Health Condition

Human blood groups (or blood types) are defined by the presence or absence of various antigens—naturally occurring substances on the surface of red blood cells that can trigger an immune response. The rhesus (Rh) blood group is one of the most important major blood groups, especially the D antigen (RhD). A person whose blood cells have the RhD antigen is called RhD positive (RhD+); someone who lacks it is RhD negative (RhD−).

Being RhD+ or RhD− can play an important role in pregnancy. Maternal RhD blood type is determined routinely in pregnancy care by serological screening (blood test),[2] but knowing the fetus's RhD blood type before birth has historically been difficult. A condition known as RhD incompatibility occurs when a pregnant person's blood type is RhD− and the fetus is RhD+.[3] This blood type incompatibility can trigger the mother's immune system to create antibodies that can potentially attack fetal red blood cells in a subsequent RhD incompatible pregnancy. To avoid this risk to future pregnancies, RhD− pregnant people typically receive an injection of Rh immunoglobulin (RhIG) prophylaxis that prevents the development of antibodies. If antibodies develop, any future pregnancies are closely monitored.

The prevalence of RhD− status varies by ethnicity. It is highest in Caucasian populations (about 15%), lower in black African populations (3% to 5%), about 1% in North American Indigenous populations, and very rare in East Asian populations.[4,5] The chance of having an RhD+ fetus is 50% for an RhD− person with a heterozygous partner (a person whose genotype includes one RhD− allele and one RhD+ allele, which means their child could inherit either a positive or negative RhD blood type).[2] Each year in Canada there are approximately 68,000 RhD− pregnancies, and it is estimated that just over 60% carry an RhD+ fetus.[6]

Clinical Need and Target Population

During pregnancy, red blood cells from the fetus may cross into the maternal blood stream spontaneously, during childbirth, or after events such as invasive prenatal testing (e.g., amniocentesis, cordocentesis, chorionic villus sampling [CVS]), pregnancy loss or termination, a ruptured ectopic pregnancy, or abdominal trauma.[3,7-10] When enough fetal blood cells enter the mother's blood stream (an event called fetomaternal hemorrhage) in an RhD incompatible pregnancy, the foreign RhD+ antigen from the fetus can, as noted above, trigger a maternal immune response that creates anti-D antibodies. This development of antibodies is referred to as alloimmunization (also called sensitization).

The incidence and volume of fetal blood entering the maternal circulation increases throughout gestation: from 5% to 15% of pregnancies having less than 0.1 mL in the first trimester, to 45% with more than 0.1 mL in the third trimester.[4] Detectable fetomaternal hemorrhage (measured by tests such as the Kleihauer–Betke [KB] acid elution test) occurs in an estimated 75% of

pregnancies.[4] The greater the volume of fetomaternal hemorrhage, the greater the antigen loads and consequently the likelihood of alloimmunization.[4] In an RhD− pregnant person, alloimmunization can result from as little as 0.1 mL of fetal cells crossing the placental barrier.[4] It is estimated that approximately 1% to 2% of RhD− pregnancies are alloimmunized with anti-D antibodies per year despite the mother receiving RhIG prophylaxis.[6,11]

Maternal RhD alloimmunization during pregnancy rarely affects the first pregnancy during which it occurs (incident pregnancy). This is because the immune response to the RhD antigen in the first pregnancy triggers development of immunoglobulin M (IgM) antibodies that cannot cross the placenta due to their high molecular weight.[4] However, the immune response to a secondary antigenic challenge (i.e., any subsequent RhD incompatible pregnancy and with fetomaternal hemorrhage) is faster and reaches a higher concentration (titre) of predominantly immunoglobulin G (IgG) antibodies. These IgG antibodies are of lower molecular weight, and thus can cross the placenta and cause hemolysis (destruction of red blood cells) in the fetus.[5,12-14] Hemolysis can lead to fetal anemia (decreased number of red blood cells) and a life-threatening blood disorder known as hemolytic disease of the fetus and newborn (HDFN) or erythroblastosis fetalis.[4] Though 44 different antibodies have been implicated in HDFN,[4] one of the most frequent causes of HDFN in Canada (and the only preventable one) is RhD incompatibility that leads to alloimmunization. RhD alloimmunized pregnancies are therefore monitored intensively throughout gestation for signs of increasing antibody titres and, if titres reach a critical level, for signs of fetal anemia as a result of hemolysis.

The prevalence of HDFN is about 1 in 21,000 live births.[5] It affects approximately 4,000 fetuses worldwide each year, of which 15% die before birth.[7] Severe fetal anemia occurs in about 10% of at-risk fetuses, is confirmed by invasive prenatal tests (e.g., cordocentesis, amniocentesis), and requires intrauterine blood transfusion.[15] About 90% of fetuses in RhD alloimmunized pregnancies have mild anemia or are unaffected. The severity of HDFN ranges from newborn jaundice (mild disease) to severe fetal anemia, fetal heart failure, hydrops fetalis (life-threatening edema in the fetus), brain damage, and intrauterine death.[8,14,15] Neonatal care for HDFN includes phototherapy for hyperbilirubinemia and jaundice (which untreated can lead to encephalopathy), monitoring hemoglobin and bilirubin levels to determine if simple or exchange blood transfusion is required, and potentially administration of intravenous immunoglobulin.[15] Before RhD was identified as a primary cause, perinatal (newborn) mortality from HDFN was on the order of 40% to 50%. With the introduction of new management options, including intrauterine and neonatal transfusions and induced late preterm delivery upon detection of elevated maternal antibody titres and fetal anemia detected by Doppler ultrasound, perinatal mortality was reduced to 15% to 20%.[4] Presently, in high-resource countries like Canada, mortality from HDFN is less than 0.5 per 1,000 live births as a result of current standard management of RhD alloimmunization.[4]

Current Management of RhD− Pregnancies

Prevention of Alloimmunization

Alloimmunization (production of anti-D antibodies) and resultant HDFN are preventable by maternal injection of Rh immunoglobulin (RhIG) prophylaxis (common trade names in Canada are WinRho and Hyper RHO; US trade name colloquially used is RhoGAM). RhIG is a blood product derived from pooled human plasma and is a valuable and limited resource.[8] RhIG is routinely given in nonalloimmunized RhD− pregnancies as anti-D prophylaxis at a dose of 300 mcg around 28 to 34 weeks' gestation. Another dose is given within 72 hours of delivery of an RhD+ baby (confirmed by cord blood typing) at a dose of 300 mcg, along with any additional

dose required, based on a test to calculate volume of fetomaternal hemorrhage.[4,5,11,14] Additional doses of RhIG are administered, as needed, after other events during the pregnancy that may trigger fetomaternal hemorrhage, such as amniocentesis, abdominal trauma, or antenatal vaginal bleeding.[4]

This routine use of RhIG prophylaxis in many developed countries has reduced the incidence of maternal RhD alloimmunization from 16% to 0.1% when given at 28 weeks' gestation and to RhD incompatible pregnancies at birth, and has reduced the prevalence of HDFN in subsequent pregnancies substantially, from 16% to 2%.[14] Routine RhIG prophylaxis has also reduced perinatal mortality from RhD alloimmunization and HDFN by 100-fold.[16] However, due to missed or inadequate dosing or failure to access or accept prophylaxis, maternal alloimmunization still occurs in 1% to 2% of RhD− pregnant people in Canada.[11]

Though effective and having a low likelihood of adverse effects, RhIG prophylaxis is not entirely without risk. Blood products can carry the risk of transmitted infection, as was seen in Ireland when many women were infected with hepatitis C from RhIG administration in the 1970s[17] and in Canada with the tainted blood scandal in the 1980s.[18] No documented cases of viral or bacterial infection due to RhIG have occurred in Canada. However, there is risk associated with RhIG of local and systemic reactions and infection, including new infections or prion diseases in the blood supply that are not yet screened for.[6] It is estimated that in a predominantly Caucasian population, as many as 40% of nonalloimmunized RhD− pregnancies receive unnecessary RhIG treatment due to unknown RhD status of the fetus.[5]

Fetal Management in Alloimmunized Pregnancies

Alloimmunized pregnancies are considered high-risk pregnancies and are managed with intensive monitoring for maternal antibody levels and fetal well-being as a precaution, should the fetus be RhD+ and at risk of HDFN.[5] Monitoring begins when the pregnant person is found to be alloimmunized, usually before 16 to 24 weeks' gestation. It includes regularly checking maternal antibody titres (e.g., monthly in the first two trimesters, biweekly in the third trimester or if there is an increase), biweekly Doppler ultrasound to measure peak systolic velocity of the fetal mid-cerebral artery (blood flow of a fetal brain artery) and, potentially, fetal blood sampling (usually via cordocentesis) if anemia is suspected.[2,11,13,19] Weekly fetal heart rate monitoring and ultrasounds may also be performed.[13]

Anti-D antibody concentrations at critical levels (e.g., titres > 1:16 or 1:32) are known to potentially lead to substantial fetal anemia and signify the need for fetal Doppler ultrasound of the mid-cerebral artery. Detection of increased peak systolic velocity (faster blood flow than normal) generally indicates fetal anemia necessitating intervention.[4,13] Alloimmunized pregnancies with signs of fetal anemia but too early for delivery will undergo fetal cord blood sampling and may need intrauterine transfusion to increase levels of red blood cells in the fetus.[8,13] Intrauterine transfusion is a procedure with an estimated 1% to 3% risk of membrane rupture or infection and is associated with poorer outcomes when done early in the second trimester.[13] The treating clinician may consider early delivery at a safe gestational age (e.g., 37 to 38 weeks) but delivery may be warranted earlier in severe cases of HDFN when weighing the risks of preterm delivery with those of continued monitoring and intrauterine transfusion, worsening anemia, and possible fetal demise.[13] Intensive fetal monitoring and any interventions are highly specialized care only available at tertiary care centres, which may necessitate travel for people living outside major urban centres.

In summary, standard care for alloimmunized RhD− pregnancies, involves frequent investigations and medical visits with maternal-fetal medicine specialists, which can be burdensome, inconvenient, and may lead to more invasive prenatal tests that carry fetal risk.[2] In nonalloimmunized RhD− pregnancies, current guidelines advise a precautionary treat-all approach with antenatal RhIG anti-D prophylaxis.[19] RhIG is an expensive resource and targeted administration could reduce unnecessary use and preserve the supply.[8] Knowing fetal RhD status in an RhD− pregnancy would prevent the need for unnecessary intensive monitoring or RhIG treatment in approximately 95% of cases with an RhD− fetus.[6]

Health Technology Under Review

In 1997, it was discovered that there is a sufficient quantity of cell-free fetal DNA (cffDNA) in maternal plasma to determine the fetus's RhD genotype (the specific genes that determine the fetus's RhD blood type).[5] The test is referred to as noninvasive fetal RhD blood group genotyping, or fetal RhD genotyping. To conduct the test, maternal blood is collected with anticoagulant, and the plasma is later separated from the cellular component (via centrifugation) and stored in proper conditions to enable DNA extraction and analysis.[2,19] Through this test, fetal blood group antigens can be determined noninvasively from 10 weeks' gestation in nonalloimmunized pregnancies[6] and 16 weeks' gestation in alloimmunized pregnancies (personal communication, Canadian Blood Services, by telephone, 11 January 2019). Prior methods of sampling fetal DNA for RhD typing, such as amniocentesis and CVS, are invasive (they involve extracting fetal cells from the placenta or amniotic sac) and carry risks.[5] Risks of amniocentesis and CVS include miscarriage (about 0.5%–1%) and fetomaternal hemorrhage, which can in turn increase maternal antibody levels.[2,5,19]

An RhD− phenotype (RhD negative blood type) can result from several genotypes (variants of the gene). The most common variants underlying RhD− phenotypes include complete RhD gene deletion, the RhD pseudogene *RHDΨ* (a nonfunctional copy of the RhD gene), and the *RhD-CE-D* hybrid gene (haplotype or group of genes inherited together).[20] Deletion of the RhD gene is the cause of RhD− status in Caucasians, whereas any of the three variants can produce RhD− status in the majority of the black African population.[20] The fetal RhD genotyping test targets fetal DNA amplification of regions of exon (parts) 7 and 10 within the RhD gene, located in the region of p36.13−p34.3 on chromosome 1, using techniques such as polymerase chain reaction (PCR, a widely used laboratory test method that copies the DNA segment of interest to allow it to be analyzed).[21] Using both exons 7 and 10 helps prevent false-positive results in fetuses with the RhD pseudogene (*RHDΨ*) and *RhD-CE-D* haplotypes because exon 7 is negative in PCR with these variants.[19,21]

Regulatory Information

Laboratories can develop and validate fetal RhD genotyping tests in-house, which do not require Health Canada approval. No noninvasive fetal RhD genotyping tests or test kits are registered in Health Canada's Medical Devices Active Licence Listing online database, as of this writing. Test kits are subject to Health Canada regulation as Class 3 medical devices. We did identify the existence of two kits and one laboratory-developed test on the international market, by an Internet search (Table 1). A clinical expert noted there is another RhD test kit made by Devyser (CE mark is pending) and used in Sweden.

Table 1: Commercially Available Tests and Kits for Noninvasive Fetal RhD Genotyping Identified

Name (Manufacturer)	Type	Description
Cell3 Direct Rhesus D Fetal Blood Group Genotyping Kit[22] (Nonacus)	Test kit	Real-time quantitative PCR No cffDNA extraction required Amplification of RhD exons 5, 7, 10
SensiGene Fetal RHD Genotyping[a] (Sequenom)	Laboratory-developed test	Proprietary SEQureDx technology/matrix-assisted laser desorption/ionization time-of-flight mass spectrometry-based nucleic acid analysis Amplification of RhD exons 4, 5, 7 and psi (ψ) pseudogene in exon 4
Free DNA Fetal Kit RhD[23] (Institut de Biotechnologie Jaques-Boy under Bio Rad label)	Test kit (CE marked)	Real-time PCR Efficient cffDNA DNA extraction method required Amplification of RhD exons 5, 7, 10

Abbreviations: cffDNA, cell-free fetal DNA; PCR, polymerase chain reaction; RhD, rhesus D blood group.
[a]Considered investigational by the following US health insurance providers: Blue Cross Blue Shield of North Carolina, AmeriGroup RealSolutions in healthcare Medical Policy & Technology Assessment Committee, Regence of Oregon and Utah, Premera Blue Cross.

Ontario, Canadian, and International Context

Noninvasive fetal RhD genotyping is not performed in any laboratory in Canada. Owing to a lack of public funding, this test is not part of current noninvasive prenatal testing (NIPT) done by Canadian laboratories performing NIPT for common fetal genetic conditions. In Ontario, current access to the test is available only for alloimmunized pregnancies through the province's Out-of-Country Prior Approval Program for diagnostic laboratory testing. After approval, maternal blood samples—almost exclusively Rh D, E, c, or Kell antigen alloimmunized pregnancies—can be sent to a laboratory in Bristol, UK. The Out-of-Country program pays for the test while the hospital covers the shipping cost (about $100 per test). In Ontario, nonalloimmunized pregnancies are not tested. Several referral centres that care for high-risk pregnant patients order fetal RhD genotyping directly through Bristol or indirectly through Mount Sinai Hospital in Toronto, a major fetal therapy unit for Ontario, so access to the test is not, in principle, limited by geography. However, local awareness that the test is available and its use in practice may differ by region or practice. Expert consultations highlighted that not all centres in the province were aware they could send samples to the UK laboratory for testing, and so have not. In addition, accessing the overseas test necessitates financial and time costs on the part of the clinician for ordering and shipping and is difficult to navigate in centres with low numbers of cases. In Ontario, there are approximately 21,000 nonalloimmunized and 1,800 alloimmunized RhD− pregnancies per year (full details in Table 25).

Genotyping the father of the fetus, to determine whether he is heterozygous, may be helpful in determining if it is possible for the fetus to be RhD+. RhD genetic testing of the father is not done routinely in Canada.[11] Paternal phenotyping (through serology) can be informative when the father is RhD−. Paternal genotype can be predicted from the father's Rh phenotype by testing their Rh D, C/c, and E/e status; the most probable genotype can be inferred based on the most likely combinations of these antigens. Importantly, paternal testing is reliant upon accurate, private disclosure of paternity. For a pregnant person, paternal testing can potentially create conflict between privacy in their relationship and the well-being of the fetus, as it requires disclosure of biological paternity.[11]

National prenatal RhD genotyping screening programs have been introduced in Denmark, Sweden, Finland, and The Netherlands.[6] Regional programs have been implemented in Belgium, France, and Germany as well.[6] A small number of specialized or national reference laboratories across Europe provide fetal RhD genotyping.[19] Most European laboratories developed quantitative PCR techniques for noninvasive fetal RhD genotyping using maternal venous blood samples.[2] In Australia, as of 2009, real-time PCR was not yet available for noninvasive fetal RhD genotyping.[2] The technology used for the test is volume dependent, and very high volumes could warrant use of next-generation (high-throughput) sequencing for analysis. In November 2016, the UK National Institute for Health and Care Excellence released diagnostic guidance recommending high-throughput noninvasive fetal RhD genotyping to guide antenatal anti-D prophylaxis in nonalloimmunized women, provided the overall cost of testing falls at or below a threshold price of £24 per test, where it is cost-effective.[8]

Terminology

We use the term “RhD” most often in this report and, as needed, specify other Rh antigens (c, C, e, and E) in context. Similarly, for simplicity, we use the terms “nonalloimmunized” and “alloimmunized” to refer to RhD non/alloimmunization, recognizing that numerous other antigens can trigger red cell alloimmunization.

Expert Consultation

We consulted with experts in the specialty areas of obstetrics, hematology, maternal-fetal medicine, pediatric hematology and newborn medicine, laboratory medicine, and health economics to help inform our understanding of aspects of the health technology, plan our methodologies, and contextualize the evidence.

PROSPERO Registration

This health technology assessment has been registered in PROSPERO, the international prospective register of systematic reviews (CRD42019128547), available at https://www.crd.york.ac.uk/PROSPERO.

CLINICAL EVIDENCE

Research Questions

1. What is the diagnostic test accuracy of noninvasive fetal RhD blood group genotyping in RhD-negative (RhD−) pregnancies?
2. What is the clinical utility of noninvasive fetal RhD blood group genotyping to:
    - Guide administration of Rh immunoglobulin prophylaxis in nonalloimmunized RhD− pregnancies?
    - Guide intensive monitoring for fetal well-being and hemolytic disease of the fetus and newborn (HDFN) in alloimmunized RhD− pregnancies?

Methods

Clinical Literature Search

To address both research questions, we performed a literature search on February 25, 2019, to retrieve studies, published from January 1, 1997, to the search date. We used the Ovid interface to search the following databases: MEDLINE, Embase, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, Health Technology Assessment, and National Health Service Economic Evaluation Database (NHSEED). We chose a date limit from 1997 to align with the scientific discovery of sufficient quantity of cell-free fetal DNA in maternal blood for fetal genotyping.[24]

Medical librarians developed the search strategies using controlled vocabulary (e.g., Medical Subject Headings) and relevant keywords. The final search strategy was peer reviewed using the PRESS Checklist.[25]

We created database auto-alerts in MEDLINE and Embase and monitored them for the duration of the assessment period. We also performed a targeted grey literature search of health technology assessment agency sites as well as clinical trial and systematic review registries. The grey literature search was updated on July 23 and 24, 2019. See Appendix 1 for our literature search strategies, including all search terms.

Diagnostic Test Accuracy (Question 1)

We conducted an overview of reviews to systematically bring together and summarize evidence from multiple systematic reviews assessing the diagnostic test accuracy of noninvasive fetal RhD blood group genotyping from maternal blood in RhD− pregnancies.

Eligibility Criteria

Studies

Inclusion Criteria

- English-language full-text publications
- Studies published between January 1, 1997, and February 25, 2019

- Systematic reviews or health technology assessments of diagnostic test accuracy studies (i.e., randomized controlled trials or cohort studies with both reference standard and genotyping tests performed in all participants)
- Reviews that clearly report search methods (e.g., databases, keywords, dates) at minimum

Exclusion Criteria

- Primary studies
- Editorials, commentaries, case reports, conference abstracts, letters
- Animal and in vitro studies
- Feasibility studies, test validation studies, laboratory protocol development studies
- Studies where results for outcomes of interest cannot be extracted
- Unpublished data

Participants

- All serologically confirmed RhD− pregnancies (alloimmunized and nonalloimmunized; singleton or multiple pregnancy)

Index Test

- Noninvasive prenatal RhD genotyping with cell-free fetal DNA in maternal blood (whole blood, plasma, or serum), including laboratory-developed tests or commercial test kits

Reference Standard

- Neonatal cord blood typing
- Genotyping or serological results from invasive tests for fetal blood typing (i.e., amniocentesis, chorionic villus sampling [CVS], cordocentesis)

Outcome Measures

- Diagnostic test accuracy
- Sensitivity
- Specificity
- Rates of true positives, true negatives, false positives, false negatives
- Inconclusive or indeterminate results
- Positive predictive value
- Negative predictive value
- Receiver operating characteristic curve

Literature Screening

A single reviewer used Covidence systematic review management software[26] to screen titles and abstracts, then obtained full text of systematic reviews that appeared eligible for the review, according to the inclusion criteria. The reviewer then examined the full-text articles to identify

systematic reviews that met the inclusion criteria. The reviewer also screened the reference lists of the included systematic reviews (815 references) for any additional relevant systematic reviews not identified through the search. We report citation flow and reasons for exclusion for full-text articles for the diagnostic test accuracy overview of reviews, according to the PRISMA statement (Figure 1).[27]

Data Extraction

We extracted relevant data on systematic review objectives, methods, included studies, risk-of-bias and quality assessment, results, and PICOTS (population, index text, reference standard, outcome, time, and setting) from the published systematic reviews. We consulted cited publications as needed for additional information on study methods and laboratory protocols only.

Evidence Synthesis

We assessed overlap of studies in the included systematic reviews using a study matrix and calculating the corrected covered area (CCA), a numerical measure by Pieper et al.[28] The CCA overlap is interpreted as slight (0–5), moderate (6–10), high (10–15), or very high (> 15).

We provide a narrative synthesis of results as analyzed and reported in included systematic reviews. Findings are presented in text and tabular formats, noting trends across systematic reviews. We did not re-analyze or meta-analyze data or conduct network meta-analysis because of a lack of detailed data reported and the risk of double-counting. Where necessary and where data were available, we calculated confidence intervals around point estimates from published meta-analyses. Where possible, we categorized findings into the following subgroups of interest: alloimmunized versus nonalloimmunized pregnancies, singleton versus multiple pregnancies, and trimester of testing.

Critical Appraisal of Evidence

We assessed risk of bias of included systematic reviews using the Risk of Bias in Systematic Reviews (ROBIS) tool (Appendix 2).[29] We report the existing determination of risk of bias for each included study and any quality assessment for the total body of evidence (e.g., Grading of Recommendations Assessment, Development, and Evaluation [GRADE]) as conducted and reported by systematic review authors.

Where no GRADE or equivalent quality assessment was provided and a sufficient level of detail was available in the published systematic review, we sought to evaluate the quality of the body of evidence within the systematic review for each outcome according to the *GRADE Handbook*.[12] GRADE judges the quality of the body of evidence based on the following considerations: risk of bias, inconsistency, indirectness, imprecision, and publication bias.

Clinical Utility (Question 2)

We separated our systematic review on the clinical utility of noninvasive fetal RhD genotyping by alloimmunized and nonalloimmunized pregnancies.

Eligibility Criteria

Studies

Inclusion Criteria

- English-language full-text publications
- Studies published between January 1, 1997, and February 25, 2019
- Comparative study designs (e.g., randomized controlled trial, two-arm cohort, case-control studies)
- Noncomparative study designs including single-arm cohorts
- Systematic reviews/meta-analyses of the above primary study types

Exclusion Criteria

- Editorials, commentaries, case reports, conference abstracts, letters
- Animal and in vitro studies
- Feasibility studies, test validation studies, laboratory protocol development studies
- Studies where results for outcomes of interest cannot be extracted
- Unpublished data

Participants

- Serologically confirmed RhD− pregnancies
    - Nonalloimmunized RhD− singleton or multiple pregnancies
    - Alloimmunized RhD− singleton or multiple pregnancies

Intervention

- Noninvasive prenatal RhD genotyping with cell-free fetal DNA in maternal blood (whole blood, plasma, or serum), including laboratory-developed tests or commercial test kits

Comparator

- Standard of care
    - Nonalloimmunized pregnancies: administer Rh immunoglobulin (RhIG) prophylaxis for all RhD− women at about 28 to 34 weeks' gestation, after any fetomaternal hemorrhage or invasive procedure, and at birth if fetus is confirmed RhD+ by neonatal cord blood typing
    - Alloimmunized pregnancies: monitor all for HDFN and fetal anemia

Outcome Measures

Nonalloimmunized pregnancies

- Unnecessary RhIG avoided
- Risk of alloimmunization
- Compliance with RhIG prophylaxis
- Maternal quality of life
- Adverse effects such as infections from or reactions to RhIG
- Implementation outcomes such as uptake of testing, uptake of RhIG
- Avoidance of cord blood RhD testing

Alloimmunized pregnancies

- Invasive procedures avoided including amniocentesis, CVS, cordocentesis, intrauterine transfusion
- Frequency of hospital visits and blood tests
- Maternal quality of life
- Adverse effects of testing or intensive monitoring interventions
- Implementation outcomes such as procedures missed

Literature Screening

A single reviewer conducted an initial screening of titles and abstracts using Covidence systematic review management software[26] and then obtained the full texts of studies that appeared eligible for review according to the inclusion criteria. A single reviewer then examined the full-text articles and selected studies eligible for inclusion. The reviewer also screened the reference lists of included studies (815 references) for any additional relevant studies not identified through the search. We report citation flow and reasons for exclusion for full-text articles for the systematic review according to the PRISMA statement (Figure 2).[27]

Preference was given to systematic reviews, aiming to select the systematic review(s) determined to be of highest methodological quality with consideration to lowest risk of bias, recency, and comprehensiveness. As there was no such systematic review available or of adequate quality to answer all of the research question(s) on clinical utility, we included primary studies.

Data Extraction

We extracted relevant data on included study characteristics, risk-of-bias and GRADE assessment, results, and PICOTS (population, intervention, comparator, outcome, time, and setting). Participant information (demographics, trimester, medical and obstetric history, RhD antigen status), test characteristics (weeks of gestation, test technique, cut-offs, laboratory protocols) and outcomes were also extracted.

Evidence Synthesis

As noted, we reviewed and report results separately for alloimmunized and nonalloimmunized pregnancies. We provide a narrative summary of results because meta-analysis was not appropriate due to clinical and methodological heterogeneity.[30] We calculated means, medians,

and other summary statistics from published data as needed. Where possible, we separated data by the following subgroups: trimester of testing, singleton versus multiple pregnancies.

Critical Appraisal of Evidence

For nonrandomized studies, we assessed the risk of bias of each included study using the Risk of Bias Assessment tool for Non-randomized Studies (RoBANS).[31] We evaluated the quality of the body of evidence for each outcome according to the *Grading of Recommendations Assessment, Development, and Evaluation* (GRADE) *Handbook*.[12] The body of evidence was assessed based on the following considerations: risk of bias, inconsistency, indirectness, imprecision, and publication bias. The overall rating reflects our certainty in the evidence.

Results: Diagnostic Test Accuracy

Clinical Literature Search

The database search of the clinical literature yielded 1,445 citations published between January 1, 1997, and February 25, 2019. After removal of duplicates, we screened 875 citations. Eight systematic reviews assessing diagnostic test accuracy were identified; however, two produced only summaries in English, with the full text published in Norwegian[32] or Swedish[33] and were therefore excluded. We included six systematic reviews reported in seven articles[20,34-39] that met our inclusion criteria. Figure 1 presents the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram for the clinical literature search for research question 1.

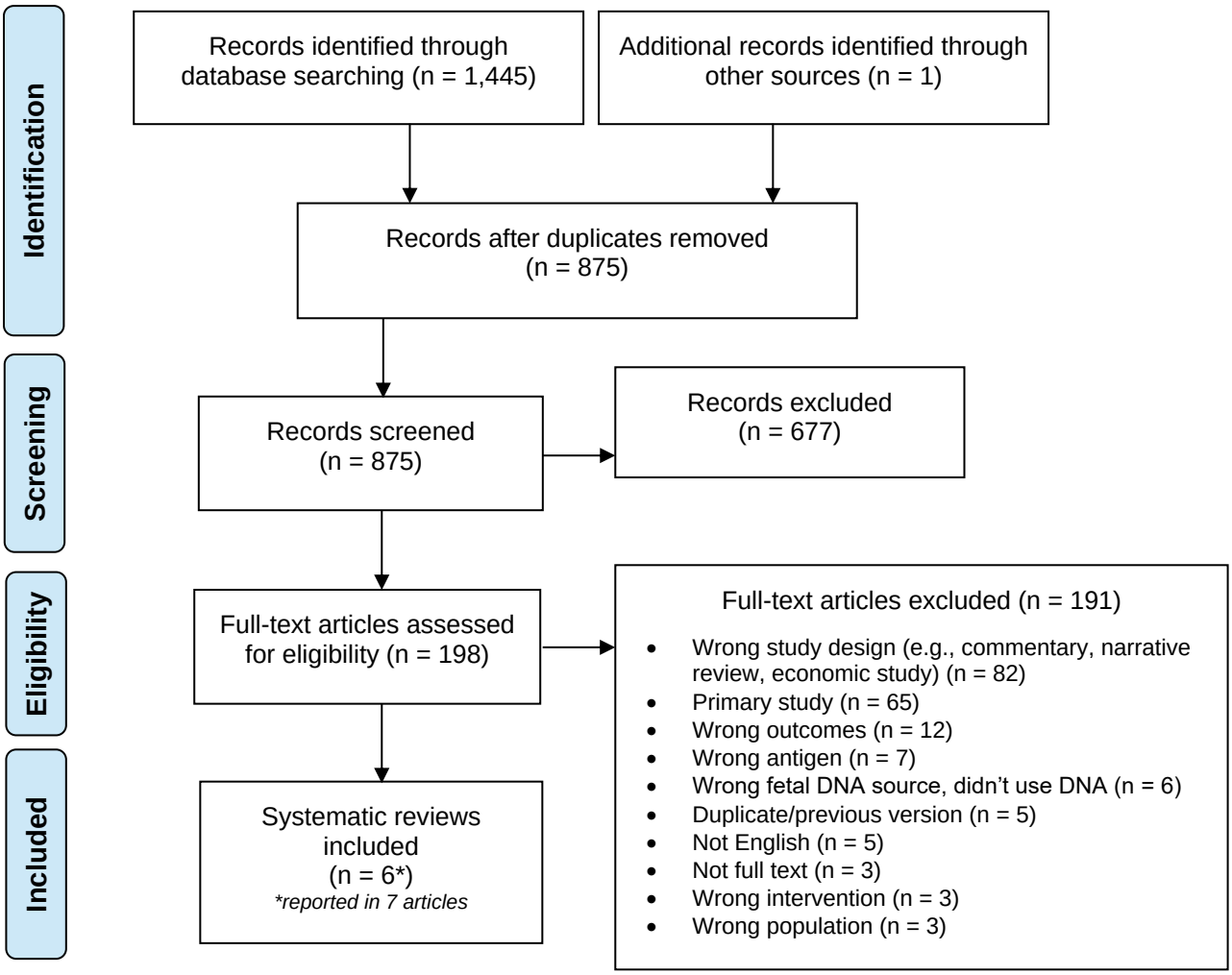

Figure 1: PRISMA Flow Diagram—Clinical Search for Research Question 1

Abbreviation: PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-analyses.
Source: Adapted from Moher et al, 2009.[27]

Characteristics of Included Systematic Reviews

Table 2 summarizes the characteristics of the six systematic reviews.[20,34-39] These reviews cover primary studies published from database inception up to 2016. Only one review had a medical librarian conduct the systematic literature search.[39] All systematic reviews assessed the accuracy of noninvasive RhD fetal blood group genotyping in RhD−pregnancies. The reviews focused on nonalloimmunized pregnancies, although only Yang et al[39] explicitly stated their eligible population was nonalloimmunized only. The reference standard for almost all the reviews was cord blood typing (serology) of the neonate. The scope of the reviews by Mackie et al[36] and Wright and Burton[38] was noninvasive prenatal or cell-free fetal DNA (cffDNA) testing broadly, but findings and data on fetal RhD blood group genotyping were available separately and we extracted these.

The measures of test performance reported by systematic reviews varied and included accuracy, sensitivity, specificity, positive and negative predictive values, likelihood ratios, and inconclusive and false result rates (false positives and false negatives). Four systematic reviews[20,34,36,39] conducted meta-analysis or hierarchical models to derive summary estimates. Among those four systematic reviews, heterogeneity was investigated using summary receiver operating characteristic curve (SROC) plots,[20,39] forest plots,[34] or both.[36] Three of the six reviews were authored by groups in the United Kingdom,[36,38,39] and the others were from China,[20] the United States,[34] and Germany/Switzerland.[35]

Overlap Between Systematic Reviews

The review by Zhu et al[20] did not provide any references for the 37 studies they included. Primary studies could thus not be identified, and we could not include this systematic review in our assessment of overlap.

A total of 89 citations were included across the five systematic reviews for which we assessed overlap. There were 70 unique citations. The corrected covered area was 0.05 or 5%, representing slight overlap between the systematic reviews. This marginal overlap is primarily explained by the literature search dates for each review and, to a lesser extent, reflects variation across systematic reviews in their eligibility criteria such as testing platforms, test timing, requirements for minimum sample sizes or full 2x2 data, and inclusion of abstracts and posters.

Risk of Bias in the Systematic Reviews

We judged risk of bias to be low in three systematic reviews[34,36,39] and high in the other three[20,35,38] mainly owing to concerns with eligibility criteria, identification and selection of studies, and data collection and study appraisal (see Appendix 2, Table A1).

Only two of the systematic reviews[36,39] assessed the risk of bias of their included studies and both used the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool.[40] The review by Mackie et al[36] was a broad review of all types of noninvasive prenatal testing with a subanalysis for fetal RhD genotyping. The authors reported that most studies were at low risk of bias overall; however, they assessed risk of bias for included studies across all tests (n = 117) and we could not abstract the results for the 30 studies on RhD. The systematic review by Yang et al[39] judged six of the eight included studies to be at low risk of bias, and two to be at high risk of bias mainly due to concerns about potential bias in patient selection, the index test, and flow and timing—three of the four key domains of the QUADAS-2 tool (full details are in Table 2 of Yang et al, 2019[39]). No systematic review conducted a quality assessment of their included body of literature. There was insufficient information reported in the reviews for us to assess the quality of the body of evidence within each systematic review using GRADE.

Table 2: Characteristics of Systematic Reviews of Diagnostic Accuracy of Noninvasive Fetal RhD Genotyping

Author, Year	Country	Scope (Population, Index Test, Reference Standard)	Literature Search	N Studies	Outcomes Reported	Main Analyses
Yang et al, 2019[39]; also reported in Saramago et al, 2018[37]	UK	**P:** RhD− nonalloimmunized singleton and multiple pregnancies **I:** RhD-NIPT using cffDNA in maternal plasma on automated robotic platform for high volumes **R:** cord blood serology or other postnatal blood test of infant	**Databases searched:** 11 **Grey literature:** ongoing, unpublished, and guidelines **Search dates:** inception–Feb 2016	8	FPR FNR	Hierarchical bivariate meta-analysis and HSROC
Mackie et al, 2017[36]	UK	**P:** RhD− singleton pregnancies (all)[a] **I:** NIPT using cffDNA in maternal blood **R:** blood sample at birth	**Databases searched:** 5 **Grey literature:** hand searched along with reference lists **Search dates:** 1997–April 13, 2015	30	Likelihood ratios Sensitivity Specificity Inconclusive results	Bivariate logistic regression model with unstructured correlation Forest plots and HSROCs
Zhu et al, 2014[20]	China	**P:** RhD− pregnancies **I:** RhD-NIPT using cffDNA in maternal blood, serum, plasma **R:** RhD blood type of fetus or at birth	**Databases searched:** 2 **Search dates:** 1996–2013	37	Accuracy SROC/AUC Sensitivity, specificity NPV, PPV FN, FP	Random effects bivariate meta-analysis Subgroup: trimester
Wright and Burton, 2009[38]	UK	**P:** All pregnancies[a] **I:** NIPT using cffDNA **R:** NR[c]	**Databases searched:** 1 **Search dates:** up to August 2008	3	Accuracy FN	Descriptive only
Legler et al, 2009[35]	Germany, Switzerland	**P:** RhD− pregnancies **I:** RhD-NIPT using cffDNA in maternal blood **R:** NR[b]	**Databases searched:** 1 **Search dates:** 2006–2008	11[c]	Accuracy Sensitivity, specificity	Descriptive only Separated by “proof-of-principle studies” (i.e., small samples) and clinical utility studies
Geifman-Holtzman et al, 2006[34]	US	**P:** RhD− pregnancies	**Databases searched:** 6	37	Sensitivity, specificity	Weighted random effects linear model/binomial distribution

Author, Year	Country	Scope (Population, Index Test, Reference Standard)	Literature Search	N Studies	Outcomes Reported	Main Analyses
		I: RhD-NIPT using cffDNA from maternal blood, plasma, serum **R:** Rh typing of fetus/newborn	**Grey literature:** abstracts from scientific forums, bibliographies of published articles **Search dates:** inception–2005		NPV, PPV	meta-analysis Hierarchical Bayesian random effects analysis using Markov chain Monte Carlo simulation Subgroups: alloimmunized, trimester

Abbreviations: AUC, area under the curve; cffDNA, cell-free fetal DNA; FN, false negative; FNR, false-negative rate; FP, false positive; FPR, false-positive rate; HSROC, hierarchical summary receiver operating characteristic curve; N, number of; NIPT, noninvasive prenatal test; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; RhD. rhesus D blood group; SROC, summary receiver operating characteristic curve; UK, United Kingdom; US, United States.

[a]Information relevant to RhD component of the review are presented; overall scope encompassed all noninvasive prenatal testing.

[b]Reference standard was not clearly stated in eligibility criteria reported.

[c]All studies included in Geifman-Holtzman et al, 2006, were excluded.[35]

Test Accuracy

Three reviews conducted meta-analyses to produce summary accuracy estimates. Table 3 shows the summary accuracy, sensitivity, specificity, positive predictive values, and negative predictive values from these systematic reviews.

Table 3: Diagnostic Accuracy Summary Estimates From Systematic Reviews of Noninvasive Fetal RhD Genotyping

Author, Year	N Samples	Accuracy % (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV or LR+ (95% CI)	NPV or LR− (95% CI)
Mackie et al, 2017[36]	10,290 tests[a]	—	99.3 (98.2–99.7)	98.4 (96.4–99.3)	LR+ 61 (22–167)	LR− 0.007 (0.003–0.186)
Zhu et al, 2014[20]	All:11,129	95.3	—	—	—	—
	Conclusive: 10,777[b,c]	98.5[b,c] (98.2–98.7)[d]	98.9 (98.6–99.1)[d]	97.7 (97.2–98.1)[d]	98.7 (98.4–98.9)[d]	98.0 (97.5–99.0)[d]
Geifman-Holtzman et al, 2006[34]	All: 3,261	91.4 (NR)	—	—	—	—
	After some exclusions: 3,184[e]	91.7 (NR)	—	—	—	—
	After all exclusions: 3,078[f]	94.8[a] (NR)	Random effects 95.4 (90.6–97.8)	Random effects 98.6 (96.4–99.5)	Random effects 99.0 (97.9–99.6)	Random effects 92.1 (80.9–97.0)
			Bayesian model 96.7 (92.5–98.9)	Bayesian model 98.9 (96.7–99.9)	Bayesian model 99.4 (98.4–99.9)	Bayesian model 92.7 (81.8–97.9)

Abbreviations: AUC, area under the curve; CI, confidence interval; LR, likelihood ratio; N, number of; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; RhD, rhesus D blood group; SE, standard error.

[a]Information relevant to RhD component of the review are presented; overall scope encompassed all noninvasive prenatal testing.

[b]352 of 11,129 samples were excluded from the analysis because they were inconclusive.[20]

[c]The summary receiver operating characteristic curve analysis provided further evidence of high diagnostic accuracy AUC 0.9937 SE(AUC) 0.0025 Q*9683 SE(Q*0.0073.).[20]

[d]Confidence interval was calculated from data available from the published systematic review.

[e]Excludes studies with < 10 samples and more than 1 sample per woman (77 samples).

[f]183 of 3,261 samples were excluded from meta-analysis for the following reasons: 49 duplicates; 28 in studies with sample sizes < 10; 106 excluded by authors of primary studies.[34]

Summary estimates of test accuracy (percent correct) ranged from 91.4% to 98.5% (Table 3). Also in Table 3, all summary estimates of sensitivity and specificity were in the high 90s (sensitivity range: 95.4%–99.3%; specificity range: 97.7%–98.9%), along with positive predictive values in the range of 98.7% to 99.4% and negative predictive values ranging from 92.1% to 98%. Accuracy estimates were influenced by whether inconclusive results were included or excluded, with slightly lower estimates (albeit all greater than 90%) when some or all inconclusive tests were included.

Two systematic reviews did not meta-analyze results, but described similar figures.[35,38] Wright and Burton[38] reported the number of samples tested in each study were 300, 563, and 1,997 with accuracy reported to be 95.7%, 99.8%, and 99.3%, respectively.[38] Legler et al[35] reported on 14 test protocols separated into what they considered as “proof of principle” test protocols (i.e., those with a small sample size, n = 8 protocols) and large cohorts (i.e., 300 to 1,869 samples, n = 6 protocols). Across the 14 test protocols in the 11 included studies, the accuracies ranged from 90.7% to 99.8%, sensitivity ranged from 99.5% to 99.8%, and specificity from 94% to 99.5%.[35]

False-Positive, False-Negative, and Inconclusive Test Results

Table 4 summarizes the findings of the three reviews that reported overall estimates of false-positive, false-negative, and inconclusive test results. The systematic review by Yang et al[39] conducted bivariate meta-analyses and presented summary estimates only for false-positive rates and false-negative rates (Table 4) because the authors judged sensitivity and specificity to be very high. The other two systematic reviews reporting false or inconclusive results reported means or medians across their included primary studies.[20,36]

Table 4: False or Inconclusive Test Results From Noninvasive Fetal RhD Genotyping

Author, Year	Scenario, n	False-Positive Rate, % (95% CI)	False-Negative Rate, % (95% CI)	Inconclusive Results, % (n/N)
Yang et al, 2019[39]; also reported in Saramago et al, 2018[37]	Inconclusive treated as positive, 8	Bivariate meta-analysis 3.86 (2.54–5.82)	Bivariate meta-analysis 0.34 (0.15–0.76)	—
	Inconclusive treated as positive, 6[a]	Bivariate meta-analysis 4.37 (2.79–6.78)	Bivariate meta-analysis 0.38 (0.15–0.94)	Range 0.4–14.3[b] Median 5.7[b]
	Excluding all inconclusive, 8	Bivariate meta-analysis 1.26 (0.87–1.83)	Bivariate meta-analysis 0.35 (0.15–0.82)	—
	Bristol studies, 3[c]	Bivariate meta-analysis 5.73 (4.58–7.16)	Bivariate meta-analysis 0.21 (0.09–0.48)	Range 3.4–12.2[b] Median 8.0[b]
Mackie et al, 2017[36]	Studies reporting inconclusive results, 13[d]	Mean across studies 3.4 Median 1.2	Mean across studies 3.4 Median 2.9	Range 0.73–15.2[b] Median 6.b[c]
Zhu et al, 2014[20]	All samples, 11,129	—	—	3.1 (352/11,129)
	Conclusive samples: 10,777[e]	1.3 (NR)	2.0 (NR)	NA

Abbreviations: CI, confidence interval; n, number of given test results; N, total number of test results; NA, not applicable; RhD, rhesus D blood group.
[a]Only 6 studies reported inconclusive results.[39]
[b]Ranges and medians were derived from data available in the published systematic review.
[c]Diagnostic test accuracy studies from the International Blood Group Reference Laboratory in Bristol, England, only.
[d]Information relevant to RhD component of the review are presented; overall scope encompassed all noninvasive prenatal testing (30 studies).
[e]352 of 11,129 samples were excluded from the analysis because they were inconclusive.[20]

The systematic review by Mackie et al[36] stated that 13 of their 30 included studies reported inconclusive results and 10 gave the following reasons, in decreasing frequency: no reason, RhD gene variant, insufficient number of markers present from pre-specified cut-off, test failure, low fetal fraction. The systematic review by Legler et al[35] commented on inconclusive results as a by-product of failed DNA amplification, which they suggest may vary depending on the DNA extraction method used and may require repeat testing.

Subgroup: Accuracy by Gestational Age/Timing

Two reviews conducted subgroup meta-analyses to estimate test accuracy by gestational age (weeks), categorized by trimester of pregnancy (Table 5).[20,34] Both found that accuracy was highest when testing was performed in the first trimester. Zhu et al[20] did not report confidence intervals for accuracy estimates and they were not calculable from data presented in that review. However, their statistical analysis excluded inconclusive results, which may lead to more optimistic estimates.

Table 5: Diagnostic Accuracy of Noninvasive Fetal RhD Genotyping by Trimester of Pregnancy

Author, Year	1st Trimester Accuracy, % (95% CI)	2nd Trimester Accuracy, % (95% CI)	3rd Trimester Accuracy, % (95% CI)
Zhu et al, 2014[20]	99 (NR)	98.3 (NR)	96.4 (NR)
Geifman-Holtzman et al, 2006[34]	90.8 (86.3–94.0)	85.0 (81.1–88.2)	85.3 (80.4–89.2)

Abbreviations: CI, confidence interval; NR, not reported; RhD, rhesus D blood group.

Subgroup: Accuracy in Alloimmunized Pregnancies

Geifman-Holtzman et al[34] reported the test accuracy of noninvasive fetal RhD genotyping in alloimmunized pregnancies to be 91.8% (95% confidence interval not reported).

Subgroup: Singleton Versus Multiple Pregnancies

No systematic reviews reported the test accuracy of noninvasive fetal RhD blood group genotyping specifically in singleton or multiple pregnancies.

Results: Clinical Utility

Clinical Literature Search

The database search of the clinical literature yielded 1,445 citations published between January 1, 1997, and February 25, 2019. After removal of duplicates, we screened 876 citations. We identified 11 studies[37,41-50] (10 observational studies and one health technology assessment) that met our inclusion criteria. We reviewed the reference lists of included studies and identified one relevant citation not captured by our literature search.[51] However, it was superseded by a more recent, included publication[44] and thus was excluded. See Appendix 3 for a list of selected studies excluded after full-text review. Figure 2 presents the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram for the clinical literature search.

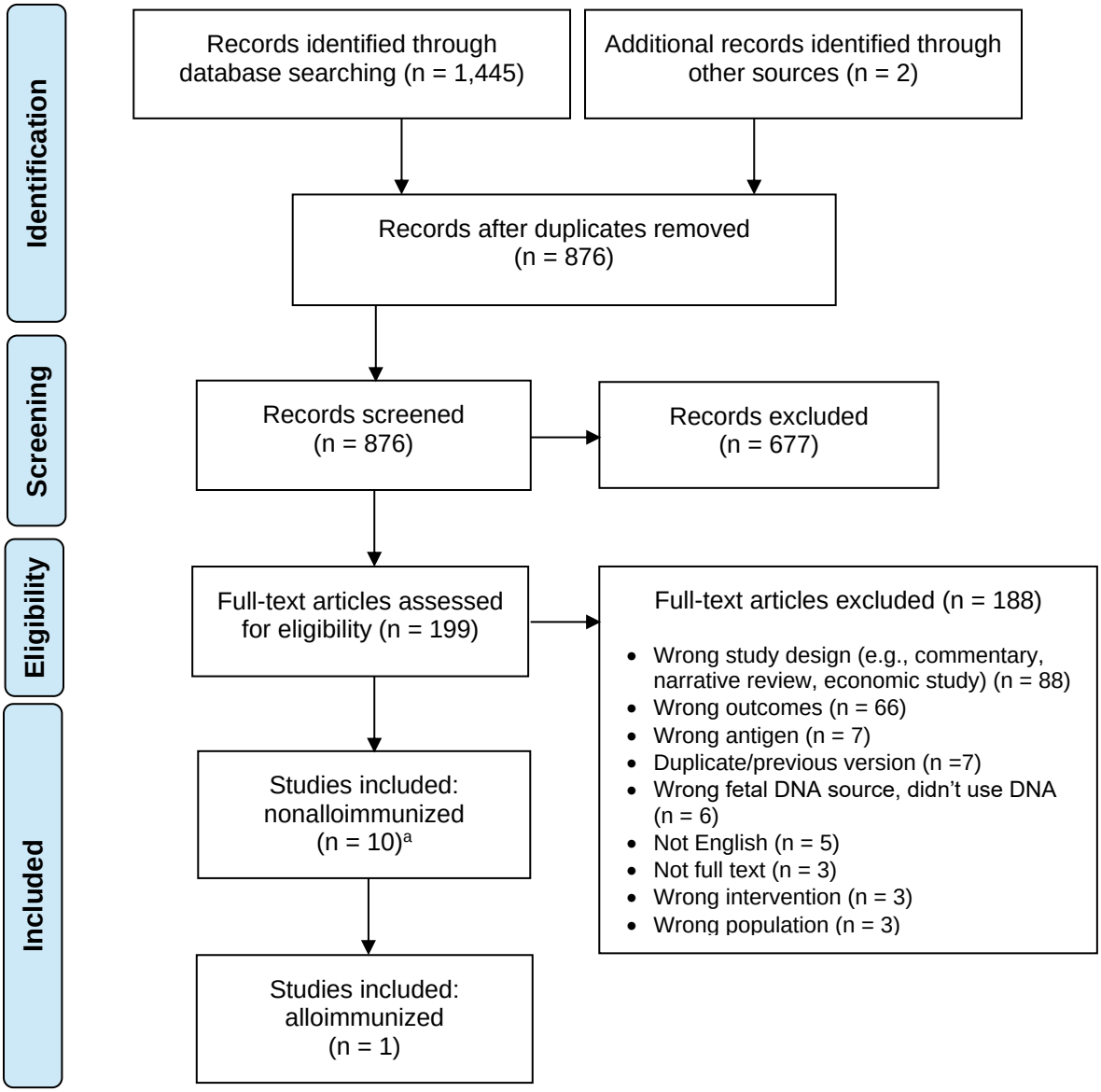

Figure 2: PRISMA Flow Diagram—Clinical Search for Research Question 2

Abbreviation: PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-analyses.
[a]Nine primary studies, one health technology assessment.
Source: Adapted from Moher et al, 2009.[27]

Characteristics of Included Studies

Of the 11 included studies, one[48] was on alloimmunized RhD− pregnancies and 10 were on the nonalloimmunized population (nine primary studies, one health technology assessment).[37,41-47,49,50] The HTA[37] included five of the same nine primary studies we included on nonalloimmunized RhD− pregnancies, while we identified an additional four relevant studies[43,44,46,47] that were published since 2016, the literature search date of the HTA. The HTA captured one citation our search did not,[51] but this study was superseded by an updated 2016 publication[44] which we included. For these reasons, we do not report results from the HTA, but rather report results directly from the included primary studies (n = 10).[41-50]

Table 6 shows the characteristics of the 10 primary studies. The studies were conducted mainly in routine health care settings in Europe and Scandinavia. Nine were single-arm nonrandomized studies, one was a prospective comparative cohort study comparing universal (control) with targeted RhIG prophylaxis (fetal RhD genotyping),[43] and one was a population-based cohort study of fetal RhD genotyping compared with historical controls.[50]

Risk of Bias in the Included Studies

Appendix 2, Table A2 shows our risk of bias assessment for the 10 nonrandomized studies. We judged three studies to be at low risk of bias on all domains[44,46,49] and two studies to be at low risk of bias on all domains except for potential selection biases introduced by inadequate control of confounding variables, for which the risk of bias was unclear.[41,50] For five studies, the risk of bias was judged to be either high or unclear for two or more domains.[42,43,45,47,48]

Table 6: Characteristics of Included Studies on Clinical Utility of Noninvasive Fetal RhD Genotyping

Author, Year	Country	Objective(s)	Study Design	Population Source	N[a]	Relevant Outcomes
Nonalloimmunized RhD− Pregnancies						
Darlington et al, 2018[43]	France	Evaluate impact of fetal RhD genotyping to determine cost, test accuracy, and management of anti-D prophylaxis	Prospective 2-arm trial	Pregnancies followed in 11 university hospital maternity clinics, 2009–2012 Control arm also recruited prospectively or identified at the end of pregnancy and consented retrospectively	Genotyping: 515 Control: 335	Unnecessary RhIG avoided Alloimmunization RhIG compliance Uptake of testing
Haimila et al, 2017[46]	Finland	Report first 2 years of fetal RhD screening and compliance with anti-D prophylaxis	Prospective cohort	Pregnancies attending maternity clinics for second routine antibody screening	10,814	Unnecessary RhIG avoided Uptake of testing
Papasavva et al, 2016[47]	Cyprus	Determine feasibility and experiences of routine fetal RhD genotyping	Prospective cohort	Pregnancies referred for testing by obstetrician during routine visit	71	Unnecessary RhIG avoided
de Haas et al, 2016[44]	The Netherlands	Report first 15 months of performance, accuracy, and compliance with national fetal RhD screening program	Prospective cohort (consecutive series)	National antenatal screening program for all pregnancies, July 4, 2011–Oct 7, 2012	32,222 pregnancies (25,789 people)	Uptake of testing
Soothill et al, 2015[49]	UK	Define potential difficulties, assess solutions, and explore savings of service program pilot	Prospective cohort	Service implementation pilot in 3 maternity service areas offering testing, April 1, 2013–Sept 30, 2013	529	Unnecessary RhIG avoided RhIG compliance
Clausen et al, 2014[41]	Denmark	Report first 2 years of national routine fetal RhD screening program	Population-based prospective cohort	As part of national RhD prophylaxis program offering fetal RhD genotyping to all RhD− pregnancies in 5 regions	5 regions: 12,668 Region 1: 690	Unnecessary RhIG avoided RhIG compliance (Region 1 only)
Tiblad et al, 2013[50]	Sweden	Estimate the incidence of alloimmunization with targeted anti-D prophylaxis via fetal RhD screening	Population-based prospective cohort with historic controls	All RhD− pregnancies in 1st trimester, Sept 1, 2009–Dec 31, 2011 Reference cohort consisted of all RhD− women giving birth in the same region, 2004–2008	Study cohort: 9,380 Reference cohort: 18,546	Unnecessary RhIG avoided Alloimmunization RhIG compliance Adverse events Uptake of testing

Author, Year	Country	Objective(s)	Study Design	Population Source	N[a]	Relevant Outcomes
Grande et al, 2013[45]	Spain	Screen mixed-ethnic population in late 2nd trimester and assess test accuracy	Population-based prospective cohort	6 health centres in Barcelona-West health district, Feb 2010–Oct 2011	302	Unnecessary RhIG avoided RhIG compliance
Damkjær et al, 2012[42]	Denmark	Assess compliance with the anti-D prophylaxis program including fetal RhD screening to target RhIG prophylaxis	Retrospective cohort	Hospital with largest obstetrics department	239	Unnecessary RhIG avoided RhIG compliance
Alloimmunized RhD− Pregnancies						
Rijnders et al, 2004[48]	The Netherlands	Validate fetal RhD genotyping from maternal plasma and offer the test to patients with medical need for it	Prospective cohort	Patients with a medical reason for RhD status determination at one hospital over 2 years	3[b]	Invasive procedures and tests avoided

Abbreviations: RhD, rhesus D blood group; RhIG, Rh immunoglobulin; N, sample size; UK, United Kingdom.

[a]Unless stated, number of pregnancies is equivalent to the number of pregnant people in the studies.

[b]Total study cohort included 24 singleton pregnancies tested for fetal sex (*SRY* gene, n = 21) because they were carriers for X-linked diseases, and 3 RhD− alloimmunized pregnancies who underwent noninvasive RhD genotyping to determine fetal status.[48]

Nonalloimmunized RhD− Pregnancies

Nine studies examined using fetal RhD genotyping to screen nonalloimmunized pregnancies for incompatibility and target the administration of anti-D prophylaxis with RhIG.[41-47,49,50] The genotyping protocols from all the studies analyzed cffDNA in maternal plasma and ran their assay in several replicates, at least duplicate or triplicate, for reproducibility. All but one[50] test targeted at least two exons on the RhD gene. Table 7 shows the timing, platforms, and other test characteristics of fetal RhD genotyping in the included studies.

Table 7: Test Characteristics of Noninvasive Fetal RhD Blood Group Genotyping From Maternal Plasma in Studies of Nonalloimmunized RhD− Pregnancies

Author, Year	Gestational Timing, Weeks	Testing Platform, DNA Extraction	Exon(s) Targeted	Test Control(s)
Darlington et al, 2018[43]	Overall: 23 ± 8 Genotyping group: 19 ± 4 Control group: 28 ± 9	Real-time PCR, Free DNA Fetal Kit RHD (Institut Biotechnologie Jaques BOY)	7, 10	If fetus is RhD− on first test, controlled by second test on a new blood sample taken 1 week later
Haimila et al, 2017[46]	Mainly 24–26, accepted from 20 onwards	Real-time PCR, QIAsymphony Automate (Qiagen)	5, 10	RhD− and RhD+ controls included; no controls for total DNA
Papasavva et al, 2016[47]	NR	Real-time PCR, QIAamp Circulating Nucleic Acid Kit (Qiagen)	4, 5, 10	*SRY* to confirm presence of male fetal DNA; *CCR5* to evaluate total DNA; RhD+, RhD− and *RHDΨ*
de Haas et al, 2016[44]	Mean 27 ± 6 days	Real-time PCR, Viral NA Large Volume Kit (Roche)	5, 7	Computer algorithm advises repeat test (same sample) if RhD− or inconclusive result
Soothill et al, 2015[49] testing protocol reported in Finning et al, 2008[52]	15–17, up to 26	Real-time PCR, MDx BioRobot (Qiagen)	5, 7	*CCR5* for total DNA, RhD+, RhD− and *RHDΨ*, and no DNA
Clausen et al, 2014[41]	25	Real-time PCR, automated DNA extraction (5 regions each with own validated test protocol)[a]	2 exons: 5, 7 or 5, 10 or 7, 10	RhD+ and RhD− control sample; *CCR5* for total DNA
Tiblad et al, 2013[50] testing protocol reported in Wikman et al, 2012[53]	From 8 onward	Real-time PCR, MagnaPure LC Total Nucleic Acid Isolation Kit – Large Volume (Roche)	4	RhD+ and RhD− control sample; GAPDH cycle threshold value for fetal DNA
Grande et al 2013[45]	24–26	Single multiplex real-time PCR; Automated DNA extraction COBAS AmpliPrep DNA/RNA Extractor (Roche)	5, 7	*DYS14* for fetal DNA in RhD− male fetuses; exon 10 and *SRY* to confirm RhD− result on second DNA Extraction
Damkjær et al 2012[42]	25 (range 23–28) Mean 27 (SD 22 days)	Real-time PCR, automated DNA extraction (each of 5 regions with own validated protocol)	2 exons: 5, 7 or 5, 10 or 7, 10	*CCR5, SOD,* or GAPDH cycle threshold value for total DNA

Abbreviations: DNA, deoxyribonucleic acid; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; RhD, rhesus D blood group; PCR, polymerase chain reaction; SD, standard deviation.

[a]In regions 1 and 3 of Denmark, DNA extraction using QIAsymphonySP (Qiagen); in regions 2 and 5, DNA extraction using MagNa Pure LC 2.0 (Roche); and in region 4, DNA extraction using MagnaPure Compact (Roche).[41]

Two studies reported the ethnic composition of their population.[44,45] In the study conducted in Spain,[45] the population was 84% Caucasian, 12% Latin American, 1.8% African, 1.1% Pakistani, and less than 1% Asian and other ethnicities. In the national screening program of the

Netherlands, most RhD− pregnant people (90.4%) were European, with 4.1% of Mediterranean origin, 4.0% other ethnicities, and less than 1% each of Black, Asian, and Hindustani.[44]

Unnecessary RhIG Prophylaxis Avoided

Eight of the included studies reported on the ability of fetal RhD genotyping to identify RhD incompatible pregnancies (i.e., an RhD− mother carrying an RhD+ fetus), allowing clinicians to target RhIG prophylaxis only to those who require it and avoid unnecessary administration of the treatment.[41-43,45-47,49,50] Table 8 summarizes these results.

Table 8: Appropriate RhIG Administration or Avoidance in RhD− Pregnancies Based on Fetal RhD Genotyping

Author, Year	Unnecessary RhIG Avoided in Pregnancies Carrying an RhD− Fetus	Unnecessary RhIG Avoided in All Pregnancies Genotyped, % (n/N)	Related Outcomes, if Applicable
Darlington et al, 2018[43]	Genotyping: 93% Control: 27%[a]	NR	Total treated appropriately[b]: Genotyping group 85% vs. control group 62% (*P* < .0001)
Haimila et al, 2017[46]	99.6% (3,626[b]/3,641)	33.7% (3,626[b]/10,814)	Unnecessary RhIG given to 0.4% (39 people) of pregnancies carrying RhD− fetus, mainly owing to inconclusive results
Papasavva et al, 2016[47]	100%[c] (18/18[b])	25.3% (18/71)	—
Soothill et al, 2015[49]	94% (17/18)	35%	6% reduction in use of RhIG per month of program (P < .001, 95% CI 4%–8%) in pregnancies carrying RhD− fetus 10% (5 people) with inconclusive results given RhIG
Clausen et al, 2014[41]	97.3%	37.1%	—
Tiblad et al, 2013[50]	100%[d]	39% (3,270/8,374)	—
Grande et al, 2013[45]	95% (90/95)	NR	5% (5/95) of pregnancies carrying RhD− fetus requested RhIG
Damkjær et al, 2012[42]	98.6% (68/69[c])	31.5% 68/216[c] genotyped	Unnecessary RhIG given to 1.2% (1/69) before genotyping; reason unknown

Abbreviations: n, number of people avoiding RhIG; N, total number of people in the study; NR, not reported; RhD, rhesus D blood group; RhIG, Rh immunoglobulin.

[a]Based on paternal genotype, determined retrospectively.

[b]Appropriate treatment defined as receiving RhIG if carrying an RhD+ fetus and not receiving RhIG if carrying an RhD− fetus. When considering a second test to confirm results, 88% in genotyping group and 63% in control group were treated appropriately.[43]

[c]Calculated from the data reported in the published article.

[d]Taken from Figure 1 (study participants flow) in Tiblad et al.[50]

Across studies, 25.3% to 39% of all RhD− pregnancies (with an RhD+ or RhD− fetus) avoided unnecessary RhIG after noninvasive fetal RhD blood group genotyping (Table 8). Among the RhD− pregnancies carrying an RhD− fetus (i.e., not RhD incompatible nor at risk for

alloimmunization), over 90% avoided unnecessary RhIG. Darlington et al[43] reported 93% of not-at-risk RhD− pregnancies avoided unnecessary RhIG in the genotyping arm, compared with only 27% in the control arm (*P* value or confidence intervals not provided). After noninvasive fetal RhD blood group genotyping in the studies, a small proportion of people (range: 0.4%–10%) received RhIG upon request[45] or when test results were inconclusive.[46,49]

We rated the GRADE certainty of the evidence for this outcome as low because observational studies not warranting upgrade considerations are judged to be of low quality by the GRADE framework (Appendix 2, Table A3).

Compliance With Targeted RhIG Prophylaxis

Five studies reported that overall compliance with the targeted RhIG prophylaxis program (the proportion of RhD incompatible pregnancies that did receive RhIG, as recommended) was over 80%.[41-43,49,50] In the two-arm study by Darlington et al,[43] 87% of the RhD genotyping group received RhIG prophylaxis compared with 82% in the control group (universal prophylaxis). They also reported that of RhD incompatible pregnancies, 65% in the genotyping group received RhIG at optimal timing (26–32 weeks' gestation) compared with 78% of the control group.[43]

As shown in Table 9, compliance ranged from 85.3% to 100% across the four single-arm cohort studies. In addition to the compliance point estimates, Damkjær and colleagues[42] also reported a significant increase in the number of people recommended and receiving RhIG prophylaxis at 29 weeks over the study period (Chi-square *P* = .04).

Table 9: Compliance With Targeted RhIG Prophylaxis as Reported in Single-Arm Cohort Studies

Author, Year	RhIG Compliance, %
Soothill et al, 2015[49]	Among pregnancies carrying RhD+ fetus: 100
Clausen et al, 2014[41]	Overall: 93.2
	At optimal timing (GA 28–30 weeks): 85.3[a]
	Both prenatal and postnatal: 78
	Overall postnatal: 99.1
	Postnatal only: 21
Tiblad et al, 2013[50]	Overall: 89.9
Damkjær et al, 2012[42]	Overall: 86
	At optimal timing (GA 28–30 weeks): 68

Abbreviations: GA, gestational age; RhIG, Rh immunoglobulin.
[a]Of samples tested at GA 24–26 weeks.[41]

We rated the GRADE certainty of the evidence for this outcome as very low, downgrading because of limitations in risk of bias (Appendix 2, Table A3).

Uptake of Noninvasive Fetal RhD Genotyping

Six studies reported the uptake of RhD genotyping.[41,42,44-46,50] All of these studies were population-based screening programs specifically for nonalloimmunized RhD− pregnancies and uptake was 84% or higher, as shown in Table 10. Testing was accepted by most pregnant people eligible for RhD− genotyping, increasing in one study from 69.7% in the first year to 97.3% in the second year.[46]

Table 10: Uptake and Acceptance of Screening Nonalloimmunized RhD− Pregnancies With RhD Genotyping

Author, Year	Testing Uptake, % (n/N)
Haimila et al, 2017[46]	Year 1: 69.7% Year 2: 97.3%
de Haas et al, 2016[44]	Overall: at least 98% First 4 weeks: 91.1% Year 1: 96.3% End of study period: 97.5%
Clausen et al, 2014[41]	84.2%[a] (581/690)[a]
Tiblad et al, 2013[50]	89% (8,374/9,380)
Grande et al, 2013[45]	94% (284/302)
Damkjær et al, 2012[42]	90% (216/239)

Abbreviations: n, number of people tested; N, total number of pregnant people; RhD, rhesus D blood group.
[a]Reported only for Region 1 after 15 months of Danish screening program.[41]

We rated the GRADE certainty of the evidence for this outcome as low because observational studies not warranting upgrade considerations are judged to be of low quality according to the GRADE framework (Appendix 2, Table A3).

Risk of Alloimmunization

One study was designed to evaluate the risk of alloimmunization using a targeted RhIG strategy via fetal RhD genotyping compared with a historical reference cohort receiving standard prophylaxis.[50] Routine prenatal administration of RhIG prophylaxis was not part of the care for the historical reference cohort; instead, RhIG was administered routinely after birth and after any potentially sensitizing events (events that increase the risk of fetomaternal hemorrhage and alloimmunization). In the reference cohort, there were 86 alloimmunizations among 18,546 women. In the genotyping cohort, 24 alloimmunizations occurred (n = 9,380 women): 14 of these (58.3%) occurred before scheduling RhIG, four (16.7%) occurred despite receiving RhIG during their pregnancy (RhIG failure), two (8.4%) occurred with unknown time or cause with antibodies found 10 months postpartum, and two occurred in women who did not receive RhD genotyping due to poor compliance early in the screening program. The final two women (8.4%) were alloimmunized during the first trimester of their subsequent pregnancy, despite having received RhIG prophylaxis during the study. Table 11 provides a summary of results. The risk of alloimmunization was 45% lower in the genotyping cohort compared with the historic reference cohort that received postnatal and antenatal RhIG prophylaxis following any potentially sensitising events.

Table 11: Relative Risk of Alloimmunization With Targeted RhIG Prophylaxis by Fetal RhD Genotyping Compared With Universal Prophylaxis

Author, Year	Genotyping Cohort Incidence, % (95% CI)	Reference Cohort Incidence, % (95% CI)	Risk Ratio (95% CI)	Absolute Risk Difference, % (NNT)
Tiblad et al, 2013[50]	0.26 (0.15–0.36)	0.46 (0.37–0.56)	0.55 (0.35–0.87)	0.20 (500)

Abbreviations: CI, confidence interval; NNT, number needed to treat; RhD, rhesus D blood group; RhIG, Rh immunoglobulin.

We rated the GRADE certainty of the evidence for this outcome as very low, downgrading because of limitations in risk of bias and indirectness (Appendix 2, Table A3).

Two other studies each reported one false-negative result from noninvasive fetal RhD genotyping in pregnancies that missed receiving RhIG prophylaxis entirely[43] or until after birth,[46] though it is not reported whether the presence of anti-D antibodies was confirmed.

Other Outcomes of Interest

No studies reported on maternal quality of life, avoidance of cord blood RhD testing, or adverse events associated with testing or RhIG in nonalloimmunized pregnancies.

Alloimmunized RhD− Pregnancies

One study provided information on the outcomes of RhD− alloimmunized pregnancies when treatment decisions were guided by noninvasive fetal RhD genotyping.[48] The study cohort included singleton pregnancies tested noninvasively for fetal sex because they were carriers for X-linked diseases (*SRY* gene, n = 21) and three RhD− alloimmunized pregnancies who underwent noninvasive fetal RhD blood group genotyping to determine fetal RhD status to guide pregnancy care. We report the study procedures and outcomes for these three RhD− alloimmunized pregnancies from the study.

In the study, fetal RhD genotyping was compared with RhD status determined by CVS, amniocentesis, or cord blood serology at birth.[48] Fetal DNA was extracted in duplicate from maternal plasma using the Qiagen minikit (Qiagen) and the assay was run to target exon 7 using real-time quantitative PCR. The RhD assay was run in triplicate and interpreted as positive (RhD+ fetus) when fetal DNA was amplified in at least two of the three replicates, and negative when none or one replicate showed a positive result. If the results of the duplicate DNA isolations were incongruent, the results of the test were classified as inconclusive.

Invasive Procedures Avoided

Of the three RhD− pregnancies, one was found via RhD genotyping to be carrying an RhD− fetus and avoided close monitoring for fetal well-being, and eventual amniocentesis.[48] The other two RhD− pregnancies were found to be carrying RhD+ fetuses and were therefore closely monitored for fetal well-being and signs of anemia.

We rated the GRADE certainty of the evidence for this outcome as very low, downgrading because of limitations in risk of bias (Appendix 2, Table A4).

Other Outcomes of Interest

The study reported no information on frequency of hospital visits and blood tests, maternal quality of life, adverse events, or procedures missed in alloimmunized pregnancies.

Discussion

Across systematic reviews, test accuracy of noninvasive fetal RhD blood group genotyping was high. Studies of the uptake of this testing and its clinical impact in practice showed positive individual and system outcomes, with many pregnant people avoiding unnecessary RhIG prophylaxis or intensive prenatal monitoring. A precautionary approach, where inconclusive test results are treated as positive (i.e., people receive RhIG prophylaxis or are intensively

monitored), also led to positive outcomes for individuals and screening programs that implemented targeted pregnancy care based on noninvasive cffDNA testing for RhD incompatibility. One clinical utility study employing noninvasive fetal RhD genotyping to target prenatal RhIG prophylaxis observed reduced incidence of alloimmunization compared with using RhIG only after birth or sensitizing events.

Our findings for diagnostic accuracy are consistent with those of earlier health technology assessments from Sweden[33] and Norway.[32] The finding of reduced incidence of alloimmunization after introduction of fetal RhD genotyping comes from a single study[50] that compared targeted prenatal use of RhIG with a cohort who received it only postnatally or after a sensitizing event. That study has limited generalizability to the Canadian context because, currently, RhD− pregnant people receive universal prenatal RhIG prophylaxis at 28 weeks' gestation, in addition to after birth. Thus, we would not expect a reduction, or probable material change, in the incidence of alloimmunization if targeted prophylaxis were adopted in Ontario.

Test accuracy appeared to be most influenced by the handling of inconclusive results, as a systematic review on the quality of these studies has noted.[54] Across systematic reviews, there was variation in the rate and analytic treatment of inconclusive test results. We noted that most test protocols involved multiple exon targets and replicates, scrutiny of negative test results using internal quality controls to confirm presence of sufficient cffDNA, and/or retesting to attempt to determine the reason for an inconclusive result (e.g., insufficient cffDNA, sample contamination, sample mix-up, presence of a rare fetal or maternal RhD gene variant). The literature indicates that noninvasive fetal RhD blood group genotyping appears to be robust for the most common RhD gene variants across multicultural populations (e.g., deletion, pseudogene *RHDΨ*, *RhD-CE-D*). However, numerous rare gene variants may be more likely to yield inconclusive test results, and these are not limited to one or another ethnic group. Further, not all RhD variants necessarily put pregnant people at risk of alloimmunization, as the relationship between genotype and phenotype is complex. People with inconclusive results on fetal RhD genotyping, or those with clinical suspicion of potentially being at risk for alloimmunization, tend to be treated as positive (using a precautionary approach) and followed closely by care providers to avoid potential harm.

From the available subgroup analyses of accuracy data, we could not clearly ascertain differential accuracy based on gestational timing. Accuracy seemed slightly higher when testing was performed in the first trimester. However, we are cautious about this subgroup analysis as it is somewhat inconsistent with the foundational biological mechanism that genotyping is based on—maternal plasma contains less cffDNA in the first trimester compared with the second or third. Similarly, we could not draw conclusions from the available data about potential variations in accuracy by ethnicity.

Strengths and Limitations

At this time we cannot draw conclusions about the following outcomes of interest because no data were found: avoided newborn cord blood RhD typing, maternal quality of life, adverse events associated with testing or RhIG in nonalloimmunized pregnancies, frequency of hospital visits and blood tests, maternal quality of life, adverse events, or procedures avoided in alloimmunized pregnancies.

There are not many overviews of reviews on diagnostic test accuracy in the literature and, to our knowledge, ours is the first to assess noninvasive fetal RhD blood group genotyping. We chose this methodological approach owing to the existence of several systematic reviews on the

accuracy of noninvasive fetal RhD genotyping published during the past 15 years. The available systematic reviews synthesized primary studies over roughly sequential periods of time, allowing us to essentially capture the entire body of literature on diagnostic accuracy up to 2016. However, by conducting an overview of reviews, it is possible we missed some studies published since that time.

We are reasonably assured in having captured the bulk of the diagnostic accuracy literature, particularly by including three systematic reviews judged to be at low risk of bias.[34,36,39] These reviews used methods that were comprehensive in their eligibility criteria, identification and selection of studies, and data collection and study appraisal, and they all conducted meta-analyses. The other three systematic reviews, which had risk of bias concerns,[20,35,38] reported accuracy estimates in support of the systematic reviews of superior quality. We were not able to conduct a GRADE assessment to determine certainty in the results on diagnostic accuracy, owing to the limited reporting of information in the systematic reviews. We are currently not aware of any published guidance to conduct quality assessments of evidence in the context of overviews of reviews.

We synthesized primary studies to assess the clinical utility of implementing noninvasive fetal RhD blood group genotyping because we identified new studies published since the existing health technology assessment by the UK National Institute for Health and Care Excellence[37] and to permit examination of the alloimmunized population. To our knowledge, ours is the first systematic review of the clinical utility of this test for alloimmunized RhD− pregnancies. We found the research on the alloimmunized population to be considerably scarce. While it is a small population thanks to effective RhIG prophylaxis programs, alloimmunization carries a burden of intensive monitoring both on pregnant people and health system resources. The available literature on alloimmunized RhD− pregnancies suggests that noninvasive fetal RhD blood group genotyping permits pregnancies that are not RhD incompatible to avoid unnecessary intensive pregnancy care and invasive procedures. Although many of the included studies measured test accuracy, it was not an outcome of interest for our clinical utility review.

Ongoing Studies

We are not aware of any ongoing studies that have potential relevance to this review.

Conclusions

Diagnostic Accuracy

Noninvasive fetal RhD blood group genotyping using cell-free fetal DNA from maternal blood in RhD− pregnancies was found to have high test accuracy, sensitivity, specificity, and positive and negative predictive values, from our overview of published systematic reviews.

Clinical Utility

The evidence suggests that noninvasive fetal RhD blood group genotyping using cell-free fetal DNA from maternal blood to guide care of RhD− pregnancies:

- Largely avoids unnecessary RhIG prophylaxis in the vast majority of nonalloimmunized RhD− pregnancies not at risk of alloimmunization (GRADE: Low)
- May lead to high compliance with targeted RhIG prophylaxis programs for nonalloimmunized RhD− pregnancies at risk of alloimmunization, but the evidence is very uncertain (GRADE: Very low)
- Leads to large uptake rates of fetal RhD genotyping of 84% or higher as part of care for nonalloimmunized RhD− pregnancies (GRADE: Low)
- May reduce the risk of alloimmunization when fetal RhD genotyping is used to target prenatal RhIG prophylaxis, compared with RhIG prophylaxis administered only after birth or potentially sensitizing events, but the evidence is very uncertain (GRADE: Very low)
- May avoid unnecessary invasive procedures in alloimmunized RhD− pregnancies not at risk of hemolytic disease of the fetus and newborn, but the evidence is very uncertain (GRADE: Very low)

ECONOMIC EVIDENCE REVIEW

Research Questions

1. What is the cost-effectiveness of noninvasive fetal RhD blood group genotyping compared with usual care for the management of nonalloimmunized RhD negative (RhD−) pregnancies?
2. What is the cost-effectiveness of noninvasive fetal RhD blood group genotyping compared with usual care for the management of alloimmunized RhD− pregnancies?

Methods

Economic Literature Search

We performed an economic literature search on February 26, 2019, to retrieve studies published from database inception until the search date. To retrieve relevant studies, we developed a search using the clinical search strategy with an economic and costing filter applied.

We created database auto-alerts in MEDLINE and Embase and monitored them for the duration of the assessment period. We also performed a targeted grey literature search of health technology assessment agency websites, clinical trial and systematic review registries, the Tufts Cost-Effectiveness Analysis Registry, and The Hospital for Sick Children (SickKids) Paediatric Economic Evaluation (PEDE) Database. The grey literature search was updated on July 23–24, 2019. See Appendix 1 for our literature search strategies, including all search terms.

Eligibility Criteria

Studies

Inclusion Criteria

- English-language full-text systematic reviews, health technology assessments, or individual-level comparative economic studies published from database inception until February 26, 2019, or later as identified via auto-alert search updates
- The following types of trial-based or model-based economic studies: cost-effectiveness analyses, cost–utility analyses, cost–benefit analyses, or cost-consequence analyses

Exclusion Criteria

- Narrative reviews, editorials, commentaries, conferences abstracts, letters, study protocols, guidelines, and unpublished studies
- Economic studies that evaluate other pre- and postnatal blood typing tests (e.g., amniocentesis, chorionic villus sampling, cord blood typing)
- Noncomparative costing studies, feasibility studies, or cost-of-illness studies

Population

- Serologically confirmed nonalloimmunized or alloimmunized RhD− pregnancies (singleton or multiple)

Interventions

- Noninvasive fetal RhD blood group genotyping (i.e., cell-free fetal DNA in maternal blood [plasma, serum, or whole blood], laboratory-developed or commercial) versus usual care (e.g., universal RhIG prophylaxis for nonalloimmunized RhD− pregnancies)

Outcome Measures

- Incremental costs, incremental effectiveness (e.g., quality-adjusted life-years [QALYs], disability-adjusted life-years [DALYs]), and incremental economic statistics such as incremental cost-effectiveness ratio (ICER) or incremental net benefit (INB) of the examined intervention versus usual care in the target populations

Literature Screening

A single reviewer conducted an initial screening of titles and abstracts using Covidence systematic review software[26] and then obtained the full texts of studies that appeared eligible for review according to the inclusion criteria. A single reviewer then examined the full-text articles and selected studies eligible for inclusion. The reviewer also examined reference lists for any additional relevant studies not identified through the search.

Data Extraction

We extracted relevant data on study characteristics and outcomes to collect information about the following:

- Source (e.g., citation information, study type)
- Methods (e.g., study design, analytic technique, perspective, time horizon, population, intervention[s], comparator[s])
- Outcomes (e.g., health outcomes, costs, incremental cost-effectiveness ratio[s])

Study Applicability and Limitations

We determined the usefulness of each identified study for decision-making by applying a modified quality appraisal checklist for economic evaluations originally developed by the National Institute for Health and Care Excellence (NICE) in the United Kingdom to inform the development of NICE's clinical guidelines.[55] We modified the wording of the questions to remove references to guidelines and to make it specific to Ontario. Next, we separated the checklist into two sections. In the first section, we assessed the applicability of each study to the research question (directly, partially, or not applicable). In the second section, we assessed the limitations (minor, potentially serious, or very serious) of the studies that we found to be directly applicable.

Results

Economic Literature Search

The economic literature search yielded 114 citations published from database inception until February 26, 2019. After removing duplicates, we identified 74 studies from database searching and 4 citations from other sources. No additional articles were identified through auto-alerts in MEDLINE or Embase or through additional search of the grey literature.

We excluded 56 articles based on information in the title and abstract and obtained 22 potentially relevant articles for full-text assessment. Eight studies met the inclusion criteria and were assessed to establish the applicability of their findings to the Ontario context. One of these studies,[56] a published journal article, presented the results of a previously conducted health technology assessment[37] (identified in our grey literature search). We decided to include solely the published article in our review as it contained updated analyses. See Appendix 4 for a list of studies excluded after full-text review. Figure 3 presents the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram for the economic literature search.

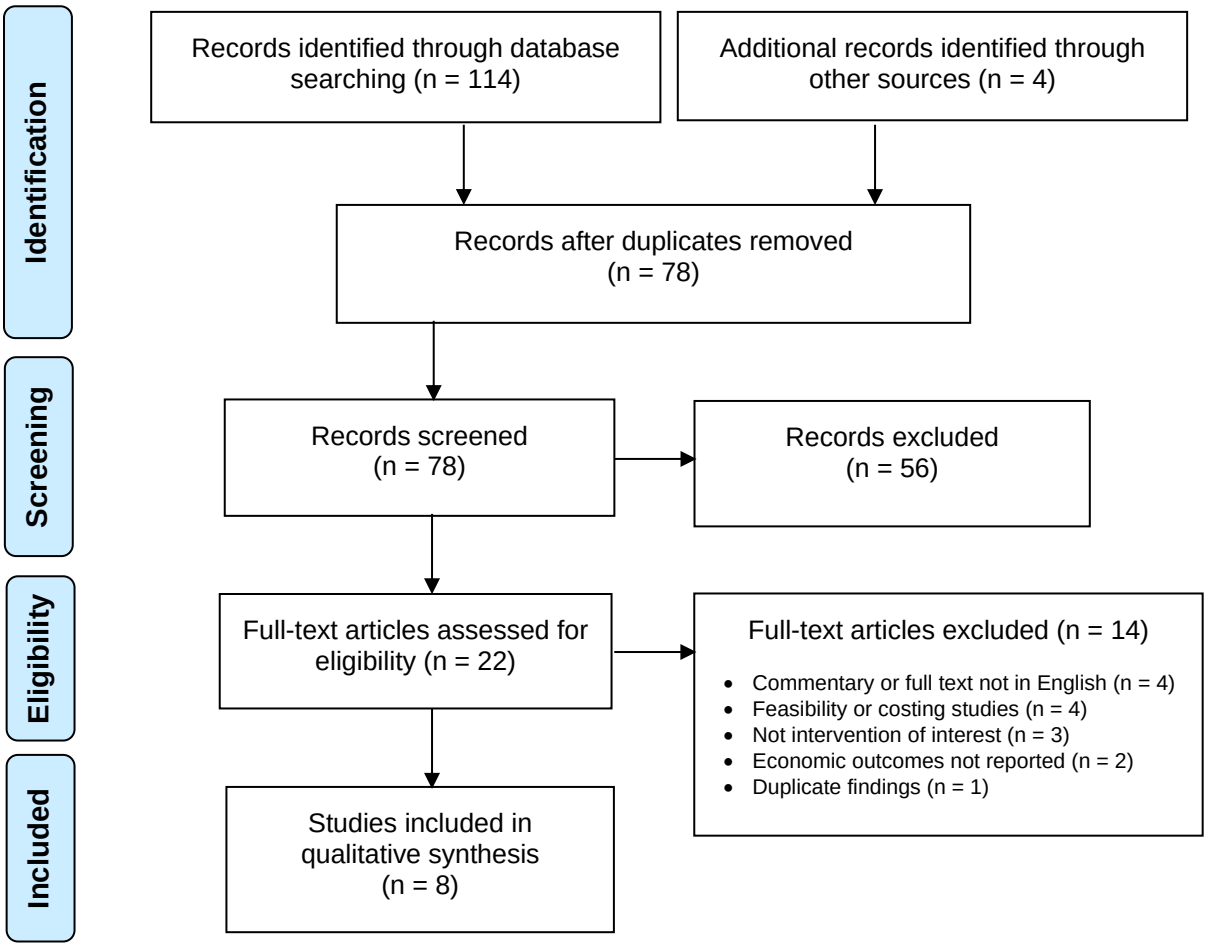

Figure 3: PRISMA Flow Diagram—Economic Search Strategy

Abbreviation: PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-analyses.
Source: Adapted from Moher et al, 2009.[27]

Overview of Included Economic Studies

Table 12 presents the characteristics, outcomes, and results of the eight included studies in detail. Below, we summarize and compare their study methods and cost-effectiveness findings.

Study Methods

Study Design, Analytic Technique, and Study Perspective

Seven studies were model-based cost-effectiveness analyses that took health care sector perspectives of the United States,[57,58] the United Kingdom,[56] Australia,[59] Sweden,[60] and Canada.[61,62] One individual-level cost-effectiveness analysis was conducted alongside a multicentre open nonrandomized clinical trial that included 11 clinics in France and was done from a health care sector perspective.[43]

Of the model-based economic analyses, three studies[58,59,62] used decision-tree models to describe a short-term clinical pathway from the beginning of the nonalloimmunized RhD− pregnancy until birth. One of these studies, a decision analysis by Hawk et al,[58] considered a clinical pathway of subsequent alloimmunized pregnancies and extrapolated the costs and clinical outcomes over several pregnancies. A US decision-tree analysis by Moise et al[57] followed the course of the index (first) nonalloimmunized pregnancy and of one subsequent alloimmunized pregnancy, without a precise definition of the study time horizon. The remaining three studies[56,60,61] simulated the costs and effects over a longer time horizon accounting for time between subsequent pregnancies. Thus, a UK cost–utility microsimulation analysis by Saramago et al[56] examined the course of at least two pregnancies (3 years apart) and accounted for the cumulative costs and health outcomes over a newborn's lifetime; a Swedish Markov cohort cost-effectiveness analysis by Neovius et al[60] analyzed the course of the index and subsequent pregnancies over a 10-year time horizon; and, lastly, a Canadian cost-effectiveness agent-based microsimulation analysis by Duplantie et al[61] analyzed the course of the index and one subsequent pregnancy over a 4-year time horizon.

Study Population

All eight studies examined nonalloimmunized RhD− pregnancies indicated for antenatal prophylaxis with anti-D Rh immunoglobulin (i.e., RhIG). Several studies[56-58,60,61] further followed usual clinical care for a cohort of women who became alloimmunized after the index pregnancy.

Intervention: Fetal RhD Genotyping

In all studies, noninvasive fetal RhD blood group genotyping was identified as a separate intervention strategy, done between 11 and 14 weeks of gestation on average. In the one Canadian study,[61] this test was also examined as a part of a more complex, mixed intervention together with paternal RhD typing via serology (i.e., "mixed screening" where fetal genotyping was done if the father was RhD positive; Table 12).

Since all studies examined nonalloimmunized RhD− pregnancies, noninvasive fetal RhD genotyping was followed by targeted (selected) prophylaxis with RhIG for index pregnancies where the testing indicated either RhD incompatibility or inconclusive results.[43,57-60,62] RhIG was administered as it would be in usual care (e.g., at 28 weeks' gestation, and possibly postpartum if RhD incompatibility was confirmed via cord blood testing). Furthermore, the UK cost-effectiveness analysis[56] examined RhIG prophylaxis in five different postpartum strategies that followed the RhD genotyping. These strategies considered changes in the use of cord blood testing and/or fetomaternal hemorrhage testing postdelivery, and consequent changes in administration of RhIG postdelivery (for details, see notes to Table 12).

Although several studies[56-58,60,61] followed the clinical care associated with alloimmunized pregnancies, only one US cost-effectiveness analysis[57] clearly accounted for the use of

noninvasive fetal RhD genotyping in an alloimmunized pregnancy. This study modeled possible reductions in resources used in the complex management of alloimmunized pregnancies with confirmed high and clinically significant antibody titers (≥ 1:16). For the remaining long-term analyses, it is unclear whether repetitive genotyping was considered after the index pregnancy to determine the fetus's blood type in subsequent pregnancies; if this was modeled, it is uncertain how it affected the change in health care resource use in alloimmunized pregnancies.

Comparators: Usual Care With Universal Prophylaxis or No Prophylaxis

In all studies, usual care considered routine prophylaxis with RhIG in all nonalloimmunized RhD− pregnant women at 28 weeks' gestation. In a majority of the studies, administration of RhIG was also considered within 72 hours after delivery in all RhD− pregnancies or conditional on results of the cord blood test. Several studies also properly accounted for administration of RhIG in case of fetomaternal hemorrhage events.[43,56,57,62] Usual care of alloimmunized pregnancies was clearly described in two studies considering complex management of RhD− women at risk of having a baby with hemolytic disease of the fetus and newborn (HDFN).[57,61]

In addition to universal antenatal administration of RhIG, two studies[57,61] also modeled an alternative comparator that included antenatal RhIG conditional on results of paternal RhD serologic testing. Finally, two model-based cost-effectiveness studies[58,60] included a strategy of no screening and no RhIG prophylaxis for nonalloimmunized pregnancies and compared it with the current usual care. This alternative strategy may not be informative for assessment of the cost-effectiveness of noninvasive fetal RhD genotyping because it is an obsolete approach that has been replaced with universal RhIG prophylaxis in developed countries.[63]

Assessment of Health Outcomes

The majority of included economic evaluations examined short-term health outcomes relevant to the mother or the baby. Four of the eight included studies reported on the rate of alloimmunization in nonalloimmunized RhD− mothers.[57,58,60,62] In this population, one economic analysis[62] also evaluated the number of RhIG doses averted with genotyping. Given the safety of RhIG,[64] no model-based study accounted for a potential risk of infection related to blood products or transfusion. Three studies reported on neonatal health outcomes related to HDFN, as follows: the number of healthy babies born,[58,59,61] the number of newborns without HDFN,[61] and the number of newborns with serious morbidity or who died due to HDFN.[58]

The UK cost-effectiveness analysis[56] was the only study to estimate QALYs of the newborn associated with long-term consequences of HDFN. In this analysis, health state utilities were assumed from a prior NICE report on the cost-effectiveness of universal prophylaxis with RhIG; that report identified limited information from a few small studies of low-birth-weight premature babies.[37,65-68] The long-term model distinguished two health states related to HDFN: one associated with minor developmental problems in newborns (e.g., squinting, myopia, delay in language and fine motor skills) continuing for about 16 years before transitioning to a well health state, and another associated with major developmental problems (e.g., cerebral palsy, bilateral deafness, severe neurodevelopmental delay), continuing over the lifetime.

Lastly, the French trial-based economic evaluation[43] reported a health care system performance outcome associated with noninvasive fetal RhD genotyping. This performance measure was reported as the percentage of nonalloimmunized RhD− women receiving appropriate pregnancy management, defined as the sum of RhD− women who were at risk of alloimmunization and received RhIG and those who were not at risk and did not receive this prophylaxis.

Assessment of Costs

All studies adopted a health care sector or payer perspective, and they all appropriately measured direct medical costs associated with universal RhIG prophylaxis or targeted prophylaxis (i.e., selective used of RhIG guided by noninvasive fetal RhD genotyping). Several studies reported the costing categories in detail and also accounted for additional use of RhIG due to potential alloimmunization events during the index nonalloimmunized pregnancy.[43,56-58,61]

The clinical care of an alloimmunized pregnancy is complex. Noninvasive fetal RhD genotyping does not change the clinical care pathway of alloimmunized pregnancies, but test results can streamline the use of expensive health care resources, saving them for those with confirmed RhD incompatibility. Costing of health care resource use in this subpopulation was thoroughly conducted in three economic evaluations[56,57,61]:

- In the lifetime UK cost-effectiveness analysis, Saramago et al[56] assumed the same list of clinical procedures used in the previous NICE report,[68] basing the frequency of use on data from the British National Registry. They accounted for intensive monitoring, Doppler scanning, and intrauterine transfusions during an alloimmunized pregnancy; for the management of babies with HDFN, they considered the use of phototherapy, exchange transfusions with hospitalization in neonatal intensive care units (NICU), and follow-up care. Lastly, they accounted for long-term costs of minor or major developmental problems over a newborn's lifetime. However, based on the information reported in that study,[37,56] it is unclear whether and how much noninvasive fetal RhD genotyping decreased the expensive treatment of babies with HDFN
- In a Canadian cost-effectiveness analysis, Duplantie et al[61] followed Canadian guidelines to account for the main procedures used during pre- and postnatal care of the second (alloimmunized) pregnancy. They costed usual care with intensive monitoring, Doppler scanning, potential use of intrauterine transfusions in case of HDFN, and high-level costing of infants born with HDFN and requiring NICU care
- In contrast, Moise et al[57] provided the most thorough costing analysis of HDFN (antenatal and postnatal costs) per pregnancy, stratified by disease severity (i.e., $33,466 USD/mild case of HDFN, $66,517 USD/moderate case, and $147,274 USD/severe case)

The mean cost of noninvasive fetal RhD genotyping substantially varied between the analyses. The UK,[56] Swedish,[60] Australian,[59] and Canadian[62] economic analyses evaluated the use of RhD genotyping as a high-throughput screening intervention in a relatively large sample of nonalloimmunized pregnancies. In those circumstances, the test cost was in a low range, below $100 per person (i.e., £46 GBP,[56] €57,[60] $46 AUD,[59] and $34 CAD[62]). In three other studies, the cost of RhD genotyping was in a high range, up to $470 per person ($399 USD,[57] $450 USD,[58] and $471 CAD[61]).

None of the studies took a societal perspective; therefore, potentially large indirect costs related with productivity loss, especially for alloimmunized pregnancies, were not accounted for in the included economic analyses.

Study Findings

Results of the included economic analyses are inconsistent with respect to the cost-effectiveness of noninvasive fetal RhD blood group genotyping (Table 12):

- Only two of the eight studies[59,62] indicated clear cost savings with noninvasive fetal RhD genotyping (i.e., this intervention had incremental benefits and lower costs compared with usual care for the majority of the examined outcomes)
- Two other studies[43,56] showed a trade-off between noninvasive fetal RhD genotyping and comparative strategies, but while both reported an ICER, only one[56] estimated the ICER expressed as additional cost per QALY gained
- The remaining four studies[57,58,60,61] showed unfavourable cost-effectiveness for noninvasive fetal RhD genotyping for most of the examined outcomes, suggesting that genotyping was less effective and more costly than alternatives

The two studies that found cost savings with noninvasive fetal RhD genotyping in nonalloimmunized pregnancies were conducted in Canada[62] and Australia.[59] In these studies, targeted prophylaxis with RhIG after genotyping was associated with lower costs, reduction of RhIG doses,[62] an equal number of alloimmunizations,[62] and a greater number of healthy babies.[59] Per-person costs of the testing, in these analyses, was relatively low (i.e., $45.5 AUD [2016] and $33.7 CAD [2013]).

One of two economic analyses that estimated ICERs was a UK lifetime cost–utility analysis by Saramago et al,[56] which compared four fetal RhD genotyping strategies guiding postpartum testing versus usual care. Inconclusive or false-negative test results and cost of genotyping were the most influential drivers of cost-effectiveness. Only one genotyping strategy was associated with a favourable estimate of the ICER: this strategy (i.e., PP5, see Table 12) assumed that postpartum cord serology would be performed in the case of a test-negative status after genotyping—i.e., an inconclusive (potentially false-negative) or compatible (an RhD− fetus) genotyping result. Compared to universal prophylaxis, noninvasive fetal RhD genotyping was less costly but also less effective, with the ICER falling within acceptable limits as compared with a UK willingness-to-pay value of £20,000 per QALY gained.

The second study that provided an ICER was an individual-level cost-effectiveness analysis in 922 nonalloimmunized French women.[43] The authors produced an estimate of benefit (ICER: €578 per percentage gain in performance) using a system quality measure, with performance defined as the percentage of appropriately treated RhD− pregnancies. This ICER is less meaningful because it is unclear how much a decision-maker would be willing to pay to achieve a percentage gain in improvement with the intervention.

Lastly, four studies[57,58,60,61] including one from Canada[61] found that universal RhIG prophylaxis in nonalloimmunized pregnancies was associated with lower costs and greater or similar effects for the majority of examined health outcomes, compared with targeted RhIG prophylaxis guided by noninvasive fetal RhD genotyping (Table 12). In addition, two of these studies[57,61] compared the cost-effectiveness of several alternatives, including paternal RhD blood typing alone[61] or combined with universal prophylaxis[57] or combined with noninvasive fetal RhD genotyping (i.e., mixed screening)[61] for both nonalloimmunized and subsequent alloimmunized pregnancies. Duplantie et al[61] found that, in alloimmunized pregnancies, paternal screening alone was cost saving with respect to the number of surviving babies (larger number of surviving babies and less costly) compared with usual care; with respect to the number of babies with HDFN, this was a cost-minimization analysis as paternal screening was less costly but equally effective as usual care. In this analysis, compared with paternal screening alone, mixed screening (which included both paternal typing and noninvasive fetal RhD genotyping) was more costly and slightly more effective with respect to HDFN (estimated ICER of

$237,000/baby without HDFN); however, it was equally effective with respect to neonatal survival. Overall, Duplantie et al[61] suggested that paternal screening alone for alloimmunized pregnancies was the best economic option. In contrast, Moise et al,[57] who carefully costed all possible clinical pathways based on the severity of HDFN, found that usual care without paternal typing was cost saving compared with both strategies they examined: (1) universal prophylaxis with paternal testing and (2) targeted prophylaxis with noninvasive fetal RhD genotyping. Of note, the authors[57] did not distinguish health outcomes by type of pregnancy (index nonalloimmunized vs. alloimmunized pregnancy) but presented results cumulatively across all pregnancies; thus, it is difficult to understand the effect of noninvasive fetal RhD genotyping in alloimmunized pregnancies for which RhIG prophylaxis is not indicated.

Sensitivity Analysis

Several studies explored the influence of the cost of noninvasive fetal RhD genotyping on cost-effectiveness results.[56-58,60,61] Saramago et al[56] conducted a threshold analysis and found that all four testing strategies (which guided further postpartum care) would be cost-effective compared with universal prophylaxis if the test cost less than £26.6 (2015 GBP), a 57% reduction of the cost used in the reference case analysis. Moise et al[57] could not identify any point at which noninvasive fetal RhD genotyping would be cost neutral compared with universal prophylaxis. In other studies,[58,60,61] the cost of noninvasive fetal RhD genotyping would need to decrease by approximately three times for targeted RhIG prophylaxis in nonalloimmunized pregnancies to be cost neutral compared with universal prophylaxis. For example, in a Canadian study,[61] genotyping needed to be less than $140 per test to be cost saving; it cost $471 in the reference case analysis.

Table 12: Results of Economic Literature Review—Summary

Author, Year, Country of Publication	Study Design, Analytic Technique, Perspective, Discounting, Time Horizon	Population	Intervention(s) and Comparator(s)	Results		
				Health Outcomes	Costs	Cost-Effectiveness
Moise et al, 2019,[57] US	Study design: Model-based CEA Analytic technique: Decision tree (2 models) Perspective: Health care sector Discounting: NA Time horizon: Short-term, pregnancy until delivery (~38 wk)	Two RhD– populations: • Nonalloimmunized pregnancies • Alloimmunized pregnancies Mean age, y: NR Female, %: 100	*Intervention:* 1) Selective strategy: targeted RhIG prophylaxis guided with noninvasive fetal RhD genotyping *Comparators:* 2) Universal with paternal strategy: universal antenatal RhIG prophylaxis based on serologic testing of father's status 3) Universal without paternal strategy: universal RhIG prophylaxis without paternal RhD testing	Number of alloimmunization cases in the total population (mean, in 3,945 878 live births in 2016): 1) Selective: 810 2) Universal with paternal: 1,030 3) Universal without paternal: 765 Mean difference, number of alloimmunization cases: • Universal without paternal vs. universal with paternal: −265 • Universal without paternal vs. selective: 45 • Universal with paternal vs. selective: 220	Currency, year: USD, NR (2016?) Total mean costs *per pregnancy* (computed separately for each population), by strategy: 1) Universal with paternal 2) Universal without paternal 3) Selective *Nonalloimmunized:* 1) $663.80; 2) $722.30; 3) $869.30 *Alloimmunized:* 1) $4.78; 2) $3.55; 3) $3.76 Mean cost difference per pregnancy: *Nonalloimmunized:* • Universal without paternal vs. universal with paternal: $58.50 • Selective vs. universal without paternal: $147 *Alloimmunized:* • Universal without paternal vs, selective: $0.21 • Universal without paternal vs. universal with paternal: $1.02 Microcosting of health care resource use for both nonalloimmunized and alloimmunized pregnancies (3 levels of severity of HDFN) Cost of genotyping, reference case: $399 per person	*Reference case:* For the total population, universal prophylaxis with RhIG without paternal testing was associated with the greatest benefits (i.e., the smallest number of alloimmunized pregnancies) and with cost savings (compared to noninvasive fetal RhD genotyping); ICER not reported *Sensitivity analyses:* PSA: not done Threshold analysis, cost of noninvasive fetal RhD genotyping (reference case = $399): Targeted screening using noninvasive fetal RhD genotyping not cost neutral (compared to universal) no matter how low the cost of the genotyping test was (test cost ranged $24–$172)

Author, Year, Country of Publication	Study Design, Analytic Technique, Perspective, Discounting, Time Horizon	Population	Intervention(s) and Comparator(s)	Results		
				Health Outcomes	Costs	Cost-Effectiveness
Darlington et al, 2018,[43] France	Study design: CEA alongside a nonrandomized open-label 2-arm multicentre clinical trial (11 clinics) Analytic technique: Individual-level CEA Perspective: Health care sector Discounting: NA Time horizon: Short-term, duration of pregnancy until delivery (~38 wk)	Nonalloimmunized RhD− women, ≥ 18 y (N = 922) Mean age, y: Intervention vs. control: 30 (SD 5) vs. 31 (SD 5) Female, %: 100 Singleton pregnancies: 97%	*Intervention:* Noninvasive fetal RhD genotyping guiding selective antenatal RhIG prophylaxis (6 hospitals, n = 586) *Comparators:* Usual care, universal prophylaxis with RhIG (Rhophylac, 300 mcg) at 28 wk gestation, within 72 h post-delivery, and as needed in case of FMH event (5 hospitals, n = 346)	Performance[a] (% of RhD− women receiving appropriate management), intervention and usual care: 88% and 64% Mean difference: 24%	Currency, year: €, 2014 (1€ = 1.2 USD) Total mean costs (related to RhD status), intervention and usual care: €591 and €542 Mean difference: €139 Individual-level microcosting of health care resource use for nonalloimmunized pregnancies Cost of genotyping, reference case: €140 per person (includes the cost of a commercial kit, materials, and labour)	*Reference case:* ICER (incremental cost per additional women appropriately treated): €578/% gain in performance *Sensitivity analyses:* PSA:60% probability of fetal genotyping being cost-effective at a theoretical WTP of €585/% gain in performance Scenario analysis (including all costs and hospital stay), ICER: €1,059/additional appropriately treated woman
Saramago et al, 2018,[56] UK (update of NICE health technology assessment[37])	Study design: Model-based CEA Analytic technique: Decision tree Perspective: Health care sector, NHS-PPS Discounting: 3.5% Time horizon: Lifetime	Nonalloimmunized RhD− women in their 1st pregnancy Mean age, y: NR Female, %: 100	*Interventions:* HT-NIPT for targeted prophylaxis guided by noninvasive fetal RhD genotyping (at 16-wk visit): 5 strategies considered impact of HT-NIPT on postpartum care[b] *Comparators:* Usual care: routine antenatal anti-D prophylaxis with either postpartum cord serology (CS) or FMH test (if CS+); postpartum RhIG guided by CS and FMH tests	Total mean QALYs, dominant intervention (HT-NIPT PP5) and usual care, per 100,000 women: 2,433,756.3 and 2,433,755.8 Mean difference: −0.05 QALY HT-NIPT postpartum strategy 5 (PP5): inconclusive results separated from test+ and test− and treated as test− in postpartum care pathway (CS, FMH, and administration of RhIG guided by these tests), assumed no adverse effects of RhIG	Currency, year: £, 2015 Total mean costs, dominant intervention (HT-NIPT PP5) and usual care, per 100,000 women: £15,221,338 and £15,983,725 Mean difference, intervention vs. usual care: −£762,387 Microcosting of health care resource use for both nonalloimmunized and alloimmunized pregnancies Cost of genotyping, reference case: £45.48 per person (for a sample of 46,000 women)	*Reference case*: All HT-NIPT strategies associated with cost savings but less effective then usual care; HT-NIPT PP5 most cost-effective and with the highest net health benefit (ICER ~£1,660,000/QALY, and above the WTP of £20,000/QALY) *Sensitivity analyses*: PSA: 96% probability of HT-NIPT PP5 strategy being cost-effective at WTP of £20,000/QALY Threshold analysis: HT-NIPT in general cost-effective if the overall genotyping test cost is £26.60 or less

Author, Year, Country of Publication	Study Design, Analytic Technique, Perspective, Discounting, Time Horizon	Population	Intervention(s) and Comparator(s)	Results		
				Health Outcomes	Costs	Cost-Effectiveness
Gordon et al, 2017,[59] Australia	Study design: Model-based CEA Analytic technique: Decision tree Perspective: Health care sector Discounting: NA Time horizon: Short-term, duration of pregnancy and birth	Nonalloimmunized RhD− women indicated for antenatal anti-D prophylaxis Mean age, y: NR Female, %: 100	*Intervention:* Noninvasive fetal RhD genotyping with targeted RhIG prophylaxis *Comparator:* Usual care, universal anti-D prophylaxis	Number of healthy babies (mean), intervention and usual care: NR Mean difference, intervention vs. usual care: NR, qualitative statement indicating a slightly higher number of healthy babies with noninvasive fetal RhD genotyping compared with usual care	Currency, year: AUD ($), NR (2016?) Total costs per healthy baby (mean), intervention and usual care: NR and $7,489 Mean difference: Unknown Mean difference, per pregnancy, intervention vs. usual care: −$24 ($7,471 vs. $7,495) Costing: major cost items for nonalloimmunized pregnancies Cost of genotyping: $45.48 per person (sample of 46,000 women)	*Reference case*: Intervention with targeted prophylaxis dominant over usual care: associated with a slightly higher number of healthy babies and slightly lower costs *Sensitivity analyses*: PSA: 97% probability of fetal genotype testing being cost-effective over usual care at a WTP of $50,000/healthy baby In 1-way deterministic analyses, key drivers of cost-effectiveness of RhD fetal genotyping were cost of anti-D prophylaxis; probability of alloimmunization in usual care; transportation cost per blood sample; and test cost
Neovius et al, 2015,[60] Sweden	Study design: Model-based CEA Analytic technique: Markov cohort model Perspective: Health care sector Discounting: 3% (costs only) Time horizon: Unclear, long-term, 10–12 y (the duration of the 1st and future pregnancy)	Nonalloimmunized RhD− women (1st pregnancy) Mean age, y: NR Female, %: 100	*Intervention:* Noninvasive fetal RhD genotyping with targeted RhIG prophylaxis *Comparators:* • Usual care: routine antenatal anti-D immunoglobulin prophylaxis (RAADP) • No RAADP (historical control) Markov 3-state model: "Not immunized", "Immunized during pregnancy", or 'Immunized from start of pregnancy"; cycle length, 40 wk;	Total number of alloimmunizations (%), intervention, usual care (RAADP) and no RAADP: 0.42%, 0.36% and 1.02% Mean difference, intervention vs. RAADP and intervention vs. no RAADP: 0.06% and −0.59%	Currency, year: €, 2014 Total costs (mean per pregnancy), intervention, usual care (RAADP), and no RAADP: €1,174.41, €1,157.96, and €1,206.61 Mean difference, intervention vs. RAADP and intervention vs. no RAADP: €16.45 and −€32.21 Costing of health care resource use for both nonalloimmunized and alloimmunized pregnancies, based on health administrative (registry) data Cost of RhD genotyping: €57.37 per person	*Reference case:* • ICER, intervention vs. RAADP: dominated by RAADP (more alloimmunizations, higher costs) • ICER, intervention vs. no RAADP: cost saving (fewer alloimmunizations, lower costs) *Sensitivity analyses:* PSA: 97% probability of intervention being cost-effective vs. no RAADP at a WTP of €10,000/averted alloimmunization Drivers of cost-effectiveness were the mean number of pregnancies per mother; screening test cost; and proportion alloimmunized

Author, Year, Country of Publication	Study Design, Analytic Technique, Perspective, Discounting, Time Horizon	Population	Intervention(s) and Comparator(s)	Results		
				Health Outcomes	Costs	Cost-Effectiveness
			time horizon accounts for 2.2 pregnancies per mother on average			2-way analysis: targeted RAADP would be cost-effective vs. universal RAADP (current usual care) if genotyping test cost €47 (16% lower than cost used in the reference case)
Teitelbaum et al, 2015,[62] Canada	Study design: Model-based CEA Analytic technique: Decision tree Perspective: Health care sector/payer (Alberta) Discounting: NA Time horizon: Short-term (duration of pregnancy and up to 1 y)	Nonalloimmunized RhD− pregnancies (N = 69,286) Mean age, y: NR Female, %: 100	*Intervention:* Noninvasive fetal RhD genotyping with targeted RhIG prophylaxis *Comparator:* Usual care: RAADP program at 28 wk gestation and any sensitizing event during the pregnancy	Total number of: • Alloimmunizations per pregnancy: 0.00018 (equal for intervention and usual care) • Alloimmunizations per RhD− pregnancy: 0.0012 (equal for both strategies) • RhIG doses per RhD− pregnancy: 1.50 (intervention) and 1.95 (usual care) Mean difference, RhIG doses per RhD− pregnancy, intervention vs. usual care: −0.45	Currency, year: CAD ($), 2013 Total mean costs per pregnancy, intervention and usual care: $67.20 and $71.43 Mean difference (per pregnancy), intervention vs. usual care: −$4.23 Costing: major cost items for nonalloimmunized pregnancies Cost of RhD genotyping: $33.68 per person	*Reference case:* ICER, intervention vs. usual care: cost saving *Sensitivity analyses:* PSA: not done 1-way deterministic analyses related to handling inconclusive results as true positive with RhIG prophylaxis (as in usual care) or exclusion of cord blood typing showed similar findings (cost saving)
Hawk et al, 2013,[58] US	Study design: Model-based CEA Analytic technique: Decision tree Perspective: Health care sector Discounting: NA Time horizon: Short-term, duration of pregnancy	Nonalloimmunized RhD− pregnancies Mean age, y: NR Female, %: 100	*Intervention:* 1) Noninvasive fetal RhD genotyping with targeted RhIG prophylaxis *Comparators:* 2) Usual care: routine antenatal anti-D prophylaxis program 3) No screening or prophylaxis	Number of new RhD alloimmunization cases per 1 million pregnancies: 1) Targeted prophylaxis: < 1 2) Usual care: 0 3) No screening/prophylaxis: 45,360 Number of deaths or serious morbidity due to HDFN per 1 million pregnancies: 1) Targeted prophylaxis: < 1 2) Usual care: 0 3) No screening/prophylaxis: 1,134 Mean difference in HDFN or alloimmunized pregnancies, targeted prophylaxis vs. usual care: dominated (usual care more beneficial)	Currency, year: USD ($), 2012 Total mean costs per pregnancy: 1) Targeted prophylaxis: $682 2) Usual care: $351 3) No screening/ prophylaxis: $5,670 Mean cost difference per pregnancy, intervention vs. usual care: $331 Costing: major cost items for nonalloimmunized pregnancies Cost of RhD genotyping, reference case: $450 per person	*Reference case:* Targeted prophylaxis vs. usual care: more costly (dominated) *Sensitivity analyses:* PSA: not done Threshold analysis indicated that targeted prophylaxis was cost neutral (compared to usual care) if genotype testing was costed at $119 Main driver of CEA results in 1-way deterministic analysis was false-negative rate associated with genotype testing (test accuracy was based on studies mainly in Caucasian populations)

Author, Year, Country of Publication	Study Design, Analytic Technique, Perspective, Discounting, Time Horizon	Population	Intervention(s) and Comparator(s)	Results		
				Health Outcomes	Costs	Cost-Effectiveness
Duplantie et al, 2013,[61] Canada	Model-based CEA Analytic technique: Agent-based state-transition discrete event analysis Perspective: Health care sector/payer (Quebec) Discounting: None Time horizon: Unclear, about 4 y, accounts for a 1st and 2nd pregnancy (about 3 y between 2 pregnancies)	Nonalloimmunized RhD− women indicated for antenatal anti-D prophylaxis Mean age, y: NR Female, %: 100	*Interventions:* • Fetal genotyping (noninvasive fetal RhD genotyping with targeted RhIG prophylaxis) • Mixed screening (determination of father's Rh type; if positive, followed by noninvasive fetal RhD genotyping with targeted RhIG prophylaxis) *Comparators:* • Rh typing of father (immunological test) followed by usual care in eligible pregnancies • Usual care, RAADP	Total number of babies without HDFN (outcome 1) and surviving infants (outcome 2) per 10,000 pregnancies across the 4 strategies, 1st pregnancy: usual care: 9,974.75 (outcome 1) and 9,810.91 (outcome 2); fetal genotyping: 9,974.86 and 9,811.18; Rh typing of father: 9,974.79 and 9,811.77; mixed screening: 9,974.63 and 9,810.81 Mean difference, 1st pregnancy, interventions vs. RAADP: fetal genotyping: 0.07 fewer babies without HDFN, additional 0.27 alive; Rh typing of father: additional 0.04 babies without HDFN, additional 0.86 alive; mixed screening: 0.04 fewer babies without HDFN, 0.10 fewer alive Outcomes 1 and 2 per 10,000 pregnancies, 2nd pregnancy: usual care: 9,912 and 9,807; fetal genotyping: 9,914 and 9,808; Rh typing of father: 9,912 and 9,808; mixed screening: 9,914 and 9,808 Mean difference, 2nd pregnancy, interventions vs. usual care: fetal genotyping: 2 more babies without HDFN, 1 more baby alive; Rh typing of father: 0 babies without HDFN, 1 more alive; mixed screening: 2 more babies without HDFN, 1 more alive	Currency, year: CAD ($), 2011 Total mean costs per 10,000 pregnancies, 4 strategies, 1st and 2nd pregnancy: usual care: $101,848,991 and $106,687,882; fetal genotyping: $103,310,771 and $107,193,950; Rh typing of the father: $101,911,011 and $106,362,892; mixed screening: $102,864,181 and $106,837,257 Mean difference per 10,000 pregnancies, interventions vs. usual care, 1st and 2nd pregnancy: fetal genotyping: $1,461,780 and $506,068; Rh typing of father: $62,020 and −$324,990; mixed screening: $1,015,190 and $149,375 Microcosting of health care resource use for both nonalloimmunized and alloimmunized pregnancies; costing does not account fully for medical costs associated with severe HDFN Cost for RhD genotyping, reference case: $471 per person	*Reference case:* ICER (recalculated), intervention vs. usual care, 1st pregnancy: usual care least expensive but less effective than paternal Rh typing (the most effective strategy of all); ICER, paternal Rh typing vs. usual care: > $1.5 million per baby without HDFN or per baby alive 2nd pregnancy: paternal Rh typing vs. usual care, less costly and more effective with respect to number of surviving babies, less costly and equally effective with respect to number of babies without HDFN; mixed screening vs. fetal genotyping, less costly and equally effective with respect to both health outcomes (cost minimization analysis); ICER (recalculated), mixed screening vs. father's Rh typing: $237,182 per baby without HDFN; equally effective and more costly with respect to the number of surviving babies *Sensitivity analyses:* PSA: not done Threshold analysis: RhD genotyping becomes cost-effective in 1st and 2nd pregnancy if test cost is < $140 (ICER not reported)

See notes next page.

Notes for Table 12:

Abbreviations: AUD, Australian dollars; CAD, Canadian dollars; CEA, cost-effectiveness analysis; cffDNA test, cell-free fetal DNA test; CUA, cost–utility analysis; FMH, fetomaternal hemorrhage; h, hour; HDFN, hemolytic disease of the fetus and newborn; HT-NIPT, high-throughput noninvasive prenatal testing; ICER, incremental cost-effectiveness ratio; NA, not applicable; NHS, National Health Service; NICE, National Institute for Health and Care Excellence; NR, not reported; PSA, probabilistic sensitivity analyses; PSS, Personal Social Service; QALY, quality-adjusted life-year; RAADP, routine antenatal anti-D prophylaxis program; RhD− , rhesus blood group D negative; RhIG, Rh immunoglobulin; SD, standard deviation; USD, United States dollars; wk, week; WTP, willingness to pay; y, year.

[a]Performance was defined as the percentage of RhD− women receiving appropriate management. Appropriate treatment was defined as the sum of RhD− women who were at risk of alloimmunization and received prophylaxis and those who were not at risk and did not receive prophylaxis.

[b]Postpartum HT-NIPT strategies were identified as PP1 to PP5.[56] PP1: postpartum cord serology and FMH testing would continue to be performed, as per current guidelines, in all women regardless of fetal RhD status identified through HT-NIPT; PP2: postpartum cord serology and FMH testing (and by implication, anti-D immunoglobulin) would be withheld if HT-NIPT identifies an RhD− fetus, but would continue to be performed if HT-NIPT was inconclusive or had identified an RhD+ fetus; PP3: postpartum cord serology would be performed if HT-NIPT of fetal RhD status identifies an RhD− fetus. FMH testing and postdelivery anti-D immunoglobulin would be administered if HT-NIPT is inconclusive or identified an RhD+ fetus; PP4: postpartum cord serology not performed in any women. FMH testing and postdelivery anti-D immunoglobulin administered if HT-NIPT is inconclusive or has identified an RhD+ fetus; PP5: postpartum cord serology would be performed if HT-NIPT identifies an RhD− fetus or if test result is inconclusive. FMH testing and postpartum anti-D immunoglobulin administered irrespective of the result of HT-NIPT and guided by either FMH or FMH and cord serology.

Applicability and Limitations of the Included Studies

Appendix 5 presents the results of the methodology checklist for economic evaluations applied to the included articles. Two of the included studies were conducted in Canada,[61,62] but owing to limitations in these studies, we considered none of the eight included studies directly applicable to our research questions and the Ontario setting (Appendix 5, Table A5).

We assessed the methodological quality of the included studies and found that five studies had potentially serious limitations,[43,57,58,60,61] two had very serious limitations,[59,62] and only one study had minor limitations[56] (Appendix 5, Table A6).

None of the included studies separated nonalloimmunized and alloimmunized pregnancies as they evaluated the cost-effectiveness of noninvasive fetal RhD genotyping over usual care, and it remains unclear if any study included the possibility of repeating the genotyping test over multiple pregnancies.

Most studies focused on nonalloimmunized pregnancies and the clinical pathway until a delivery (i.e., a short-term time horizon over approximately 38 weeks). However, many did not consider the costs of potential sensitizing events, which could be associated with a longer hospital stay or increased use of RhIG. In the majority of long-term studies, costing approaches partially captured the complex management of HDFN. Only one study used a comprehensive costing approach that considered all relevant costs associated with the clinical management and consequences of RhD alloimmunization in the United States, but it presented the results cumulatively for both nonalloimmunized and alloimmunized pregnancies.[57]

Neither of the two Canadian studies[61,62] had model structures that fully reflect clinical care pathways relevant to the Ontario setting (e.g., they did not consider the more severe forms of HDFN and the potentially large resource use associated with that care).

We also found limitations in the way most of the included cost-effectiveness analyses reported their findings. Only one lifetime model-based economic evaluation[56] assessed changes in QALYs and reported the corresponding ICER as cost per QALY gained.

Lastly, most of the studies did not address decision-making and parameter uncertainty, but conducted a deterministic (threshold) analysis to determine how much the cost of genotyping would need to decrease so that targeted prophylaxis with RhIG, guided by the fetal RhD genotyping results, could be considered cost neutral or cost saving compared with universal prophylaxis.

Discussion

The economic literature in our review did not clearly establish the cost-effectiveness of noninvasive fetal RhD blood group genotyping to guide pregnancy management for nonalloimmunized or alloimmunized RhD− pregnancies, versus usual care (universal RhIG prophylaxis or universal intensive monitoring, respectively). Two economic evaluations[59,62] in nonalloimmunized pregnancies found noninvasive fetal RhD genotyping to be cost saving (i.e., higher benefits and lower costs) versus universal RhIG prophylaxis. Despite important differences in care for nonalloimmunized and alloimmunized pregnancies, none of the included model-based studies that evaluated cost-effectiveness of this intervention in both populations reported additional benefits for alloimmunized pregnancies. This could be partially explained by

differences in modeling of fetal RhD genotyping in nonalloimmunized or alloimmunized pregnancies, over longer time horizons.

As noted, neither of the two model-based Canadian studies[61,62] fully reflected clinical care pathways relevant to the Ontario setting, particularly the intensive management of alloimmunized pregnancies.

Targeted prophylaxis with RhIG guided by results of noninvasive fetal RhD genotyping has been endorsed by NICE in the United Kingdom since 2009.[37,52,69] In their examination of the long-term cost-effectiveness of this intervention, Saramago et al[56] aimed to determine if noninvasive fetal RhD genotyping had additional benefits by looking at changes in the frequency of postpartum testing (i.e., cord blood serology and testing for fetomaternal hemorrhage). They showed that genotype testing that guided postpartum screening was cost-effective for one of five compared strategies, but also that the cost-effectiveness depended on several factors: the number and types of tests that were offset in postnatal care, the cost of the genotyping test, and the rate of false-negative or inconclusive results. Given all this, the long-term cost-effectiveness analysis by Saramago et al[56] is not directly applicable to our research questions nor to the Ontario setting.

The cost of noninvasive fetal RhD genotyping differed substantially among the analyses (e.g., per-test costs ranging from $34 CAD [2013] to $470 CAD [2011]). When the test was considered as a high-throughput intervention,[56,59,60,62] the per-test cost in the reference case analysis was relatively low (< $100 CAD). This reduction in the test cost could be explained by the relatively large number of samples required for population-based screening of all RhD− nonalloimmunized pregnancies.

In summary, the cost-effectiveness of noninvasive fetal RhD genotyping for both nonalloimmunized and alloimmunized pregnancies remains unclear for Ontario. We may expect a decrease in the use of RhIG in nonalloimmunized pregnancies. In this case, targeted prophylaxis with RhIG would need to be counterbalanced by a low cost of the genotyping test. The cost of the test could be leveraged through the existing laboratory infrastructure with automated platforms that could handle high-throughput screening across Canada (as is done in the United Kingdom). Thus far, Ontario has been outsourcing requests for noninvasive fetal RhD genotyping in alloimmunized pregnancies to the United Kingdom at a cost of around or above $600 per test (expert consultation, oral and email communications, B. de Vrijer, MD, N. Shehata, MD, Y. Lin, MD, N. Okun, MD, January 2019). As mentioned, unlike Ontario, the United Kingdom has a national reference laboratory in Bristol and sustainable funding for its infrastructure that enables implementation of the NICE recommendation for targeted screening with noninvasive fetal RhD genotyping.[37,52,69]

Finally, given that some RhD− pregnant people may live in remote or rural areas of Ontario, it would be important to understand the influence of indirect costs on the cost-effectiveness of noninvasive fetal RhD genotyping, particularly in alloimmunized pregnancies. Thus far, no economic study has examined the cost-effectiveness of this intervention from a societal perspective.

Conclusions

Our economic evidence review found inconsistent results on the cost-effectiveness of noninvasive fetal RhD blood group genotyping for nonalloimmunized or alloimmunized RhD negative pregnancies. Some studies indicated that, in nonalloimmunized pregnancies, universal RhIG prophylaxis with or without paternal RhD screening may be less costly and as effective as

targeted prophylaxis guided by the results of noninvasive fetal RhD genotyping. Few other studies suggested the contrary. No study examined the cost-effectiveness of the targeted use of this intervention for alloimmunized pregnancies. Thus, it remains unknown whether the savings from targeted management of alloimmunized pregnancies after noninvasive fetal RhD genotyping offsets the potentially dire consequences of HDFN.

Consequently, we undertook a full economic evaluation to address a policy question: whether public funding could be recommended for noninvasive fetal RhD genotyping in Ontario for the management of nonalloimmunized and alloimmunized RhD− pregnancies.

PRIMARY ECONOMIC EVALUATION

Noninvasive fetal RhD blood group genotyping has both benefits and risks. On one hand, fetal RhD genotyping can help prevent unnecessary treatment and intensive pregnancy monitoring in nonalloimmunized and alloimmunized pregnancies. Studies suggest that approximately 40% of RhD negative (RhD−) pregnant people carry RhD− fetuses.[6] In these nonalloimmunized pregnancies, the administration of Rh immunoglobulin (RhIG) prophylaxis would be unnecessary.[6] Reducing unnecessary use of RhIG may be desirable because, as a blood product, RhIG may be associated with a risk of infection, although this is extremely rare in Canada.[64,70-73] In addition, some studies suggest a potential future shortage of this blood product.[6] In alloimmunized pregnancies, usual care includes intensive clinical monitoring and management (e.g., frequent physician visits with more frequent fetal middle cerebral artery (MCA) Doppler ultrasound scans, potential cordocentesis, and potential hospitalizations for aggressive treatment and intrauterine transfusions), along with postpartum follow-up care. Noninvasive fetal RhD genotyping could direct scarce health care resources to only those with RhD incompatibility. Lastly, by reducing unnecessary testing and hospital visits, noninvasive fetal RhD genotyping may lead to less anxiety and better quality of life in pregnant people with no RhD incompatibility (email and oral communications, B. de Vrijer, MD, N. Shehata, MD, Y. Lin, MD, N. Okun, MD, G. Clarke, MD, June 26–July 9, 2019).

On the other hand, a potential risk of noninvasive fetal RhD genotyping is false-negative results, although the rate is low (see Clinical Evidence). In nonalloimmunized pregnancies, those with false-negative results could miss RhIG prophylaxis, possibly resulting in alloimmunization. In alloimmunized pregnancies, if intensive monitoring and care are not undertaken because of a false-negative genotyping test, babies may develop hemolytic disease of the fetus and newborn (HDFN) with potentially serious but rare long-term consequences.

Our economic evidence review found inconsistent results regarding the cost-effectiveness of noninvasive fetal RhD blood group genotyping for RhD− pregnancies and the study findings were not applicable to the Ontario setting. Therefore, we conducted a primary economic evaluation.

Research Questions

1. What is the cost-effectiveness of noninvasive fetal RhD blood group genotyping compared with usual care for the management of nonalloimmunized RhD− pregnancies from the perspective of the Ontario Ministry of Health?
2. What is the cost-effectiveness of noninvasive fetal RhD blood group genotyping compared with usual care for the management of alloimmunized RhD− pregnancies from the perspective of the Ontario Ministry of Health?

Methods

The information presented in this report follows the reporting standards set out by the Consolidated Health Economic Evaluation Reporting Standards Statement (CHEERS).[74] The methodological approaches follow the recent recommendations set out by the of the Canadian Agency for Drugs and Technologies in Health (CADTH) Guidelines for the Economic Evaluation of Health Technologies, fourth edition,[75] and align with our organization's Health Technology Assessments Methods and Process Guide.[76]

Type of Analysis

We performed both cost-effectiveness and cost–utility analyses to estimate the costs and outcomes of noninvasive fetal RhD blood group genotyping and usual care for nonalloimmunized or alloimmunized RhD− pregnancies.

Outcomes of Interest

For the cost-effectiveness analysis, we estimated health outcomes using the following natural units:

- Health outcomes in nonalloimmunized pregnancies
    - Probability of maternal alloimmunization
    - Number of RhIG injections
    - Probability of having a live baby with no developmental problems
- Health outcomes in alloimmunized pregnancies
    - Probability of hospitalization with intrauterine transfusions (IUTs)
    - Probability of neonatal intensive care hospitalizations (including complex care and exchange transfusions)
    - Probability of having a baby with HDFN
    - Probability of having a live baby

For both populations, we also estimated short-term direct medical costs (i.e., costs associated with the pregnancy) and long-term direct medical costs (i.e., costs associated with health care utilization of a baby born, over the model's time horizon).

For the cost-effectiveness analysis, the incremental cost-effectiveness ratio (ICER) is expressed as an additional cost ($) per additional change in the health outcome: for instance, additional cost per RhIG injection avoided or additional cost per hospitalization avoided.

For the cost–utility analysis, we estimated quality-adjusted life-years (QALYs) for a baby born, over the model's time horizon. Thus, this ICER is expressed as an additional cost per one newborn's QALY gained. This outcome may be more appropriate for decision-making related to allocation of resources for various technologies across different conditions and is suggested by the Canadian guidelines for economic evaluation,[75] among others.

Target Population

The study population was pregnant people with serologically confirmed nonalloimmunized (research question 1) or alloimmunized (research question 2) RhD− pregnancies.

Perspective

We conducted the reference case analysis from the perspective of the Ontario Ministry of Health. We also conducted a scenario analysis from the societal perspective and considered the cost of lost productivity (i.e., value of time that a pregnant person spent seeking or receiving care).

Interventions and Comparators

Table 13 summarizes the interventions and comparators evaluated in the economic analysis.

Table 13: Interventions and Comparators Evaluated in the Primary Economic Model

Intervention	Comparators	Patient Population
Noninvasive fetal RhD blood group genotyping in: • Nonalloimmunized pregnancies: genotyping followed by targeted RhIG prophylaxis in those with identified RhD incompatibility[a] • Alloimmunized pregnancies: genotyping followed by targeted intensive monitoring and possible treatment of those with identified RhD incompatibility[a]	Usual care: • Nonalloimmunized pregnancies: universal prophylaxis with RhIG • Alloimmunized pregnancies: universal intensive monitoring ± treatment	Nonalloimmunized and alloimmunized RhD− pregnancies

Abbreviations: RhD, rhesus D blood group; RhIG, Rh immunoglobulin;
[a]Maternal RhD status negative and fetal RhD status positive.

As shown in Table 13, the intervention strategy and usual care depend on the population. We describe them in detail below.

Intervention: Noninvasive Fetal RhD Blood Group Genotyping

- **Nonalloimmunized RhD− pregnancies—**All pregnant people are screened for RhD status as part of routine bloodwork during pregnancy (e.g., at gestational week 12 and thereafter; email and oral communications, B. de Vrijer, MD, N. Shehata, MD, N. Okun, MD, G. Clarke, MD, July 2019). If the fetal RhD status is positive (i.e., potential RhD incompatibility between mother and fetus), the pregnant person receives RhIG prophylaxis (1500 IU or 300 mcg) in gestational weeks 28 to 34 and up to 72 hours after delivery, following confirmation that the newborn is RhD+.[11] RhIG is also administered in case of any fetomaternal hemorrhage (FMH) event that could cause maternal alloimmunization (possible FMH events are described below, under usual care). [6,11,77,78]
- **Alloimmunized RhD− pregnancies—**All pregnant people are screened for anti-D antibodies as part of routine bloodwork during pregnancy. If maternal RhD status is confirmed negative and fetal RhD status is positive (i.e., potential RhD incompatibility after fetal RhD genotyping), frequent monitoring and possible complex care and management of complications associated with HDFN is provided according to current Canadian and North American guidelines[6,79] and as described under Usual Care, below.

Usual Care

- **Nonalloimmunized RhD− pregnancies—**Universal prophylaxis with RhIG of 300 mcg is given to all RhD− pregnant people between gestational weeks 28 and 34 (first injection) and within 72 hours after delivery (second injection) following a positive newborn test result (via cord blood sampling for RhD status). RhIG is also administered in case of FMH events. If FMH events occur before 12 weeks' gestation, a smaller dose of 120 mcg is given. At later gestational ages, a dose of 300 mcg is recommended after any of the following events[11,78]:
    - Miscarriage or abortion
    - Ectopic pregnancy
    - Vaginal bleeding at any time during the pregnancy
    - Fetal death
    - Potential bleeding due to invasive procedures (e.g., cordocentesis)
    - An external cephalic version of a breech fetus
    - Blunt trauma to the abdomen

- **Alloimmunized RhD− pregnancies—**All receive intensive monitoring and management:[11,80-82]
    - Serial maternal anti-D antibody titer and clinic visits start after confirmation of first alloimmunized pregnancy
    - If the titer in the first alloimmunized pregnancy is below a critical level (< 1:16), it is measured monthly in the first and second trimesters and biweekly in the third trimester. In this case, serial fetal MCA Doppler ultrasound monitoring is done monthly to measure peak systolic velocity
    - An anti-D antibody titer over 1:16 is considered critical and requires maternal-fetal medicine specialist care. In this case (i.e., first alloimmunized pregnancy), or if a prior pregnancy is associated with HDFN, prenatal and neonatal care could include the following procedures:
        - Serial fetal MCA Doppler ultrasound monitoring is done biweekly to measure peak systolic velocity;[83] the procedure is repeated more frequently (e.g., twice weekly) when there are signs of fetal anemia. If elevated MCA Dopplers are persistent, referral is initiated for cordocentesis (sampling of fetal blood from the umbilical cord). As cordocentesis is an invasive procedure associated with risk of fetal loss, this procedure is usually performed in a setting that allows immediate blood transfusion if fetal anemia is confirmed by fetal blood testing (email communication, B de Vrijer, MD, July and October 2019)
        - If fetal anemia (i.e., HDFN) is confirmed via Doppler monitoring, short-term hospital admission for fetal IUT is required, and in most cases, more than one transfusion would be required during the pregnancy (email communication, B de Vrijer, MD, July and October 2019)
        - Labour (or Caesarean delivery) most often occurs at 38 weeks' gestation or earlier, depending on signs of fetal anemia (because monitoring the signs of fetal anemia becomes less accurate after 38 weeks)
        - The newborn undergoes various laboratory procedures and treatments, depending on the severity of their HDFN; if HDFN is very severe and the

neonate needs top-up or exchange transfusions, they will require admission to a neonatal intensive care unit (NICU) for complex care

Discounting and Time Horizon

We used a 10-year time horizon in our reference case analysis to account for long-term costs and outcomes. In a scenario analysis, we used a lifetime horizon. We applied an annual discount rate of 1.5% to both costs and effects (including QALYs).[75] All costs were expressed in 2019 Canadian dollars.

Model Structure: Reference Case Analysis

We developed probabilistic, Markov microsimulation models to evaluate the cost-effectiveness of noninvasive fetal RhD blood group genotyping compared with usual care. We modelled this separately for nonalloimmunized and alloimmunized RhD− pregnancies.

Our intervention strategy was a diagnostic test: noninvasive fetal RhD blood group genotyping. In our models, people who receive this test could have positive, negative, or inconclusive results. We treated inconclusive results the same as positive results. Positive results could be true positive or false positive. Similarly, negative results could be true negative or false negative. These diagnostic outcomes and the consequent clinical pathways were embedded in the intervention strategy of the Markov models:

- In a nonalloimmunized RhD− pregnancy, only a positive or inconclusive test result would lead to the pregnant person receiving antenatal RhIG prophylaxis (targeted antenatal RhIG prophylaxis)
- In an alloimmunized RhD− pregnancy, only a positive or inconclusive test result would lead to antenatal intensive monitoring and complex clinical care

As illustrated in Figures 4 and 5, we created different Markov health states to represent RhD− pregnant people and their babies after delivery.

For nonalloimmunized RhD− pregnancies, our model included three health states (Figure 4):

- **Nonalloimmunized pregnancy**—All RhD− pregnant people in this health state would receive standard clinical care over 38 weeks of pregnancy—clinical visits and laboratory tests associated with routine prenatal and postnatal care including delivery. Under the usual care strategy, all would receive RhIG prophylaxis at 28 weeks' gestation and 72 hours post-delivery if the cord blood sampling result was positive, and additional RhIG injections in the case of FMH events (see Interventions and Comparators, above, and Figure 6, Clinical Pathways). In the intervention strategy, in case of a false-negative result, universal RhIG prophylaxis or prophylaxis due to a potential sensitizing event would be missed; however, at delivery, cord blood testing would indicate the incompatibility and RhIG prophylaxis would be provided. We simplified our model and did not account for any increased risk from use of RhIG because this blood product has a strong safety record and an extremely low excess risk of infection.[64,73] After birth, the model does not accumulate any further effects or costs of the pregnant person
- **Baby with no developmental problems**—This health state captures QALYs and costs associated with a baby born with no developmental problems[84,85]

- **Death**—This health state captures background mortality of babies born and followed over the model's time horizon

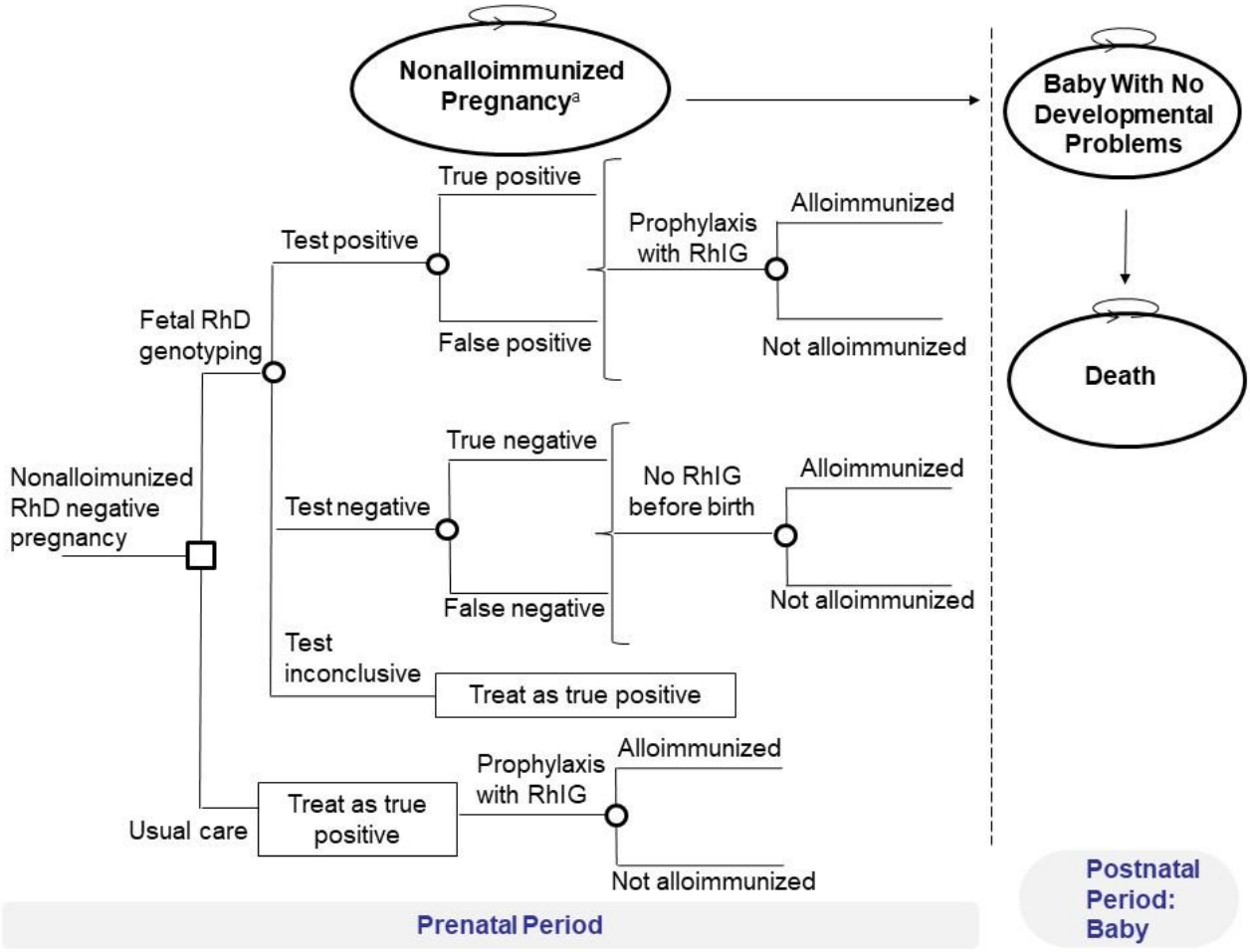

Figure 4: Markov Model Structure, Nonalloimmunized Pregnancies: Simplified Schematic

Abbreviations: RhD, rhesus D blood group; RhIG, Rh immunoglobulin.
[a]Diagnostic intervention with the use of noninvasive fetal RhD blood group genotyping is embedded in the Markov model.

For alloimmunized RhD− pregnancies, our model included five health states (Figure 5). Compared to the model for nonalloimmunized pregnancy, two new health states capture the potential long-term costs and consequences of HDFN:

- **Alloimmunized pregnancy**—As previously described, alloimmunized pregnant people could experience a fetal loss or the fetus could develop HDFN followed by complex care to treat the disease and prevent complications. Thus, pregnant people in this health state would receive intensive monitoring and complex clinical care over 38 weeks of pregnancy (as previously described, this includes frequent clinical visits and laboratory tests to monitor and identify the critical anti-D antibody titer and consequently to initiate more frequent MCA Doppler ultrasound screening, and if necessary management of complications of HDFN occurring during the pregnancy and perinatal period; see Interventions and Comparators, above, and Figure 6). In the intervention strategy, more frequent monitoring and complex treatment were modeled in pregnancies with test-positive results (i.e., RhD incompatibility)

further confirmed with findings of the critical anti-D antibody titer (through serial titer screening). Less intensive monitoring was continued for those with test-negative results (and lack of finding of the critical anti-D antibody titer result). A false-negative test result would be identified at delivery after cord blood testing and appropriate treatment of HDFN would be initiated

- **Baby with no developmental problems**—This health state is the same as in the model for nonalloimmunized pregnancies
- **Baby with minor developmental problems**—This health state accounted for QALYs and costs associated with a baby with minor developmental problems due to HDFN (e.g., squinting, myopia, minor delay in language and fine motor skills).[37,68,86] We assumed these minor developmental problems would last about 16 ± 5 years before a person transitioned to the previously described ("no developmental problems") healthier Markov state[37]
- **Baby with major developmental problems**—This health state accounted for QALYs and costs associated with a person having major developmental problems due to HDFN, such as severe neurodevelopmental delays including cerebral palsy.[37,68,86,87] We also modeled an increased risk of death for people with major developmental problems[37,88]
- **Death**—This health state captures background mortality of babies born with no or minor developmental problems and excess mortality for those with major developmental problems, followed over the model's time horizon

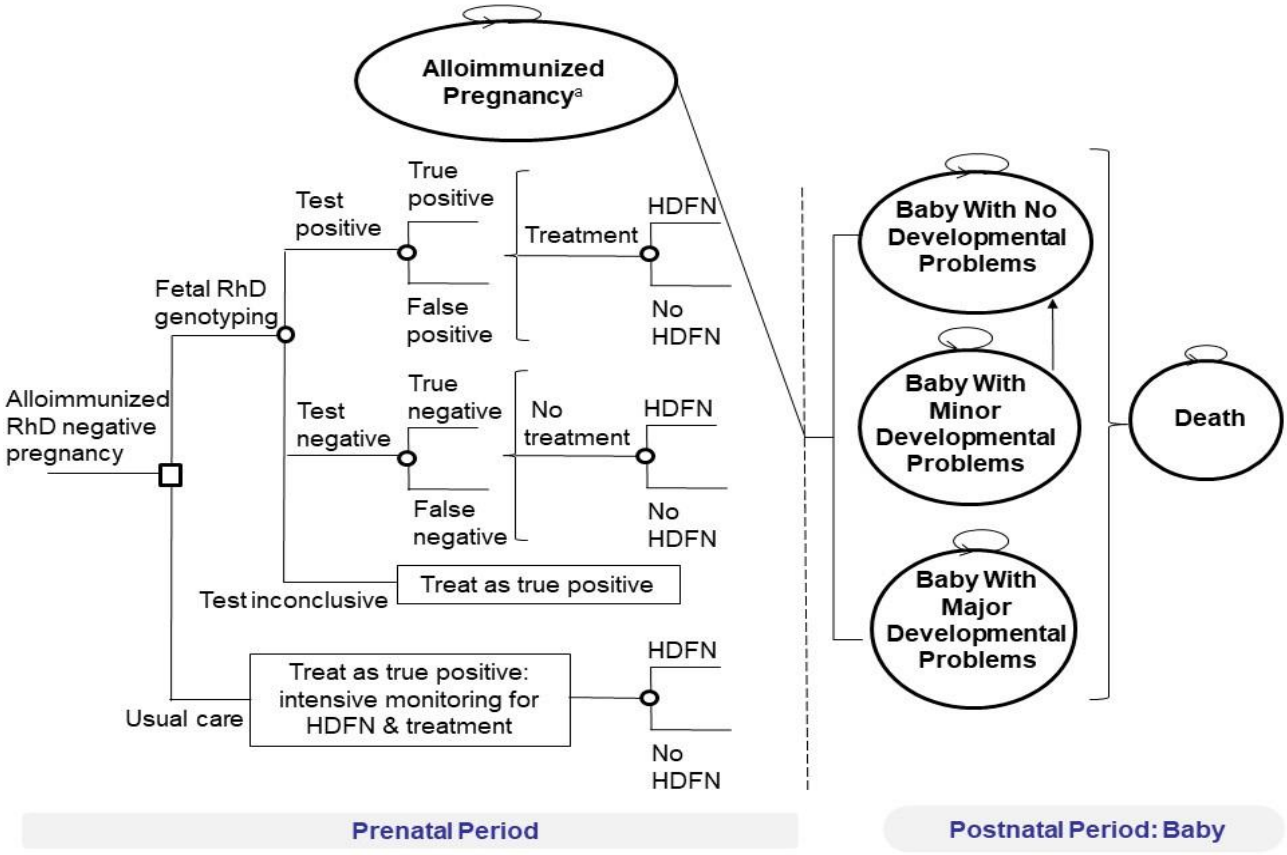

Figure 5: Markov Model Structure, Alloimmunized Pregnancies: Simplified Schematic

Abbreviations: HDFN, hemolytic disease of the fetus and newborn; RhD, rhesus D blood group.
[a]Diagnostic intervention with the use of noninvasive fetal RhD blood group genotyping is embedded in the Markov model.

We used tracker variables to count and track various events such as fetal loss, maternal alloimmunization, and number of RhIG injections. We accounted for serial use of MCA Doppler scanning, number of hospitalizations with IUT, and NICU admissions (newborn), number of babies alive, number of babies affected by HDFN, and number of babies with minor and major neurodevelopmental problems.

We used a short weekly cycle to model events that could alter the course of a nonalloimmunized or alloimmunized RhD− pregnancy, and to reflect appropriate use of health care resources—specifically, any event requiring timely RhIG prophylaxis or a change in intensity of monitoring. We also applied half-cycle correction to balance the distribution of people transitioning between health states.

Figure 6 presents the usual care clinical pathway for both nonalloimmunized and alloimmunized RhD− pregnancies (see details in Intervention and Comparators, above).

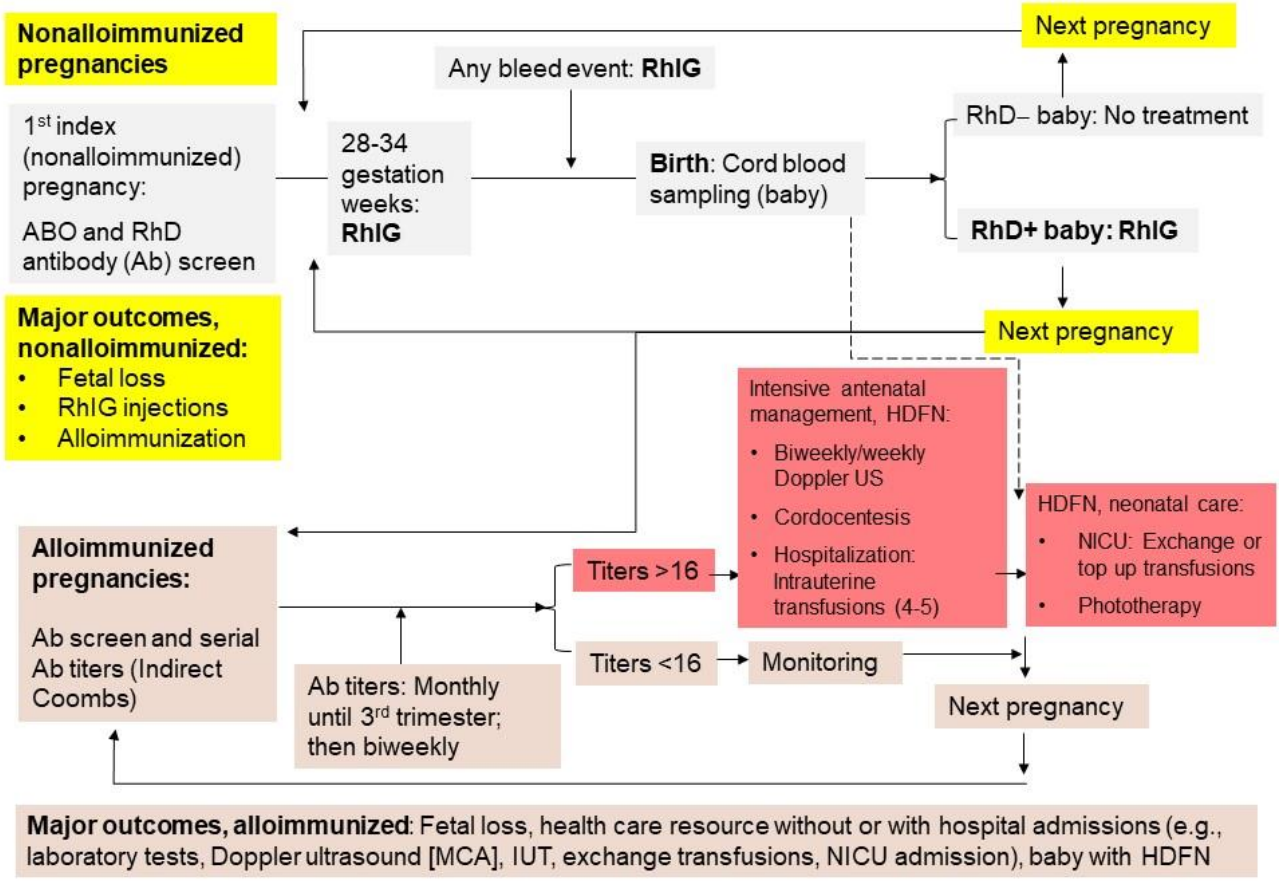

Figure 6: Clinical Pathways—Usual Care: Nonalloimmunized and Alloimmunized Pregnancies

Abbreviations: Ab, anti-D antibodies (serial titres); ABO, ABO blood group system; Doppler ultrasound (MCA), serial fetal middle cerebral artery Doppler ultrasound monitoring; HDFN, hemolytic disease of the fetus and newborn; IUT, intrauterine fetal transfusions; NICU, neonatal intensive case unit; RhD, rhesus D blood group; RhIG, Rh immunoglobulin; US ultrasound.

Main Assumptions: Reference Case Analysis

We used the following structural and parameter modeling assumptions:

- The accuracy of noninvasive fetal RhD blood group genotyping for nonalloimmunized and alloimmunized pregnancies was the same and was used to guide management according to standard clinical practice
- Inconclusive results of noninvasive fetal RhD genotyping were treated as positive
- Compliance (adherence) with RhIG prophylaxis was full; we explored lower compliance in sensitivity analysis
- In alloimmunized pregnancies, no patients dropped out from intensive monitoring and management
- Because of potential ethical issues associated with revealing nonpaternity,[6,11] we considered paternal screening in scenario analysis only
- In nonalloimmunized pregnancies, for simplicity, a 300-mcg dose of RhIG was assumed to be administered for any FMH event, regardless of gestational age
- We did not account for any potential risk of infection due to use of RhIG. The risk of infection associated with this blood product is extremely low due to rigorous safety procedures established by the Canadian Blood Services[64,70-73]
- We did not account for the use of amniocentesis because it may not typically be done in RhD− pregnancies; also, it has been replaced with serial Doppler ultrasound screening in tertiary care centres in which alloimmunized pregnancies are monitored and managed[83] (email communication, B. de Vrijer, MD, July 2019)
- Per-sample cost of noninvasive fetal RhD genotyping was the same for singleton and multiple pregnancies
- Noninvasive fetal RhD genotyping for nonalloimmunized and alloimmunized RhD− pregnancies would be conducted in an Ontario laboratory. A scenario analysis considered that the test for nonalloimmunized pregnancies would be done by the Canadian Blood Services reference laboratory (email communication, G. Clarke, MD, July 2019). Another scenario considered outsourcing this test to the UK laboratory in Bristol via the Ontario Ministry of Health Out-of-Country Prior Approval Program
- Given the limited availability of health state utilities and costs related to complications of HDFN in newborns in Ontario, we used available studies in similar populations and prior modeling studies to inform our model parameters for alloimmunized RhD− pregnancies (see details in Health State Utilities and Cost of Clinical Pathways, below). We examined the impact of these parameters in several scenario analyses

Clinical Outcome and Utility Parameters

We populated our cost-effectiveness model with the clinical and utility parameters described below. These parameters are associated with the natural and clinical course of nonalloimmunized and alloimmunized pregnancies, prevention of maternal alloimmunization, treatment and consequences of HDFN complications, and the accuracy of noninvasive fetal RhD blood group genotyping.

Natural History and Clinical Pathway

We identified the model parameters from various published sources such as our clinical evidence review, studies identified in clinical expert consultations, current clinical practice guidelines, previous model-based economic evaluations, and our economic evidence review.

According to the literature,[37] about 40% of RhD− pregnant people carry an RhD− fetus (i.e., no RhD incompatibility) and, in these cases, the administration of RhIG (in nonalloimmunized pregnancies) or intensive monitoring (in alloimmunized pregnancies) would be unnecessary. As mentioned, we assumed full compliance or adherence for the intervention and for prophylaxis with RhIG at 28 weeks and after delivery or after any bleeding event that could result in maternal alloimmunization. However, in addition to studies included in our clinical evidence review, other studies found that the compliance could be lower for noninvasive fetal RhD genotyping[89] and adherence to regular universal RhIG prophylaxis[49] or prophylaxis after a bleed could be lower as well.[37] We explored these parameter assumptions in sensitivity analyses.

Table 14 presents information on parameter inputs used in the reference case analysis. We describe additional estimates used in probabilistic scenarios, below (see Sensitivity Analysis).

Table 14: Clinical Parameter Inputs Used in the Economic Model—Reference Case Analysis

Model Parameters	Mean (SE) [a,b]	Source
Probability of RhD incompatibility	0.61	Saramago et al, 2018[37]
Probability of inconclusive results after genotyping (treated as those with RhD incompatibility)	0.067 (0.004)	Saramago et al, 2018[37]; Yang et al, 2019[39]
Nonalloimmunized Pregnancy		
Probability of having a miscarriage with hospitalization	0.047 (0.003)	Saramago et al, 2018[37]
Probability of having one potential alloimmunization event (FMH event), any time of pregnancy	0.155 (0.005)	Saramago et al, 2018[37]
Probability of alloimmunization without antenatal and postnatal RhIG prophylaxis	0.12	Johnson et al, 2017[6]; Fung Kee Fung and Eason, 2003[11]; Teitelbaum et al, 2015[62]; Urbaniak,1998[16]; Bowman, 1985[90]
Probability of alloimmunization with routine prophylaxis, antenatal and postnatal RhIG	0.002	Fung Kee Fung and Eason, 2003[11]; Teitelbaum et al, 2015[62]; Bowman, 1978[91]; Bowman et al, 1978[92]
Probability of alloimmunization, postnatal RhIG only	0.016 (0.0004)	Bowman, 1978[91]; Bowman et al, 1978[92]; Teitelbaum et al, 2015[62]
Probability of RhD+ neonate after cord blood sampling	0.63	Moise et al, 2019[57]
Alloimmunized Pregnancy		
Probability of miscarriage during pregnancy with hospitalization	0.047 (0.003)	Saramago et al, 2018[37]
Probability of fetal loss after an invasive procedure (e.g., cordocentesis)	0.050 (0.01)	Pilgrim et al, 2009[68]
Probability of having a critical antibody titer > 1:16 in the first alloimmunized pregnancy (10% risk of HDFN with this titer)	0.43	Moise et al, 2019[57]
Probability of mild HDFN	0.90	Daniels et al, 2004[5]
Probability of IUTs, severe HDFN	0.26	Moise et al, 2019[57]

Model Parameters	Mean (SE) [a,b]	Source
Probability of survival of fetus after IUTs	0.974 (0.0012)[c]	Lindenburg et al, 2012[93]
Probability of delivery (labour), Caesarean birth	0.29	BORN, 2016[94]; CIHI, 2013[95]
Probability of exchange transfusion, severe HDFN (neonatal care)	0.028 (0.0023)	Howard et al, 1997[96]
Probability of combined phototherapy with other treatments in NICU (e.g., exchange transfusions), severe HDFN	1.00	Lieberman et al, 2016[97]
Long-Term Consequences of HDFN		
Probability of minor developmental problems in a person surviving HDFN	0.06 (0.02)	Pilgrim et al, 2009[68]
Duration of minor developmental problems, years	16.0 (5.0)	Pilgrim et al, 2009[68]
Probability of long-term major developmental problems due to severe HDFN	0.048 (0.0027)	Lindenburg et al, 201[93]
Background mortality	Ontario lifetables	Statistics Canada, 2011[98]
Excess mortality in people with major developmental problems, age-adjusted SMR (risk ratio)	1.8	Lauer and McCallion, 2015[88]

Abbreviations: BORN, Better Outcomes Registry and Network; CIHI, Canadian Institute for Health Information; FMH, fetomaternal hemorrhage (bleeding); HDFN, hemolytic disease of the fetus and newborn; IUT, intrauterine transfusion; NICU, neonatal intensive care unit; RhD, rhesus D blood group; RhIG, Rh immunoglobulin; SE, standard error; SMR, standardized mortality ratio.

[a]Beta distributions were assigned to probability estimates in probabilistic sensitivity analysis where applicable. Standard error of the mean (SE) was estimated from 95% confidence intervals. Two parameters of the beta distribution (α, β) were derived from the mean and SE (stated for each model parameter). Formulas for these calculations, derived from the mean and SE, are: $\alpha = ([Mean^2] \times [1 - Mean])/([SE^2] - Mean)$; $\beta = ([\{1 - Mean\} \times \{1 - Mean\}] \times Mean)/([SE^2] - 1)$.

[b]Standard errors were estimated whenever data were available; those input parameters presented with the point estimates were treated as fixed.

[c]Survival estimated from the data reported by Lindenburg et al, 2012,[93] for the perinatal period, after removing deaths due to fetal loss (to prevent double counting).

Intervention Effects

Our clinical evidence review provided the diagnostic test accuracy of noninvasive fetal RhD genotyping for nonalloimmunized pregnancies. In Table 15, we present results of a bivariate hierarchical meta-analysis of eight individual studies by Yang et al[39] in high-throughput prenatal testing that were identified in our clinical review. An estimate of the between-study correlation between sensitivity and specificity was −0.32 in the analysis that treated inconclusive results as test positive, and 0.46 in the analysis that excluded inconclusive results.[37] We assumed the test accuracy of noninvasive fetal RhD genotyping for alloimmunized pregnancies to be the same as for nonalloimmunized pregnancies due to the lack of high-quality evidence in this population (our clinical review identified only one study, by Geifman-Holtzman et al[34]); this assumption was confirmed with clinical experts.

Table 15: Effectiveness of Noninvasive Fetal RhD Genotyping

Model Parameters	Sensitivity, %, Mean (95% CI) [a,b]	Specificity, %, Mean (95% CI) [a,b]	Source
Diagnostic test accuracy, inconclusive results treated as T+[a]	99.7 (99.2–99.9)	96.1 (94.2–97.5)	Calculated from Yang et al, 2019[39] (see Table 4, Clinical Evidence)
Diagnostic test accuracy, excluding inconclusive results[a,c]	99.7 (99.2–99.9)	98.7 (98.2–99.1)	Calculated from Yang et al, 2019[39] (see Table 4, Clinical Evidence)

Abbreviations: CI, confidence interval; FNR, false-negative rate; FPR, false-positive rate; RhD, rhesus D blood group; T+, test positive.
[a]Sensitivity and specificity with 95% CI were calculated from false-positive and false-negative results reported in Table 4 (see Clinical Evidence) based on data reported in Yang et al[39] (1−FNR and 1−FPR and their upper and lower bounds, respectively): 1) treating inconclusive results as positive (8 studies): FNR = 0.34%, 95% CI 0.15 to 0.76, and FPR = 3.86%, 95% CI 2.54 to 5.82; and 2) excluding all inconclusive results (8 studies): FNR = 0.35, 95% CI 0.15 to 0.82, and FPR = 1.26, 95% CI 0.87 to 1.83.
[b]In probabilistic sensitivity analysis, given that the estimate of the between-study correlation is small, we simplified and did not model accuracy of the test by assigning the bivariate normal distribution to sensitivity and specificity but assigned beta distributions.
[c]Used in scenario analyses.

Health State Utilities

Health state utilities measure health-related quality of life and reflect the strength of preference for specified health states. We performed a targeted literature search in MEDLINE for health state utilities associated with the intervention on March 12, 2019, to retrieve studies published from January 01, 1997, until the search date. We based the search on the population of the clinical search strategy with a methodologic filter applied to limit retrieval to utility values.[75] See Appendix 1 for literature search strategies, including all search terms. We also examined the health state utilities reported in the model-based economic evaluations identified from the economic evidence review.

In our analysis, we focused on estimating the QALYs associated with postnatal outcomes—specifically, those associated with being born with HDFN. We did not estimate utilities for the fetus in the antenatal health states (i.e., nonalloimmunized or alloimmunized pregnancy), given a lack of data and the difficulties associated with accurate estimation of utility values for an unborn child.[99] We also did not model maternal QALYs given an unclear utility value of fetal loss due to HDFN in women with RhD incompatibility. Lastly, we did not distinguish utility associated with inaccurate (false-positive, false-negative) genotyping results because our review of the quantitative preferences evidence and the accompanying qualitative review by CADTH[1] did not identify any specific input values that could be considered in our modeling study.

Table 16 presents the health state utilities included in our cost–utility model. Utilities associated with short-term minor developmental problems (e.g., squinting, myopia, delay in language and fine motor skills), as identified in two health technology assessments by the UK National Institute for Health and Care Excellence (NICE), were derived from several studies of premature babies with low birth weight.[37,65-68,86] It appears that for two postnatal health states—no developmental problems and minor developmental problems—the 2018 model-based analysis by NICE[37] considered the utility values of an Ontario-based study by Saigal et al[86] in young adult survivors of extremely low-birth-weight (n = 143) and low-birth-weight (n = 130) infancy. Saigal et al[86] compared health state utilities (by standard gamble) and self-perceived health status (by Health Utility Index 2) between these two groups. Despite significant differences in neurosensorial impairment and greater burden of morbidity in extremely low-birth-weight survivors (27% vs 2%), the mean utility scores between the two groups were not significantly different (extremely low-birth-weight survivors: 0.85, 95% CI 0.81–0.89; low-birth-weight survivors: 0.88, 95% CI 0.84–0.92; *P* value = .32).

An 8-year population-based cohort study from the Netherlands examined the probability of various long-term developmental problems including cerebral palsy or severe neurodevelopmental delay in babies surviving intrauterine transfusions after being treated for severe HDFN.[93] For the health state of a person both with long-term major developmental problems, we also considered health state utilities associated with cerebral palsy and other major developmental problems. We identified an Ontario study that elicited health state utilities from 199 people with cerebral palsy (youth and adults) at six treatment centres, using the HUI3 (Health Utility Index Mark III) instrument.[87] Based on the Netherlands study by Lindenburg et al,[93] we estimated about 42% of people with long-term developmental impairments could have cerebral palsy resulting from severe HDFN. We assumed that health state utilities related to other major developmental issues were the same as reported in prior NICE reports assessing similar interventions for RhD− pregnancies.[37,68] Given variability in the severity of conditions associated with major developmental delays, including cerebral palsy,[87,100] we tested our parameter assumptions in sensitivity analyses.

Table 16: Health State Utility Parameters: Reference Case Cost–Utility Analysis

Model Parameter: Utilities	Mean (SE)[a]	Source
No developmental problems	0.88 (0.02)	Pilgrim et al, 2009[68]; Saramago et al, 2018[37]; Saigal et al, 2006[86]
Minor developmental problems	0.85 (0.02)	Pilgrim et al, 2009[68]; Saramago et al, 2018[37]; Saigal et al, 2006[86]
Major developmental problems:		
• Overall	0.42 (0.03)	Pilgrim et al, 2009[68]; Saramago et al, 2018[37]
• Cerebral palsy	0.30 (0.03[b])	Young et al, 2010[87]

Abbreviation: SE, standard error.
[a]Beta distributions were assigned in probabilistic sensitivity analysis. Two parameters of the beta distribution (α, β) were derived from the mean and SE (stated for each model parameter).
[b]Estimated from the reported sample size (N = 199) and standard deviation (SD = 0.419).

Cost Parameters

Cost of Noninvasive Fetal Rh Genotyping: Nonalloimmunized and Alloimmunized Pregnancies

Table 17 presents per-sample cost estimates of noninvasive fetal RhD blood group genotyping used in our reference case analysis. These estimates are based on laboratory practices at Mount Sinai Hospital in Ontario. Our detailed costing methods are described in Appendix 6.

For nonalloimmunized pregnancies, the cost of noninvasive fetal RhD genotyping was $247.34 per sample and included the RhD testing only. For alloimmunized pregnancies, the cost was $328.19 per sample and included several tests: RhD alone, RHCE (c, C, and E alleles), Kell (K-antigen), and RhD Asian variant (RhDc) (email communication, R. Kandel, MD, and G. Charames, PhD, July 2019).

These cost estimates captured the costs of labour and consumables (with respect to blood sample collection, plasma preparation, and extraction of cell-free DNA, test interpretation and reporting, and quality assurance and quality control) and the cost of transporting the samples.[101]

Table 17: Per-Sample Cost Estimate of Fetal RhD Genotyping by Population

Parameter Description	Unit Cost, $	Quantity	Total Cost, $[a]	Source
Nonalloimmunized Pregnancies				
Fetal RhD genotyping[b]	242.09	1	242.09	Expert consultation
Transportation	52.50	10 samples	5.25	Tsiplova et al, 2017[101]
Total per-sample cost			247.34	
Alloimmunized Pregnancies				
Fetal RhD genotyping[b]	242.09	1	242.09	Expert consultation
Fetal Rh genotyping: +3 tests[c]	26.95	3	80.85	Expert consultation
Transportation	52.50	10 samples	5.25	Tsiplova et al, 2017[101]
Total per-sample cost			328.19	

Abbreviation: RhD, rhesus D blood group.
[a]Gamma distributions were assigned in probabilistic sensitivity analysis, assuming the standard error to the mean cost of 25%. Two parameters of the gamma distribution (α, λ) were derived using the following formulas: α = (Mean^2)/(SE^2); λ= Mean/([Mean x SE]^2).
[b]This is a lab-developed and validated test for Ontario (email communication, R. Kandel, MD, and G. Charames, PhD, July 2019).
[c]Additional 3 genotyping tests are required for alloimmunized pregnancies, for RHCE (c, C, and E), Kell, and RhDc Asian variant.

In a scenario analysis, we assumed the cost of genotyping for any RhD− pregnancy would be provided by the Out-of-Country Prior Approval Program because this test has sometimes been covered in Ontario, solely for alloimmunized pregnancies and on case-by-case basis. Currently, all samples are sent to the reference laboratory in Bristol, UK. For this scenario, we assumed the cost would be $510 to $710 per sample.

In another scenario analysis, we assumed that testing for nonalloimmunized pregnancies would be conducted by the Canadian Blood Services (CBS) reference laboratory in Alberta.[6] With a national screening program for nonalloimmunized pregnancies, the cost of noninvasive fetal RhD genotyping could be substantially lower due to large volume and the availability of high-throughput, automated, real-time polymerase chain reaction methods at the CBS lab (email and oral communication,
G. Clarke, MD, July 2019). No additional cost would be required to transport the samples, given the highly developed transportation network at the CBS (oral communication, G. Clarke, MD, July 2019). The CBS per-sample cost estimate was unavailable when we conducted this analysis. However, we assumed it would be about $125, half the cost estimated for testing in nonalloimmunized RhD− pregnancies in Ontario.

Cost of Clinical Pathways: Nonalloimmunized and Alloimmunized Pregnancies

Nonalloimmunized Pregnancies

For health care resource use associated with nonalloimmunized pregnancies, we obtained cost information from the published literature and clinical experts. Table 18 presents the unit cost and frequency of use for various diagnostic tests and procedures associated with clinical care of nonalloimmunized pregnancies. (See Appendix 7 for original data used to estimate standard errors.)

Table 18: Per-Case Input Cost Estimates—Clinical Care in Nonalloimmunized Pregnancies

Parameter Description	Unit Cost, $, Mean (SE) [a,b]	Frequency	Source
Diagnostic and Laboratory Procedures			
Blood group – ABO and Rh phenotype: maternal, neonatal, or paternal (lab fee)	6.81	1	L493, MOH Schedule of Benefits for Laboratory Services[102]
Indirect anti-human globulin test (Indirect Coombs) (lab fee)	6.81	10	L495, MOH Schedule of Benefits for Laboratory Services[102]
Fetomaternal hemorrhage, Kleihauer stain or Kleihauer-Betke (KB) test (lab fee)	9.31	1	L431, MOH Schedule of Benefits for Laboratory Services[102]
RhIG Administration			
Anti-D Ig, 1500 IU, 300 mcg	81.00 (20.25)	2	Duplantie et al, 2013[61]
RhIG administration, first injection, sole administration (professional fee)	6.75	1	G373, OHIP Schedule of Benefits[103]
RhIG administration, each additional injection (professional fee)	3.89	NA	G372, OHIP Schedule of Benefits[103]
Administration of RhIG, ambulatory (procedure)	263.64 (70.39)	1	8ZZ70HABW, OCCI (2017/18)[104]
Administration of RhIG, inpatient, after cord blood sampling or invasive procedure (procedure)	190.20 (5.16)	1	8ZZ70HABW; ICD: O36013, OCCI (2016/17)[104]
Maternal and Fetal Clinical Care			
Initial interview and initial minor assessment (professional fee)	77.20 + 33.70	2	P003 and P004, OHIP Schedule of Benefits[103]
Antenatal visits, 12 in total (4 in weeks 15–28, and 8 in weeks 29–42) (professional fee)	45.15	12	P005, OHIP Schedule of Benefits[103]
Obstetrical ultrasound (procedure)	408.14 (2.02)	3	5AB03JA, OCCI (2017/18)[104]
Miscarriage/abortion: medical management (OB-GYN), early pregnancy, abortion, initial service (professional fee)	161.15	1	A920, OHIP Schedule of Benefits[103]
Termination of pregnancy (procedure, day surgery)	1,337.58 (35.13)	1	5CA89GA, OCCI (2017/18)[104]
Delivery, vaginal, occurs 71% of the time[94,95] (professional fee)	498.70	1	P006, OHIP Schedule of Benefits[103]
Delivery, Caesarean, occurs 29% of the time[94,95] (professional fee)	579.80	1	P018, OHIP Schedule of Benefits[103]
Delivery, vaginal, occurs 71% of the time[94,95] (procedure)	3,603.73 (48.89)	1	CGM P562, OCCI (2017/18)[104]
Delivery, Caesarean, occurs 29% of the time[94,95] (procedure)	9,213.22 (844.54)	1	CGM P559, OCCI (2017/18)[104]
Cord blood sampling (procedure)	772.54 (24.28)	1	5MD11TA, OCCI (2017/18)[104]

Parameter Description	Unit Cost, $, Mean (SE) [a,b]	Frequency	Source
Postnatal care, clinical visits (6 in total: 3 clinic and 3 outpatient) (professional fee)	103.05	6	A901+H261/P005+H261, OHIP Schedule of Benefits[103]

Abbreviations: CGM, Case Group Mix grouper code; ICD, International Classification of Diseases code; MOH, Ontario Ministry of Health; NA, not applicable; OB-GYN, obstetrician-gynecologist; OCCI, Ontario Case Costing Initiative; OHIP, Ontario Health Insurance Plan; RhIG, Rh immunoglobulin; SE, standard error.

[a]All costs are in 2019 Canadian dollars. The original data are presented in Appendix 7.

[b]Input parameters presented as the point estimates were treated as fixed (i.e., physician fees or laboratory fees) and were not assigned the gamma distribution. Standard errors were calculated whenever it was possible (see Appendix 7); otherwise, SEs were assumed to be 25% of the mean cost. For the inputs with calculated SEs, we assigned the gamma distributions in probabilistic sensitivity analysis. Two parameters of the gamma distribution (α, λ) were derived from the mean and SE. Formulas for these calculations are: α = (Mean^2)/(SE^2); λ= Mean/([Mean x SE]^2).

Alloimmunized Pregnancies

Alloimmunized pregnancies are considered high risk because of the possibility of the fetus developing HDFN. Intensive monitoring and treatment for these pregnancies are most often provided in tertiary care centres or hospitals equipped with fetal-maternal units and highly specialized health care personnel. The use of noninvasive fetal RhD genotyping does not change the course of HDFN or its clinical pathway (email and oral communications, B. de Vrijer, MD, N. Shehata, MD, N. Okun, MD, July 2019).

We obtained the type and frequency of standard resource use required for the monitoring and treatment of HDFN in Ontario through expert consultation (Table 19). The most severe cases of HDFN associated with the highest use of health care resource use are infrequent (email communications, B. de Vrijer, MD, N. Shehata, MD, N. Okun, MD, July 2019, and the Ontario Case Costing Initiative database[104]).

Table 19: Per-Case Input Cost Estimates—Clinical Care in Alloimmunized Pregnancies

Parameter Description	Unit Cost, $, Mean (SE)[a,b]	Frequency	Source
Diagnostic and Laboratory Procedures			
Maternal type/screen/anti-D: blood group – ABO and Rh phenotype, lab fee	6.81	1	L493, MOH Schedule of Benefits for Laboratory Services[102]
Maternal type/screen/anti-D titer: blood group – any other antigen (lab fee)	4.14	1	L494, MOH Schedule of Benefits for Laboratory Services[102]
Maternal type/screen/anti-D titer (professional fee)	7.76	1	G035, OHIP Schedule of Benefits[103]
Serial maternal anti-D titers, lab fee: if below critical level (< 1:16), monthly titers 1st and 2nd trimester, biweekly in 3rd trimester until 36 weeks; if titer becomes critical, no need for further screening (lab fee)	7.76	1	L471, MOH Schedule of Benefits for Laboratory Services[102]
Titer – serial tube single antigen (professional fee)	7.76	1	G035, OHIP Schedule of Benefits[103]
Maternal and Fetal Clinical Care			
Initial interview and initial minor assessment (professional fee)	77.20 + 33.70	2	P003 and P004, OHIP Schedule of Benefits[103]
Maternal (fetal medicine physician) assessment, high-risk pregnancy (professional fee)	74.70	1 (20 minutes)	P002, OHIP Schedule of Benefits[103]
Antenatal visits, depending on severity of HDFN; at least 12 if titer is not critical; if critical, visits follow biweekly fetal MCA Doppler ultrasound (professional fee)	45.15	1	P005, OHIP Schedule of Benefits[103]
Obstetrical ultrasound (procedure)	408.14 (2.02)	3	5AB03JA, OCCI (2017/18)[104]
Fetal MCA Doppler ultrasound: if titer is not critical, monthly; if titer is critical, biweekly and twice a week before IUT (professional fee)	30.00 + 26.55	1	J167+J160, OHIP Schedule of Benefits[103]
Fetal MCA Doppler ultrasound screening, fee: at frequency described above (procedure)	701.37 (71.13)	1	5AB03GS, OCCI (2017/18)[104]
Maternal care, severe HDFN, 6 days (procedure)	6,351.79 (1,493.01)	1	ICD: O36231, OCCI (2015/16)[104]
IUT, initial or subsequent, severe HDFN (professional fee)	186.9	1	G280, OHIP Schedule of Benefits[103]
IUT, severe HDFN, 3–5 times (procedure)	2,817.55 (218.02)	1	5FD72HAU1, OCCI (2017/18)[104]
Medical management (OB-GYN), early pregnancy, abortion, initial service (professional fee)	161.15	1	A920, OHIP Schedule of Benefits[103]
Termination of pregnancy, (procedure, day surgery)	1,337.58 (35.13)	1	5CA89GA, OCCI (2017/18)[104]

Parameter Description	Unit Cost, $, Mean (SE)[a,b]	Frequency	Source
Delivery			
Delivery, vaginal, occurs 71% of the time[94,95] (professional fee)	498.70	1	P006, OHIP Schedule of Benefits[103]
Delivery, Caesarean, occurs 29% of the time[94,95] (professional fee)	579.80	1	P018, OHIP Schedule of Benefits[103]
Delivery, vaginal, occurs 71% of the time[94,95] (procedure)	3,603.73 (48.89)	1	CGM P562, OCCI (2017/18)[104]
Delivery, Caesarean, occurs 29% of the time[94,95] (procedure)	9,213.22 (844.54)	1	CGM P559, OCCI (2017/18)[104]
Fetomaternal hemorrhage, Kleihauer stain or Kleihauer-Betke (KB) test (lab fee)	9.31	1	L431, MOH Schedule of Benefits for Laboratory Services[102]
Cord blood sampling (procedure)	772.54 (24.28)	1	5MD11TA, OCCI (2017/18)[104]
Neonatal Clinical Care			
Phototherapy, 1 day, in ward (procedure, not NICU)	1,900.09 (28.69)	1	1YZ12JADQ, CGM P581, P585,P588, P589, P598, P599, OCCI (2015/16)[104]
NICU, initial for newborn (professional fee)	136.40	1	G556: ICU/NICU admission assessment fee, OHIP Schedule of Benefits[103]
NICU, initial day 1, level A (professional fee)	358.00	1	G600, OHIP Schedule of Benefits[103]
NICU, prolonged stay, level A, 2–30 days (professional fee)	178.95	1	G601, OHIP Schedule of Benefits[103]
NICU, level II, inpatient, 7.5 hospital days (procedure)	9,431.16 (4,063.46)	1	CGM P590, P598, P599, OCCI (2017/18)[104]
Exchange transfusion, NICU, severe HDFN (professional fee)	205.45	1	G275, OHIP Schedule of Benefits[103]
Exchange transfusion, NICU, severe HDFN (procedure)	2,549.99 (195.18)	1	CGM P557 plus multiple codes related to this procedure: 1LZ19HHU1A, 1LZ19HHU1J, 1LZ19HHU9A, 1LZ19HMU1, 5FD72HAU1, 5FD72HAU4, 5FD72HAU9, OCCI (2017/18)[104]
Outpatient MD charge pediatric assessment and clinical visits after discharge from NICU (severe HDFN) (professional fee)	57.90	12	H261, OHIP Schedule of Benefits[103]

Abbreviations: CGM, Case Group Mix grouper code; HDFN, hemolytic disease of the fetus and newborn; ICD, International Classification of Diseases code; IUT, intrauterine transfusion; MCA, middle cerebral artery; MOH, Ontario Ministry of Health; NICU, neonatal intensive care unit; OCCI, Ontario Case Costing Initiative; OHIP, Ontario Health Insurance Plan; SE, standard error.

[a]All costs are in 2019 Canadian dollars. The original data are presented in Appendix 7.

[b]Input parameters presented as the point estimates were treated as fixed (i.e., physician fees or laboratory fees) and were not assigned the gamma distribution. Standard errors were calculated whenever possible (see Appendix 7); otherwise, SEs were assumed to be 25% of the mean cost. For the inputs with calculated SEs, we assigned the gamma distributions in probabilistic sensitivity analysis.

Long-Term Consequences of HDFN

If maternal alloimmunization occurs during a nonalloimmunized pregnancy, our model assumes that a baby born from this pregnancy would not be at risk of HDFN and, therefore, would not have any developmental problems associated with that condition. We estimated the costs associated

with a baby born without developmental problems using the current Canadian pediatric recommendations on early-years child development screening.[84,85] We made a simplifying assumption that, on average, in the first 5 years of a child's life, two physician visits would occur annually (A263, Schedule of Benefits[103]: $77.70 per visit); from age 6 onwards, one physician visit per year would occur (A003/K269, Schedule of Benefits[103]: $77.20).

In the next alloimmunized pregnancy, severe forms of HDFN could be associated with long-term developmental complications and disabilities.[37,68,88] Table 20 presents annual mean costs associated with minor or major developmental problems as complications of severe HDFN.

Lunsky et al[105] conducted a secondary analysis using the Ontario administrative databases for a cohort of adults with intellectual and developmental disabilities, aged 18 to 64 years, who received disability income support. The study included individuals with mental retardation, autism, fetal alcohol syndrome, various genetic disorders, and other conditions associated with life-long limitations in cognitive and adaptive functioning or organ system diseases. The authors provided costs for two groups: one with the highest health care expenditure (top 10%) and another with the lowest health care costs (< 90th percentile). In the "baby with minor developmental problems" health state, we assumed these individuals would incur annual costs associated with the low-cost group (< 90th percentile) and calculated estimates based on the reported data ($867 for females and $721 for males [2009/10 CAD][105]).

In the "baby with major developmental problems" health state, we assumed there would be a mix of individuals with major developmental problems (58%) and cerebral palsy (42%), using data from a long-term study by Lindenburg et al[93] (we made a similar assumption for the model's health state utilities). To cost this most severe health state, we used two literature sources:

- For individuals with major developmental problems without cerebral palsy (58%), we used the mean annual health care costs of adults with intellectual and developmental disabilities in the top 10% cost group ($18,397 [2009/10 CAD] for females and $20,921 [2009/10 CAD] for males) as described by Lunsky at al[105]
- For individuals with cerebral palsy (42%), we based our cost estimates on the results of a previous health technology assessment.[106] We assumed that half of this group received usual care and the other half received dorsal rhizotomy, given eligibility for this neurosurgical treatment[106]

However, due to uncertainty around the parameter assumptions of the cost inputs, we tested the influence of these parameters on the cost-effectiveness results.

Table 20: Annual Per-Case Cost Estimates Associated With Long-Term Care of People With Intellectual and Developmental Disabilities

Parameter Description	Mean Cost, $ (SE)[a,b]	Source
Minor developmental problem	916.55 (229.14)[c]	Lunsky et al, 2019[105]
Major developmental problem, in general	23,207.35 (5,801.84)[d]	Lunsky et al, 2019[105]
Major developmental problem, cerebral palsy		
• Usual care	3,001.41 (750.35)[e]	Health Quality Ontario, 2017[106]
• Dorsal rhizotomy	161,615.19 (40,403.80)[e]	Health Quality Ontario, 2017[106]

Abbreviation: SE, standard error.

[a]All costs are in 2019 Canadian dollars.

[b]Gamma distributions were assigned in probabilistic sensitivity analysis, assuming standard error to the mean cost of 25%.

[c]Based on data reported in Table 1[105] we estimated the costs for both sexes; females accounted for about 40% of the low-cost group, and we estimated 2009/10 CAD for both sexes of $779.4. We converted this cost input to 2019 CAD using the Canadian Consumer Price Index (CPI): (137 [2019]/116.5 [2010]) * 779.4 = 916.55.

[d]Based on data reported in Table 1 in Lunsky et al,[105] we estimated the costs for both sexes; females accounted for about 47% of the high-cost group, and we estimated 2009/10 CAD for both sexes of $ 19734.72. We converted this cost input to 2019 CAD using the CPI ratio: (137 [2019]/116.5 [2010]) * $19,734.72 = $23,207.35.

[e]The original data were reported in 2016 CAD, with the cost of usual care being $2,813 and the cost of dorsal rhizotomy, $151,470. We converted these to 2019 CAD using the CPI ratio: (137 [2019]/128.4 [2016]).

Internal Validation

Formal internal validation was conducted by the secondary health economist. This included testing the mathematical logic of the model and checking for errors and accuracy of parameter inputs and equations.

Analysis

We conducted separate reference case and sensitivity analyses for the two target populations. Our reference case analysis used the most likely set of input parameters and model assumptions, confirmed through numerous expert consultations. Our sensitivity analyses explored how the results are affected by varying input parameters and model assumptions. As mentioned, the primary outcome of our economic evaluation was the ICER, reported as the incremental cost per outcome averted or gained (e.g., RhIG injections, hospitalizations with IUT or NICU admission, newborn's QALY over their lifetime or the model time horizon).

Following the CADTH guidelines,[75] we assigned distributions for model parameters and used probabilistic sensitivity analysis (PSA) to address parameter uncertainty. We presented the probability of each testing strategy being cost-effective over a range of willingness-to-pay values on a cost-effectiveness acceptability curve. We also presented uncertainty qualitatively, in one of five categories defined by the Ontario Decision Framework[107]: highly likely to be cost-effective (80%–100% probability of being cost-effective), moderately likely to be cost-effective (60%–79% probability of being cost-effective), uncertain if cost-effective (40%–59% probability of being cost-effective), moderately likely to not be cost-effective (20%–39% probability of being cost-effective), or highly likely to not be cost-effective (0%–19% probability of being cost-effective).

We ran the two-loop PSAs in the reference case and sensitivity analyses over a 10-year time horizon, sampling 10 million simulations (100 x 100,000 trials) in the reference case analysis and 5 million in scenario analyses (100 x 50,000 trials). We also completed the PSA analyses over a lifetime time horizon for both populations in our scenario analyses.

Analyses were conducted using TreeAge Pro 2019 R2.[108] Costs were reported in 2019 Canadian dollars. Where 2019 costs were unavailable, we used the Consumer Price Index to adjust to 2019 Canadian dollars.[109]

Sensitivity Analysis: Scenarios

Table 21 outlines all scenario analyses. These scenarios analyses addressed our main assumptions related to values of model input parameters and structural model assumptions.

Table 21: Probabilistic Sensitivity Analysis Scenarios: Changes in Structural and Parameter Assumptions

Parameter/Assumption: Population of Interest	Reference Case Analysis	Scenario Analysis: Major Changes in Parameter Values or Assumptions
Inclusion of paternal phenotyping: both populations	Maternal testing only: intervention with noninvasive fetal RhD genotyping	Paternal RhD testing, assuming patient consent; paternal testing included in both strategies (intervention and usual care)
Probability of inconclusive results after genotyping: both populations	0.067 (0.004)[37,39]	2% to 10%[37]
Test accuracy: both populations	Sn: 99.7%; Sp: 96.1% [39]	Sn: 99.7%; Sp: 98.7% (see Table 15)[39]
Probability of alloimmunization: nonalloimmunized population only		
• RhIG at 28 weeks and birth	0.002[62]	0.0031 (95% CI 0.0021 – 0.0040)[57]
• RhIG after birth only	0.016[62]	0.0067 (95% CI 0.0050 – 0.0084)[57]
• No RhIG	0.12	0.16[6,11]
Compliance with testing and RhIG: nonalloimmunized population only		
• Fetal RhD genotyping	100%	Assumed 0.78 for the scenario analysis, based on the range reported in our clinical review: 0.78 to 0.93 (Clausen et al, 2014[89])
• RhIG	100%	Probability of receiving universal prophylaxis at 28 weeks and birth: 0.99 (Soothill et al, 2015[49]; Saramago et al, 2018[37]) Probability of receiving RhIG after a potentially alloimmunization event: 0.958 (0.006)[37]
Analysis perspective: alloimmunized population only	Ministry of Health perspective	Societal perspective
Cost of noninvasive RhD fetal genotyping: both populations	Estimated (Table 17)	Estimated, provided by OOC-PA Program (Table 22) Lower cost: nonalloimmunized pregnancies (Table 22)
Cost of cord blood sampling: both populations	Procedure cost: $772 (SE: $24) (Tables 18 and 19)	Lab fee cost solely: $6.81 (Tables 18 and19)
Probability of long-term neurodevelopmental problems: alloimmunized population only	0.048 (SE: 0.003)[93]	3 times smaller than reference case estimate

Parameter/Assumption: Population of Interest	Reference Case Analysis	Scenario Analysis: Major Changes in Parameter Values or Assumptions
Long-term costs and HSUs of the state associated with major developmental problems: alloimmunized population only	HSUs and costs, combined for major developmental problems and cerebral palsy (see Table 16 and Table 20): HSUs = 0.42[37] and 0.30[87]	Scenario A assumed: • HSUs of major developmental problems in general: 0.42 (SE: 0.03)[37] • Costs of major developmental issues in general (costs of cerebral palsy excluded) Scenario B assumed: • HSUs of major developmental problems in general: 0.42 (SE: 0.03)[37] • Twice-lower costs of major developmental issues (costs of cerebral palsy excluded) Scenario C assumed: • HSUs of major developmental problems in general: 0.42 (SE: 0.03)[37] • 10-times higher costs of major developmental issues (costs of cerebral palsy excluded) Scenario D assumed: • HSUs of major developmental problems of 0.67 (SE:0.03),[87] based on upper-end HSUs for people with cerebral palsy • Twice-lower the reference case cost estimate (including Ontario's estimates for costs of cerebral palsy)
Discount rate: both populations	1.5%	5%
Time horizon: both populations	Long-term: 10 years	Shorter: 1 year, 5 years, and lifetime
Multiple pregnancies: nonalloimmunized population only	Nonalloimmunized population only, one pregnancy	Approximation, time horizon: 10 years and lifetime 2 pregnancies: first nonalloimmunized pregnancy followed by either a nonalloimmunized or alloimmunized pregnancy

Abbreviations: CI, confidence interval; HSU, health state utilities; NA, not applicable; OOC-PA Program, Out-of-Country Prior Approval Program; RhD, rhesus D blood group; RhIG, Rh immunoglobulin; SE, standard error; Sn, sensitivity; Sp, specificity.

Scenario: Paternal RhD Testing in Both Clinical Pathways

In this scenario, we included paternal RhD phenotyping in the clinical pathways for both nonalloimmunized and alloimmunized pregnancies. The fetus's RhD status depends on the probability of the father being homozygous D or heterozygous D. However, a number of concerns about paternal testing have been raised because of the possibility of unknown paternity in a pregnancy.[6] In our model, we assumed a nonpaternity rate of 3%, based on data in the literature and estimates from previous economic analyses.[57,61] In this scenario, we assumed the probability of the father being homozygous D to be 48%, and 52% for heterozygous D.[57] If the father is found

to be homozygous D (two RhD+ alleles), all fetuses are affected; that is, all babies will have RhD incompatibility (RhD+ baby, RhD− mother) and there is no need for fetal RhD genotyping. In this case, all RhD− mothers would require either RhIG prophylaxis (in nonalloimmunized pregnancies) or intensive monitoring (in alloimmunized pregnancies). If the father is heterozygous D (one RhD+ allele and one RhD− allele), then noninvasive fetal RhD genotyping would be required (as 50% of fetuses would have D antigen). Consequently, introduction of paternal screening, which is not routinely recommended in Canada due to ethical concerns,[6,11] would result in a smaller number of samples proceeding to noninvasive fetal RhD genotyping.

Scenario: Societal Perspective

In this scenario analysis, we used a limited approach to addressing societal perspective and accounted for the cost of lost productivity: specifically, patient time costs. We did this solely for the alloimmunized population, given that this high-risk population is intensively managed during pregnancy. Based on information available from a prior health technology assessment,[110] we assumed pregnant people would not be able to work on days they had clinical visits (i.e., about 8 hours per day). We used the median annual income in Ontario ($33,840 per year plus 30% benefits, or $22.67 per hour) to estimate the cost of lost productivity. This cost was incurred during every visit an alloimmunized RhD− person had over an average 38-week pregnancy.

Scenario: Cost of Noninvasive Fetal RhD Blood Group Genotyping

Table 22 presents per-sample cost estimates used in several scenario analyses. As explained earlier (see Cost Parameters), one scenario analysis assumed that screening of nonalloimmunized population would be done by the Canadian Blood Services, at a cost of $125 per sample. Currently, the test is not covered in Canada or Ontario for nonalloimmunized pregnancies. In other scenarios, we assumed testing for alloimmunized pregnancies would be routinely covered through the Out-of-Country Prior Approval Program.

Finally, given the findings of our economic evidence review and the wide range of test costs used in various studies, we conducted a threshold analysis to establish a break-even point at which noninvasive fetal RhD genotyping became cost neutral compared to usual care.

Table 22: Scenario Analyses: Varying the Per-Sample Costs of Noninvasive Fetal RhD Genotyping

Parameter Description	Unit Cost, $[a]	Quantity	Total Cost, $[a,b]	Source
Nonalloimmunized Pregnancies				
Test done in national CBS lab	125	1	125	Assumption
Alloimmunized Pregnancies				
Test done in Bristol, UK (OOC-PA)	450–560	1	450–560	Expert consultations[c]
Transportation of samples (OOC-PA)	60–150	1	60–150	Expert consultations[c]
Total cost (OOC-PA)	**510–710**	**1**	**510–710**	

Abbreviations: CBS, Canadian Blood Services, OOC-PA, Out of Province Prior Approval Program.
[a]All costs are in 2019 Canadian dollars.
[b]Gamma distributions were assigned in probabilistic sensitivity analysis, assuming standard error to the mean cost of 25%.
[C]Expert consultations (oral and email communications, B de Vrijer, MD, N Shehata, MD, Y Lin, MD, N Okun, MD, January 2019).

Scenario: Utilities and Costs of the "Baby With Major Developmental Problems" Health State

To test various assumptions related to health state utilities and costs associated with long-term consequences of HDFN, we conducted four different scenario analyses (presented in Table 21 as scenarios A to D). In general, to make our cost-effectiveness analyses directly applicable to the Ontario setting, we aim to use Ontario-specific estimates to inform model parameters. There is limited evidence on the costs and health state utilities associated with long-term complications of HDFN in babies born by alloimmunized RhD− pregnant people. Therefore, for the "baby with major developmental problems" health state, we used cost and utility data from Ontario-based studies in similar populations where possible. The utility parameter assigned for major developmental problems in general is similar to the one used in other modeling studies (e.g., Pilgrim et al, 2009,[68] Saramago et al, 2018[37]). Furthermore, we included people with cerebral palsy as part of this most severe state assuming that people with cerebral palsy may have the most severe form of this disease. In their study, Young et al[87] showed that utilities of people with cerebral palsy may range between 0.08 (0.25) and 0.67 (0.32), depending on the severity of their disease; this was tested in our scenario analyses.

Scenario: Modeling Subsequent Pregnancies in the Nonalloimmunized Population

In these scenarios, we firstly approximated the cost-effectiveness of the intervention for two pregnancies over the 10-year time horizon. We considered a population of RhD− individuals whose first nonalloimmunized pregnancy was followed by either a nonalloimmunized or alloimmunized pregnancy. We assumed the probability of alloimmunization to be 0.003 in one analysis (Moise et al[57]) and 0.016 in another analysis (Fung Kee Fung et al[11]), and the chance of the second pregnancy was assumed to be 0.62 (Johnson et al,[6] Yang et al[39]). The mean incremental costs and benefits were based on data determined in our two reference case analyses. Next, we included additional scenario analyses with two pregnancies, one using a lower cost for the genotyping test in nonalloimmunized pregnancies, and another using the lifetime time horizon.

We used the following steps to estimate the overall ICER across two pregnancies:

- First pregnancy
    - In our reference case analyses, we determined the mean incremental costs and mean incremental QALYs for the first nonalloimmunized or alloimmunized pregnancy, denoted as:

        ΔC1_nonallo and ΔC1_allo

        ΔE1_nonallo and ΔE1_allo
- Second pregnancy
    - The next pregnancy could be either nonalloimmunized or alloimmunized based on propagation of alloimmunization from the first pregnancy
    - We accounted for the probability of alloimmunization, denoted as p_allo, and for the chance of second pregnancy, denoted as p_next_pregnancy
    - We estimated the mean incremental costs (ΔC2), mean incremental QALYs (ΔE2) for the second pregnancy, as follows:

        $\Delta C2 = p\_next\_pregnancy * (\Delta C1\_nonallo * (1 - p\_allo) + \Delta C1\_allo * p\_allo)$

        $\Delta E2 = p\_next\_pregnancy * (\Delta E1\_nonallo * (1 - p\_allo) + \Delta E1\_allo * p\_allo)$

- Overall incremental costs and effects and the ICER were calculated as follows:

  $\Delta C\_overall = \Delta C1\_nonallo + \Delta C2$; $\Delta E\_overall = \Delta E1\_nonallo + \Delta E2$

  $ICER = \Delta C\_overall \div \Delta E\_overall$

Results

Our economic evaluation estimated the cost-effectiveness of the use of noninvasive fetal RhD genotyping compared with usual care for two different RhD− populations. Usual care is defined as universal RhIG prophylaxis for all nonalloimmunized pregnancies and universal intensive and complex monitoring and care for alloimmunized pregnancies. Tables 23a and 23b present the results of our cost-effectiveness and cost–utility analyses for a cohort of RhD− individuals with nonalloimmunized pregnancies. Tables 24a and 24b present the results of our cost-effectiveness and cost–utility analyses for a cohort of RhD− individuals with alloimmunized pregnancies.

Reference Case Analysis

Nonalloimmunized Pregnancies

In nonalloimmunized pregnancies, compared with usual care noninvasive fetal RhD genotyping was associated with a slightly higher probability of maternal alloimmunization, fewer RhIG injections, and similar probability of having a live baby with no developmental problems (Table 23a).

For the intervention strategy, after excluding the cost of genotyping ($247), we estimated that short-term direct medical costs associated with pregnancy were lower (about $8,718) than for usual care ($8,812). Long-term costs were similar for both strategies (about $980).

Table 23a: Health Outcomes in Nonalloimmunized Pregnancies: Usual Care and Noninvasive Fetal RhD Genotyping

Strategy	Outcomes[a]		
	Probability of Maternal Alloimmunization, Mean (95% CrI)	Number of RhIG Injections, Mean (95% CrI)	Probability of Having a Live Baby,[b] Mean (95% CrI)
Usual care	0.00205 (0.0018–0.0023)	1.7951 (1.7915–1.7987)	0.9471 (0.9454–0.9485)
Noninvasive fetal RhD genotyping	0.00220 (0.0019–0.0025)	1.4273 (1.4208–1.4342)	0.9472 (0.9459–0.9483)

Abbreviations: CrI, credible interval; RhD, rhesus D blood group; RhIG, Rh immunoglobulin.
[a]All outcomes estimated per person (per one pregnancy).
[b]This is a baby with no developmental issues.

Applying the incremental changes in the health outcomes and incremental cost ($154.08, Table 23b), we found that, compared with usual care, noninvasive fetal RhD genotyping would cost an additional $416 per RhIG injection avoided (ICER [incremental costs ÷ incremental effect]: $154.08/−0.36777).

As shown in Table 23b, over a 10-year time horizon, noninvasive fetal RhD genotyping for nonalloimmunized RhD− pregnancies was not cost-effective at willingness-to-pay values of $50,000 or $100,000 per QALY gained.

Table 23b: Cost–Utility Analysis, Nonalloimmunized Pregnancies: Usual Care and Noninvasive Fetal RhD Genotyping

Strategy	Mean Costs, $[a] (95% CrI)	Mean QALYs (95% CrI)	Incremental Costs,[b] $ Mean (95% CrI)	Incremental QALYs[c] Mean (95% CrI)	ICER: $/QALY Gained
Usual care	9,792.15 (9,778; 9,804)	7.1344 (7.1223; 7.1453)			
Noninvasive fetal RhD genotyping	9,946.23 (9,934; 9,957)	7.1351 (7.1258; 7.1444)	154.08 (139; 169)	0.00068 (−0.01328; 0.01459)	227,354

Abbreviations: CrI, credible interval; ICER, incremental cost-effectiveness ratio; QALY, quality-adjusted life-year; RhD, rhesus D blood group.
[a]All costs are in 2019 Canadian dollars. All costs and effects were discounted at 1.5%.
[b]Incremental cost (per person) = mean cost (intervention) − mean cost (usual care).
[c]Incremental effect (per person) = mean effect (intervention) − mean effect (usual care).
Note: Results may appear incorrect due to rounding.

Figures 7a and 7b represent the uncertainty around the estimated ICER generated in the probabilistic sensitivity analysis. Figure 7a shows a spread of the simulated ICERs across the cost-effectiveness plane and uncertainty around the ICER estimate.

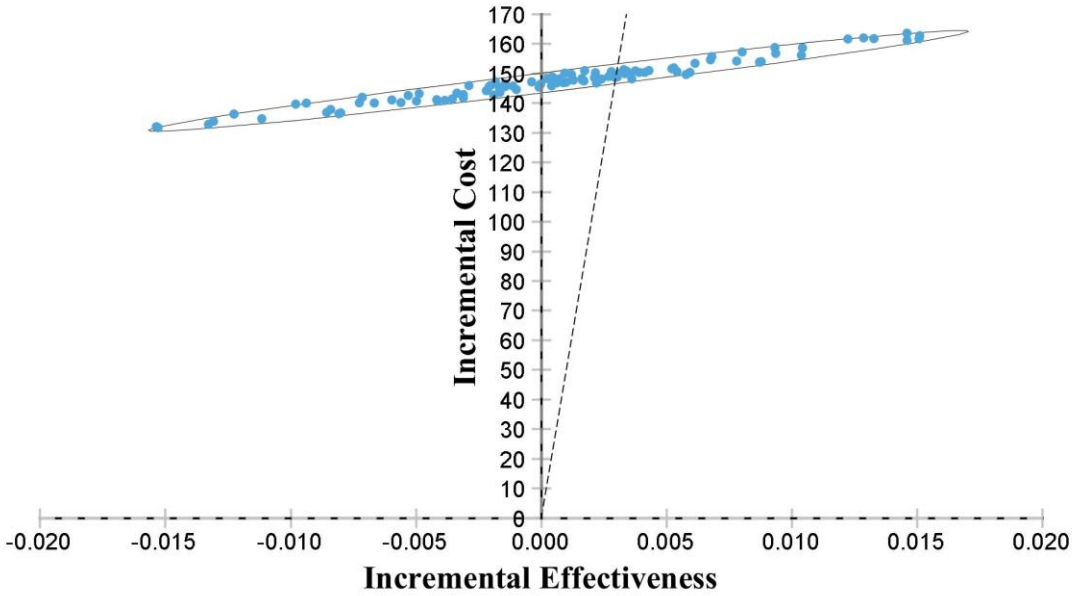

Figure 7a: Scatter Plots of Simulated Pairs of Incremental Costs and Effects in the Cost-Effectiveness Plane: Noninvasive Fetal RhD Genotyping vs. Usual Care

Note: Effectiveness is expressed in quality-adjusted life years (QALYs). Negative QALYs indicate that the intervention was associated with worse quality-adjusted survival. The diagonal dashed line that crosses the origin indicates a willingness-to-pay of $50,000 per QALY gained. A 95% confidence ellipse covers 95% of the estimated joint density and was used to represent uncertainty around the incremental cost-effectiveness ratio estimated in the probabilistic sensitivity analysis.

Figure 7b presents the probability of cost-effectiveness of noninvasive fetal RhD genotyping versus usual care over 10 years. This probability was 33% at a willingness-to-pay of $50,000 per QALY gained, reaching 47% at a willingness-to-pay of $100,000 per QALY gained. This means that the cost-effectiveness of noninvasive fetal RhD genotyping was uncertain.[107]

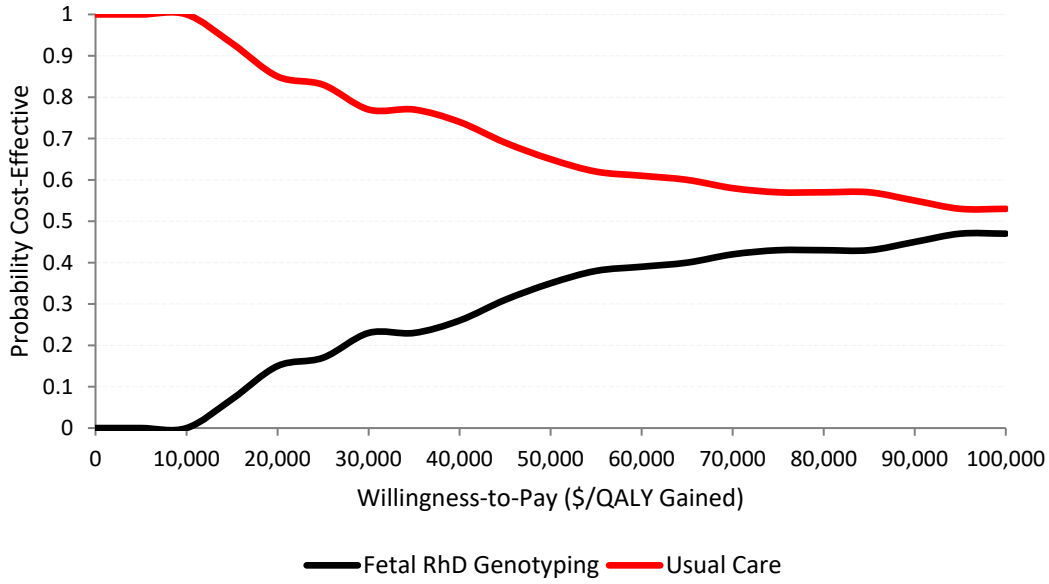

Figure 7b: Cost-Effectiveness Acceptability Curve, Nonalloimmunized Pregnancies: Noninvasive Fetal RhD Genotyping vs. Usual Care

Abbreviation: QALY, quality-adjusted life-year.

Alloimmunized Pregnancies

In alloimmunized pregnancies, noninvasive fetal RhD genotyping was associated with lower resource use during the pregnancy (lower probability of hospitalizations with IUTs or use of NICU) and better clinical outcomes compared with usual care (Table 24a).

In the intervention strategy, after excluding the cost of genotyping (about $328), we estimated short-term direct medical costs associated with an alloimmunized pregnancy to be about $23,123 and long-term costs to be about $1,402. As expected, in usual care, the short-term costs were about 1.28 times higher, about $29,597, and the long-term costs were about $1,537.

Table 24a: Health Outcomes in Alloimmunized Pregnancies: Usual Care and Noninvasive Fetal RhD Genotyping

	Outcomes[a]			
Strategy	Probability of Hospitalization With IUTs, Mean (95% CrI)	Probability of NICU Hospitalization, Mean (95% CrI)	Probability of Having a Live Baby, Mean (95% CrI)	Probability of Having a Baby With HDFN Mean (95% CrI)
Usual care	0.0479 (0.0467–0.0490)	0.0013 (0.0011–0.0016)	0.9295 (0.9278-0.9309)	0.0812 (0.0799-0.0830)
Noninvasive fetal RhD genotyping	0.0241 (0.0233–0.0250)	0.0007 (0.0005–0.0009)	0.9537 (0.9523-0.9552)	0.0597 (0.0584-0.0615)

Abbreviations: CrI, credible interval; HDFN, hemolytic disease of fetus and newborn, IUT, intrauterine transfusion; NICU, neonatal intensive care unit with aggressive treatment and exchange transfusions for babies with very severe HDFN; RhD, rhesus D blood group.

[a]All outcomes estimated per person (per one pregnancy).

Note: Results may appear incorrect due to rounding.

As shown in Table 24b, over a 10-year time horizon, noninvasive fetal RhD genotyping was cost saving for alloimmunized pregnancies. Compared with usual care, the intervention was dominant compared with usual care (lower costs of about $6,280 and increased QALYs of about 0.19).

Table 24b: Cost–Utility Analysis, Alloimmunized Pregnancies: Usual Care and Noninvasive Fetal RhD Genotyping

Strategy	Mean Costs, $[a] (95% CrI)	Mean QALYs (95% CrI)	Incremental Costs,[b] $, Mean (95% CrI)	Incremental QALYs,[c] Mean (95% CrI)	ICER: $/QALY Gained
Usual care	31,133.54 (31,088; 31,170)	6.9851 (6.972; 6.996)			
Noninvasive fetal RhD genotyping	24,853.14 (24,821; 24,890)	7.1706 (7.160; 7.182)	−6280.40 (−6,325; −6,229)	0.1855 (0.1711; 0.2009)	Dominant

Abbreviations: CrI, credible interval; ICER, incremental cost-effectiveness ratio; QALY, quality-adjusted life-year; RhD, rhesus D blood group.

[a]All costs are in 2019 Canadian dollars. All costs and effects were discounted at 1.5%.

[b]Incremental cost = mean cost (intervention) − mean cost (usual care).

[c]Incremental effect = mean effect (intervention) − mean effect (usual care).

Note: Results may appear incorrect due to rounding. The intervention strategy is considered dominant (or cost saving) when it is associated with lower costs and better effects compared with usual care. Negative costs indicate savings.

Figure 8 shows that noninvasive fetal RhD genotyping was found to be dominant in alloimmunized pregnancies. This means it was highly likely[107] for this intervention to be cost-effective compared with usual care for any willingness-to-pay value.

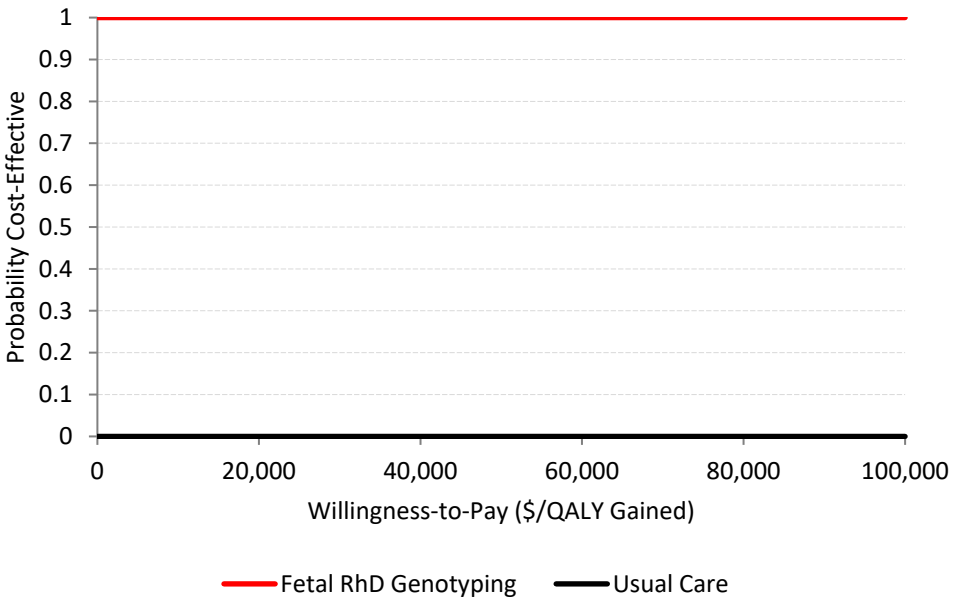

Figure 8: Cost-Effectiveness Acceptability Curve, Alloimmunized Pregnancies: Noninvasive Fetal RhD Genotyping vs. Usual Care

Abbreviation: QALY, quality-adjusted life-year.

Sensitivity Analysis

We analyzed more than 14 different scenarios to examine the parameter and structural model uncertainty. The ICER and incremental net benefit (INB) estimates for all scenarios are presented in Appendix 8, Table A10.

Nonalloimmunized Pregnancies

In nonalloimmunized pregnancies, three factors changed the cost-effectiveness of noninvasive fetal RhD blood group genotyping: the per-sample testing cost, inclusion of paternal screening, and the time horizon.

Our threshold analysis on the cost of testing found that, at a per-sample cost of $88 or less, the intervention strategy would be cost neutral or cost saving. In a probabilistic scenario assuming a cost of $66 ± $16.50, noninvasive fetal RhD genotyping was cost-effective at willingness-to-pay of $50,000 per QALY gained (incremental net benefit > 0); however, uncertainty in this result remained relatively large, with the probability of cost-effectiveness being 57%.

Next, when we assumed the use of paternal RhD screening in both strategies, noninvasive fetal RhD genotyping dominated usual care and was associated with lower costs and greater incremental effects. Noninvasive fetal RhD genotyping was moderately likely to be cost-effective compared with usual care, with a probability of cost-effectiveness of 63% at a willingness-to-pay of $50,000 per QALY (see Appendix 8, Table A10; cost-effectiveness acceptability curve figure not shown).

Also, when we modeled noninvasive fetal RhD genotyping versus usual care over a lifetime time horizon, the ICER was below $50,000 per QALY gained; based on the cost-effectiveness

acceptability curve, uncertainty about cost-effectiveness remained (the probability of the intervention being cost-effective was 57% at a willingness-to-pay of $100,000 per QALY gained).

Our last scenarios addressed a structural assumption of the model by examining the cost-effectiveness of noninvasive fetal RhD genotyping for two pregnancies over 10-year time horizon in initially nonalloimmunized RhD− pregnant people. After accounting for alloimmunization during the first pregnancy, we estimated that noninvasive fetal RhD genotyping versus usual care would be associated with mean incremental costs of about $237 and mean incremental effects of about 0.0014, yielding an ICER over $164,000 per QALY gained. However, if the cost of the testing for nonalloimmunized populations was $66 (± $16.50), noninvasive fetal RhD genotyping was less costly (−$56) and similarly effective compared with usual care (0.0014 QALYs).

Alloimmunized Pregnancies

In alloimmunized pregnancies, all results of the scenario analyses were consistent with our reference case findings: noninvasive fetal RhD genotyping was associated with lower costs and greater QALYs than usual care.

Discussion

We conducted a full economic evaluation to determine the cost-effectiveness of noninvasive fetal RhD blood group genotyping versus usual care for the management of nonalloimmunized or alloimmunized RhD− pregnancies in Ontario.

In the reference case analysis that simulated the course of one nonalloimmunized pregnancy over 10 years, we found a slightly higher probability of maternal alloimmunization in the intervention strategy (possibly resulting, for example, from false-negative results), but noninvasive fetal RhD genotyping was associated with fewer RhIG injections (ICER: $416 per one RhIG injection avoided). Our cost–utility analysis showed that, compared with usual care (universal RhIG prophylaxis), noninvasive fetal RhD genotyping (guiding targeted RhIG prophylaxis) was more costly (incremental mean cost: $154, 95% CrI: $139 to $169) and similarly effective (0.0007 QALYs, 95% CrI: −0.01 to 0.01). This very small difference in QALYs could be due to chance alone. Therefore, focusing solely on this ICER estimate may not be as meaningful for decision-making. In probabilistic scenario analyses, we further showed that noninvasive fetal RhD genotyping could be more cost-effective than usual care if the per-sample testing cost were about three to four times lower than the reference case cost of about $247. Lastly, noninvasive fetal RhD genotyping was associated with smaller costs and slightly larger effects than usual care when paternal RhD screening was included.

In the reference case analysis that simulated the course of an alloimmunized pregnancy over 10 years, we found noninvasive fetal RhD genotyping was associated with fewer hospitalizations involving IUTs and NICU admissions. In our cost–utility analysis, noninvasive fetal RhD genotyping was associated with lower mean costs (−$6,280, 95% CrI: −$6,325 to −$6,229) and increased mean QALYs (0.19, 95% CrI: 0.17 to 0.20), compared with usual care. We found this intervention was highly likely to be cost-effective over commonly used willingness-to-pay values in all scenario analyses.

With respect to nonalloimmunized pregnancies, our findings are consistent with the findings of our clinical evidence review and with published economic studies that indicate noninvasive fetal RhD genotyping would prevent unnecessary use of RhIG[62] and that paternal screening combined with this intervention may be a more favourable option in terms of cost savings.[61] Our results also

agree with findings of the UK health technology assessment showing the intervention could be cost-effective over a lifetime horizon.[37] Introducing this genotyping test in Ontario may be considered valuable because it could enable a better, more targeted approach to RhIG prophylaxis and thus reduce the use of RhIG and alleviate the potential future shortage of this blood product.[6]

Strengths and Limitations

Our modeling study provided new insights and filled a gap in the literature regarding the long-term benefits and costs of noninvasive fetal RhD genotyping for both nonalloimmunized and alloimmunized RhD− pregnancies in Ontario. As with any modeling study, our analyses are limited by parameter and structural model assumptions.

For example, the diagnostic test accuracy (i.e., sensitivity and specificity) was assumed to be the same for two target populations and not dependent on ethnicity or race (given that our clinical evidence review did not find evidence to suggest such differences). The false-negative rate for this genotyping test is relatively small, and our modeling approaches incorporated current local clinical treatment pathways that assist in a precautionary approach in the presence of clinical suspicion and fast detection of a possible misclassification. This ensures appropriate management and care of nonalloimmunized or alloimmunized pregnant people.

Next, there is very limited evidence on the health state utility associated with the most severe long-term complications of HDFN—major developmental problems. In the reference case, we mostly used health state utilities as determined in the health technology assessments by NICE.[37,68] Some of these utilities were based on Ontario cohorts. In addition, we considered utilities associated with cerebral palsy, elicited in an Ontario cohort.[87] These might be considered worst-case values as there is a spectrum of utilities associated with differences in the severity of cerebral palsy[87] and in how people with this condition experience their health-related quality of life.[86] Consequently, we conducted several scenario analyses with increased health state utility values, but noninvasive fetal RhD genotyping remained highly cost-effective in alloimmunized populations.

Also, we used the most recent long-term findings of the LOTUS study to estimate the proportion of neonates who would have long-term HDFN complications after receiving IUT.[93] It is possible that we overestimated the probability of long-term complications (4.8%), although this estimate is similar to the probability (5%) used in the 2018 NICE study by Saramago et al.[37] As expected, our model for alloimmunized pregnancies estimated an extremely small number of people transitioning to the health state of major developmental problems. Cost-effectiveness results remained robust in our scenario analysis when we used a lower rate of HDFN complications.

Next, the costs associated with the health state of major developmental problems due to HDFN were based on the most recent Ontario data.[105,106] We assumed that these costs, which were estimated for people with intellectual and developmental disabilities or with cerebral palsy, could correspond to total health care expenditures for people with major developmental problems due to HDFN. It is possible that we overestimated these expenditures. Consequently, we conducted probabilistic scenario analyses with lower treatment costs for major developmental problems to examine changes in the cost-effectiveness findings and confirmed the conclusions of our reference case analysis.

Furthermore, about 17% of pregnant people in Ontario receive their prenatal care from midwives.[94] Current regulations allow midwives to order and administer RhIG and provide care

for nonalloimmunized RhD− pregnancies, but regulations with respect to genetic testing are not clear.[111] As a result, we did not conduct a formal costing analysis examining the course of pregnancy care by various types of health care professionals. However, if publicly funded, noninvasive fetal RhD genotyping in nonalloimmunized RhD− pregnancies could be made available to all health care professionals who provide pregnancy care in Ontario.

Lastly, using our reference case closed-cohort Markov models, we were unable to accurately estimate long-term outcomes of mothers and babies over multiple pregnancies. Therefore, we developed two models and separated the outcomes of nonalloimmunized and alloimmunized pregnancies. However, we approximated the cost-effectiveness of noninvasive fetal RhD genotyping for two pregnancies in a cohort of RhD− individuals with a first nonalloimmunized pregnancy, by applying the incremental effects and costs of our two cost–utility analyses. In this scenario, given a small rate of alloimmunization that affects the second pregnancy, the cost-effectiveness of noninvasive fetal RhD genotyping remained uncertain in this population (estimated ICERs > $164,000/QALY over a 10-year time horizon). Finally, we conducted an analysis assuming a lower threshold cost for the genotyping test in nonalloimmunized pregnancies ($66): over a
10-year time horizon, we showed that noninvasive fetal RhD genotyping was less costly and similarly effective compared with usual care.

Generalizability

The findings of our economic analysis are generalizable to nonalloimmunized and alloimmunized RhD− pregnancies in Ontario. These populations are eligible for either universal prophylaxis with RhIG (current clinical care for nonalloimmunized pregnancies) or intensive monitoring and management of alloimmunization and potential complications of HDFN (current clinical care for alloimmunized pregnancies).

Conclusions

For the management of nonalloimmunized RhD− pregnancies, noninvasive fetal RhD blood group genotyping would generally not be considered a cost-effective strategy, compared to usual care, unless the cost of testing is much lower than what is currently proposed. For the management of alloimmunized RhD− pregnancies, noninvasive fetal RhD blood group genotyping is cost saving.

BUDGET IMPACT ANALYSIS

Research Questions

1. What is the potential 5-year budget impact for the Ontario Ministry of Health of publicly funding noninvasive fetal RhD blood group genotyping for nonalloimmunized RhD negative (RhD−) pregnancies?
2. What is the potential 5-year budget impact for the Ontario Ministry of Health of publicly funding noninvasive fetal RhD blood group genotyping for alloimmunized RhD− pregnancies?

Methods

Analytic Framework

We estimated the budget impact of publicly funding noninvasive fetal RhD blood group genotyping using the cost difference between two scenarios: (1) current clinical practice without public funding for this test (the current scenario) and (2) anticipated clinical practice with public funding for this test (the new scenario). Figure 9 presents the budget impact model schematic.

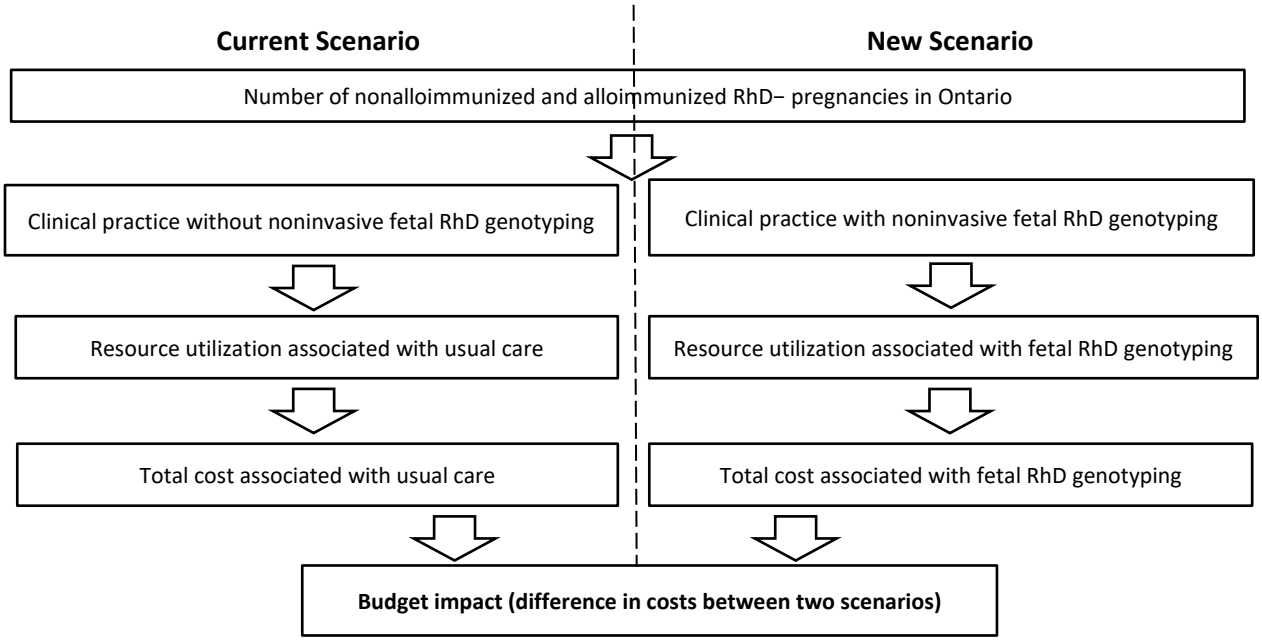

Figure 9: Budget Impact Model Schematic

We conducted a reference case analysis and several sensitivity analyses. Our reference case analysis represents the analysis with the most likely set of input parameters and model assumptions. Our sensitivity analyses explored how the results are affected by varying input parameters and model assumptions. We used cost outputs from our cost-effectiveness models to estimate the budget impact in the two target populations.

Key Assumptions

The assumptions used in our cost-effectiveness analysis also apply to this budget impact analysis. In addition, we considered the following:

- Uptake of the new intervention is expected to be high, similar to the high adherence with universal prophylaxis with Rh immunoglobulin (RhIG) currently provided as standard of care
- Only a small percentage of alloimmunized pregnancies in Ontario are currently tested with noninvasive fetal RhD genotyping, outsourced to a UK laboratory via the Ontario Ministry of Health Out-of-Country Prior Approval Program; therefore, we assumed no use of this test in the current scenario for both populations
- Start-up and implementation costs such as training, laboratory renovation, and credentialing were not included

Target Population

The population of interest are pregnant people with serologically confirmed RhD− blood type, who could be either nonalloimmunized or alloimmunized to RhD antigen.

Annually about 15% of all Caucasian pregnancies are RhD− in Ontario or Canada.[6] Based on blood donor data from the Canadian Blood Services (CBS), an RhD− pregnancy occurs in about 17.9% in Aboriginal Canadians, 8.9% in Black Canadians, and 4.2% in Asian and South-Asian Canadians, with an overall estimate for the whole blood donor population of about 26% (written communication, G Clarke, MD; data estimated from CBS blood donor data, July 2019).

Due to the lack of data on Ontario pregnancies by ethnicity, we assumed an overall percentage of RhD− pregnancies of about 15% for Ontario for the reference case analysis.[6]

Based on the literature data, maternal alloimmunization may occur in 1% to 2% of RhD− pregnant people in Canada.[11] In Ontario, the annual rate of Rh alloimmunization is 1.3%.[112]

We used these rates to calculate the number of people with nonalloimmunized and alloimmunized pregnancies who might be eligible for noninvasive fetal RhD genotyping over the next 5 years. (Table 25)

Table 25: Number of RhD− Pregnant People Eligible for Noninvasive Fetal RhD Genotyping in Ontario, 2019 to 2023

Year	Estimated No. of Live Births in Ontario[a]	No. of Nonalloimmunized RhD− Pregnancies[b]	No. of Alloimmunized Pregnancies[c]
2019	139,999	21,000	1,820
2020	141,399	21,210	1,838
2021	142,813	21,422	1,857
2022	144,241	21,636	1,875
2023	145,684	21,853	1,894

Abbreviations: No., number; RhD, rhesus D blood group.
[a]Based on 2017 Statistics Canada data and assuming 1% annual growth in population.
[b]15% of all live births in Ontario.
[c]Assuming 1.3% of pregnant people have been alloimmunized with Rh antigens (i.e., RhD alone, RHCE [c, C, and E alleles], KEL, and RhDc [Asian variant]).

Current Intervention Mix

As mentioned (see Key Assumptions), we assumed no use of noninvasive fetal RhD genotyping in the current scenario.

Uptake of Noninvasive Fetal RhD Blood Group Genotyping

We assumed the uptake of noninvasive fetal RhD genotyping would be 80% in year 1 and increase by 5% each year, reaching 100% in year 5. We made this conservative assumption of achieving full access over 5 years, given that this test could prevent unnecessary use of RhIG prophylaxis in nonalloimmunized RhD compatible pregnancies and reduce current health care spending in alloimmunized pregnancies. Also, our clinical review indicated a high uptake rate and similarly high rates for neonatal screening in general have been achieved for some Ontario regions.[94] Tables 26a and 26b present the number of people estimated to receive usual care or noninvasive fetal RhD genotyping over the next 5 years.

Table 26a: Total Volumes (Years 1–5), Nonalloimmunized Pregnancies: Reference Case Analysis

	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Current Scenario: Nonalloimmunized Pregnancies						
Usual care	21,000	21,210	21,422	21,636	21,853	107,120
Fetal RhD genotyping	0	0	0	0	0	0
Total volume	**21,000**	**21,210**	**21,422**	**21,636**	**21,853**	**107,120**
Future Scenario[a]: Nonalloimmunized Pregnancies						
Usual care	4,200	3,181	2,142	1,082	0	10,605
Fetal RhD genotyping	16,800	18,028	19,280	20,554	21,853	96,515
Total volume	**21,000**	**21,210**	**21,422**	**21,636**	**21,853**	**107,120**

Abbreviation: RhD, rhesus D blood group.
[a]Uptake increases by 5% each year, from 80% in year 1 to reach full access in year 5 (future scenario).

Table 26b: Total Volumes (Years 1–5), Alloimmunized Pregnancies: Reference Case Analysis

	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Current Scenario: Alloimmunized Pregnancies						
Usual care	1,820	1,838	1,857	1,875	1,894	9,284
Fetal RhD genotyping	0	0	0	0	0	0
Total volume	**1,820**	**1,838**	**1,857**	**1,875**	**1,894**	**9,284**
Future Scenario[a]: Alloimmunized Pregnancies						
Usual care	364	276	186	94	0	920
Fetal RhD genotyping	1,456	1,562	1,671	1,781	1,894	8,364
Total volume	**1,820**	**1,838**	**1,857**	**1,875**	**1,894**	**9,284**

Abbreviation: RhD, rhesus D blood group.
[a]Uptake increases by 5% each year, from 80% in year 1 to reach full access in year 5 (future scenario).

Resource Use and Costs

We used inputs on health care resource use and costs from our cost-effectiveness analyses, applying them for a period of one year. We estimated per-case costs associated with noninvasive fetal RhD genotyping (i.e., testing costs), short-term costs incurred during the pregnancy, and long-term costs incurred by a newborn after delivery and until the end of the year. Table 27 presents total annual costs of usual care and of care that includes noninvasive fetal RhD genotyping for both populations. We used these costs in our budget impact calculations. All costs are reported in 2019 Canadian dollars.

Table 27: Annual Per-Case Costs Used in Budget Impact Calculations: Reference Case Analysis

	Nonalloimmunized Pregnancies, $[a]		Alloimmunized Pregnancies, $[a]	
	Fetal RhD Genotyping	Usual Care	Fetal RhD Genotyping	Usual Care
Testing costs	247.34	0	328.15	0
Short-term costs	8,804.04	8,897.73	23,298.99	29,786.09
Long-term costs	42.46	42.45	57.98	66.25
Total costs	**9,093.85**	**8,940.18**	**23,685.12**	29,852.34

Note: Results may appear inexact due to rounding.
[a]All costs are in 2019 Canadian dollars.

Internal Validation

The secondary health economist conducted formal internal validation. This process included checking for errors and ensuring the accuracy of parameter inputs and equations in the budget impact analysis.

Analysis

In the reference case analysis, we estimated the 5-year budget impact of noninvasive fetal RhD genotyping for nonalloimmunized or alloimmunized RhD− pregnancies. For each population, we calculated total costs and budget impact by applying the cost estimates from Table 27 to our target population volumes (Tables 26a and 26b).

We conducted sensitivity analyses to examine the influence of the following factors on the budget impact of the reference case analysis:

- **Scenario 1.** Smaller gradual uptake rates for the intervention, in both target populations. In this scenario we assumed a substantially smaller uptake, starting at 3% and an increasing by 3% each year, to 15% in year 5 (Appendix 9, Tables A11 and A12). We also tested the impact of initial uptake starting at 15% and increasing annually by 5%, to 35% in year 5 (Appendix 9, Tables A13 and A14). We commonly use these lower uptake rates in assessing novel technologies in Ontario, and they are also supported by the variability in uptake of neonatal screening across Ontario[94]
- **Scenario 2.** Change in the cost of the intervention
    - Lower per-sample cost of $125 for noninvasive fetal RhD genotyping for the nonalloimmunized population in Ontario (see Table 22)

    - Higher per-sample costs of noninvasive fetal RhD genotyping for both populations, if the test continued to be outsourced to the Bristol, UK, laboratory via the Out-of-Country Prior Approval Program (see Table 22 for the range of this cost). As in the reference case, we assumed no uptake of the intervention in the current scenario
- **Scenario 3.** Inclusion of paternal RhD screening in the clinical pathways
- **Scenario 4.** A larger population eligible for noninvasive fetal RhD genotyping (Table 28). We estimated the budget impact of making the test available for RhD− pregnancies across Canada, assuming that one reference laboratory would conduct screening for nonalloimmunized pregnancies. For this large sample, we also tested lower uptake, starting at 15% of pregnancies in year 1 and increasing by 5% per year, to 35% uptake in year 5

The reference-case uptake rate (80% increasing to 100%) was changed only in the first scenario analysis and in the secondary analysis of scenario 4.

Table 28: Number of RhD− Pregnant People Eligible for Noninvasive Fetal RhD Genotyping in Canada, 2019 to 2023

Year	Estimated No. of Live Births in Canada[a]	No. of Nonalloimmunized RhD− Pregnancies[b]	No. of Alloimmunized Pregnancies[c]
2019	376,291	56,444	4,892
2020	380,054	57,008	4,941
2021	383,854	57,578	4,990
2022	387,693	58,154	5,040
2023	391,570	58,736	5,090

Abbreviation: RhD, rhesus D blood group.
[a]Based on 2017 Statistics Canada data and assuming 1% annual growth in population.
[b]15% of all live births in Canada.
[c]Assuming 1.3% of pregnant people have been alloimmunized with Rh antigens (i.e., RhD alone, RHCE [c, C, and E alleles], KEL, and RhDc [Asian variant]).

Results

Reference Case

Nonalloimmunized Pregnancies

Table 29 presents the budget impact of publicly funding noninvasive fetal RhD blood group genotyping for nonalloimmunized pregnancies. Adopting noninvasive fetal RhD genotyping to guide RhIG prophylaxis for nonalloimmunized pregnancies at a high uptake of 80% in year 1, increasing to 100% in year 5, would lead to cost increases of about $2.6 million in year 1 to about $3.4 million in year 5. The total 5-year budget impact was about $14.8 million.

The increase in costs with the use of fetal RhD genotyping is offset by savings from the resulting reduction in other short-term costs during pregnancy. As shown in Table 29, the cost of testing accounts for most of the estimated budget impact. This cost ranged from $4.2 million in year 1 to $5.4 million in year 5, leading to a total 5-year cost of $23.9 million.

Table 29: Budget Impact Analysis Results—Reference Case, Nonalloimmunized Pregnancies

	Total Costs and Budget Impact, $ Million[a]					
	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Current Scenario						
Total costs	**187.74**	**189.62**	**191.52**	**193.43**	**195.37**	**957.68**
Cost of genotyping	0.00	0.00	0.00	0.00	0.00	0.00
Short-term costs	186.85	188.72	190.61	192.51	194.44	953.13
Long-term costs	0.89	0.90	0.91	0.92	0.93	4.55
Future Scenario						
Total costs	**190.32**	**192.39**	**194.48**	**196.59**	**198.72**	**972.51**
Cost of genotyping	4.16	4.46	4.77	5.08	5.41	23.87
Short-term costs	185.28	187.03	188.80	190.59	192.39	944.09
Long-term costs	0.89	0.90	0.91	0.92	0.93	4.55
Budget Impact						
Total budget impact (BI)	**2.58**	**2.77**	**2.96**	**3.16**	**3.36**	**14.83**
BI: Cost of genotyping	4.16	4.46	4.77	5.08	5.41	23.87
BI: Short-term costs	−1.57	−1.69	−1.81	−1.93	−2.05	−9.04
BI: Long-term costs	0.0002	0.0002	0.0002	0.0002	0.0003	0.0012

Note: Results may appear inexact due to rounding. Negative costs indicate savings.
[a]All costs are in 2019 Canadian dollars.

Alloimmunized Pregnancies

Table 30 presents the budget impact of publicly funding noninvasive fetal RhD genotyping for alloimmunized pregnancies. In this population, adopting noninvasive fetal RhD genotyping would lead to net cost savings of about $51.5 million over 5 years, due to a reduction in health care resource use during pregnancy and after the birth. The cost of testing ranged from about $0.5 million in year 1 to about $0.6 million in year 5, when full access to fetal RhD genotyping is achieved. The total budget impact associated with the cost of this test was about $2.7 million over the next 5 years.

Table 30: Budget Impact Analysis Results—Reference Case, Alloimmunized Pregnancies

	Total Costs and Budget Impact, $ Million[a]					
	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Current Scenario						
Total costs	**54.30**	**54.84**	**55.41**	**55.94**	**56.51**	**277.00**
Cost of testing	0.00	0.00	0.00	0.00	0.00	0.00
Short-term costs	54.18	54.72	55.28	55.82	56.39	276.39
Long-term costs	0.12	0.12	0.12	0.12	0.12	0.61
Future Scenario						
Total costs	**45.33**	**45.22**	**45.11**	**44.97**	**44.84**	**225.47**
Cost of testing	0.48	0.51	0.55	0.58	0.62	2.74
Short-term costs	44.75	44.60	44.45	44.28	44.11	222.18
Long-term costs	0.11	0.11	0.11	0.11	0.11	0.55
Budget Impact						
Total budget impact (BI)	**−8.97**	**−9.62**	**−10.29**	**−10.97**	**−11.67**	**−51.52**
BI: Cost of testing	0.48	0.51	0.55	0.58	0.62	2.74
BI: Short-term costs	−9.44	−10.12	−10.83	−11.54	−12.27	−54.21
BI: Long-term costs	−0.01	−0.01	−0.01	−0.01	−0.01	−0.06

Note: Results may appear inexact due to rounding. Negative costs indicate savings.
[a]All costs are in 2019 Canadian dollars.

Sensitivity Analysis

Nonalloimmunized Pregnancies

Table 31 presents findings for the scenario analyses for nonalloimmunized pregnancies. For all scenarios, we show changes in the total costs and in the cost of testing, the most important cost item.

As expected, the 5-year budget impact was smaller if either the uptake of noninvasive fetal RhD genotyping or the per-sample cost of testing was lower (Scenarios 1a, 1b, and 2a). Inclusion of paternal screening in both the intervention and usual care strategies resulted in cost savings (Scenario 3). The 5-year budget impact increased with higher test costs, assuming noninvasive fetal RhD genotyping would be provided through the Out-of-Country Prior Approval Program (Scenarios 2b and 2c).

Table 31: Budget Impact Analysis Results—Sensitivity Analysis, Nonalloimmunized Pregnancies

	Budget Impact, $ Million[a]					
	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Reference Case						
Budget impact	**2.58**	**2.77**	**2.96**	**3.16**	**3.36**	**14.83**
Budget impact: cost of testing[b]	4.16	4.46	4.77	5.08	5.41	23.87
Scenario 1a: Uptake of Fetal RhD Genotyping: Increment of 3% per Year (Year 1, 3%; Year 5, 15%)						
Current scenario: total costs	187.74	189.62	191.52	193.43	195.37	957.68
Current scenario: cost of testing[b]	0.00	0.00	0.00	0.00	0.00	0.00
Future scenario: total costs	187.84	189.82	191.81	193.83	195.87	959.17
Future scenario: cost of testing[b]	0.16	0.31	0.48	0.64	0.81	2.40
Budget impact	**0.10**	**0.20**	**0.30**	**0.40**	**0.50**	**1.49**
Scenario 1b: Uptake of Fetal RhD Genotyping: Increment of 5% per Year (Year 1, 15%; Year 5, 35%)						
Current scenario: total costs	187.74	189.62	191.52	193.43	195.37	957.68
Current scenario: cost of testing[b]	0.00	0.00	0.00	0.00	0.00	0.00
Future scenario: total costs	188.23	190.27	192.34	194.43	196.54	961.81
Future scenario: cost of testing[b]	0.78	1.05	1.32	1.61	1.89	6.65
Budget impact	**0.48**	**0.65**	**0.82**	**1.00**	**1.18**	**4.13**
Scenario 2a: Lower Cost of Fetal RhD Genotyping ($125 per sample)						
Current scenario: total costs	187.74	189.62	191.52	193.43	195.37	957.68
Current scenario: cost of testing[b]	0.00	0.00	0.00	0.00	0.00	0.00
Future scenario: total costs	188.27	190.18	192.12	194.08	196.05	960.70
Future scenario: cost of testing[b]	2.10	2.25	2.41	2.57	2.73	12.06
Budget impact	**0.53**	**0.56**	**0.60**	**0.64**	**0.68**	**3.02**
Scenario 2b: Higher Cost of Fetal RhD Genotyping ($510 per Sample, via OOC-PA)						
Current scenario: total costs	187.74	189.62	191.52	193.43	195.37	957.68
Current scenario: cost of testing[b]	0.00	0.00	0.00	0.00	0.00	0.00
Future scenario: total costs	194.74	197.13	199.54	201.99	204.46	997.86
Future scenario: cost of testing[b]	8.57	9.19	9.83	10.48	11.14	49.22
Budget impact	**6.99**	**7.51**	**8.03**	**8.56**	**9.10**	**40.18**
Scenario 2c: Higher Cost of Fetal RhD Genotyping ($710 per Sample, via OOC-PA)						
Current scenario: total costs	187.74	189.62	191.52	193.43	195.37	957.68
Current scenario: cost of testing[b]	0.00	0.00	0.00	0.00	0.00	0.00
Future scenario: total costs	198.10	200.73	203.40	206.10	208.83	1,017.16
Future scenario: cost of testing[b]	11.93	12.80	13.69	14.59	15.52	68.53
Budget impact	**10.35**	**11.11**	**11.88**	**12.67**	**13.47**	**59.48**
Scenario 3: Paternal Screening (Included in Both Strategies)						
Current scenario: total costs	187.33	189.20	191.09	193.00	194.94	955.56
Current scenario: cost of testing[b]	0.00	0.00	0.00	0.00	0.00	0.00
Future scenario: total costs	185.88	187.64	189.43	191.23	193.05	947.23
Future scenario: cost of testing[b]	1.17	1.26	1.35	1.43	1.53	6.74

	Budget Impact, $ Million[a]					
	Year 1	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Total**
Budget impact	**–1.45**	**–1.56**	**–1.67**	**–1.78**	**–1.89**	**–8.34**
Scenario 4a: Canadian Sample, up to 15% Uptake of Fetal RhD Genotyping						
Current scenario: total costs	504.62	509.66	514.76	519.91	525.11	2,574.05
Current scenario: cost of testing [b]	0.00	0.00	0.00	0.00	0.00	0.00
Future scenario: total costs	504.88	510.19	515.56	520.98	526.46	2,578.06
Future scenario: cost of testing[b]	0.42	0.85	1.28	1.73	2.18	6.45
Budget impact	**0.26**	**0.53**	**0.80**	**1.07**	**1.35**	**4.01**
Scenario 4b: Canadian Sample, up to 35% Uptake of Fetal RhD Genotyping						
Current scenario: total costs	504.62	509.66	514.76	519.91	525.11	2,574.05
Current scenario: cost of testing[b]	0.00	0.00	0.00	0.00	0.00	0.00
Future scenario: total costs	505.92	511.41	516.97	522.59	528.27	2,585.16
Future scenario: cost of testing[b]	2.09	2.82	3.56	4.32	5.08	17.87
Budget impact	**1.30**	**1.75**	**2.21**	**2.68**	**3.16**	**11.11**

Abbreviations: RhD, rhesus D blood group; OOC-PA, Out-of-Country Prior Approval Program.
[a]All costs are in 2019 Canadian dollars.
[b]Cost of testing captured the costs of labour and consumables (i.e., blood sample collection, plasma preparation, and extraction of cell-free DNA, test interpretation and reporting, and quality assurance and quality control) and the cost of transporting the samples (details presented in Appendix 6).
Note: Results may appear inexact due to rounding. Negative costs indicate savings.

Alloimmunized Pregnancies

Table 32 presents scenario analyses findings for alloimmunized pregnancies. We found cost savings in all scenarios; thus, we present the budget impact related to the cost of testing.

As expected, the budget impact associated with the cost of testing decreased with a lower uptake of the intervention or with paternal screening (Scenarios 1a, 1b, and 4). Compared with total cost savings in the reference case, the 5-year savings would be smaller if this genetic test were provided via the Out-of-Country Prior Approval Program (Scenarios 2a and 2b), as the cost of testing would be two to three times higher.

Table 32: Budget Impact Analysis Results—Sensitivity Analysis, Alloimmunized Pregnancies

	Budget Impact, $ Million[a]					
	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Reference Case						
Budget impact	**−8.97**	**−9.62**	**−10.29**	**−10.97**	**−11.67**	**−51.52**
Budget impact: cost of testing[b]	0.48	0.51	0.55	0.58	0.62	2.74
Scenario 1a: Uptake of Fetal RhD Genotyping: Increment of 3% per Year (Year 1, 3%; Year 5, 15%)						
Current scenario: total costs	54.30	54.84	55.41	55.94	56.51	277.00
Current scenario: cost of testing[b]	0.00	0.00	0.00	0.00	0.00	0.00
Future scenario: total costs	53.97	54.16	54.38	54.56	54.76	271.82
Future scenario: cost of testing[b]	0.02	0.04	0.05	0.07	0.09	0.28
Budget impact	**−0.34**	**−0.68**	**−1.03**	**−1.39**	**−1.75**	**−5.18**
Scenario 1b: Uptake of Fetal RhD Genotyping: Increment of 5% per Year (Year 1, 15%; Year 5, 35%)						
Current scenario: total costs	54.30	54.84	55.41	55.94	56.51	277.00
Current scenario: cost of testing[b]	0.00	0.00	0.00	0.00	0.00	0.00
Future scenario: total costs	52.62	52.57	52.55	52.47	52.43	262.64
Future scenario: cost of testing[b]	0.09	0.12	0.15	0.18	0.22	0.77
Budget impact	**−1.68**	**−2.27**	**−2.86**	**−3.47**	**−4.08**	**−14.36**
Scenario 2a: Higher Cost of Fetal RhD Genotyping ($510 per sample, via OOC-PA)						
Current scenario: total costs	54.30	54.84	55.41	55.94	56.51	277.00
Current scenario: cost of testing[b]	0.00	0.00	0.00	0.00	0.00	0.00
Future scenario: total costs	45.60	45.50	45.42	45.30	45.19	227.00
Future scenario: cost of testing[b]	0.74	0.80	0.85	0.91	0.97	4.27
Budget impact	**−8.70**	**−9.34**	**−9.99**	**−10.65**	**−11.32**	**−50.00**
Scenario 2b: Higher Cost of Fetal RhD Genotyping ($710 per sample, via OOC-PA)						
Current scenario: total costs	54.30	54.84	55.41	55.94	56.51	277.00
Current scenario: cost of testing[b]	0.00	0.00	0.00	0.00	0.00	0.00
Future scenario: total costs	45.89	45.81	45.75	45.65	45.57	228.67
Future scenario: cost of testing[b]	1.03	1.11	1.19	1.26	1.34	5.94
Budget impact	**−8.41**	**−9.03**	**−9.66**	**−10.29**	**−10.94**	**−48.33**
Scenario 3: Paternal Screening (Included in Both Strategies)						
Current scenario: total costs	45.84	46.30	46.78	47.23	47.71	233.85
Current scenario: cost of testing[b]	0.00	0.00	0.00	0.00	0.00	0.00
Future scenario: total costs	43.15	43.41	43.68	43.93	44.20	218.38
Future scenario: cost of testing[b]	0.13	0.14	0.15	0.16	0.18	0.77
Budget impact	**−2.69**	**−2.89**	**−3.09**	**−3.30**	**−3.50**	**−15.48**
Scenario 4a: Canadian Sample, up to 15% Uptake of Fetal RhD Genotyping						
Current scenario: total costs	145.96	147.42	148.88	150.37	151.87	744.50
Current scenario: cost of testing[b]	0.00	0.00	0.00	0.00	0.00	0.00
Future scenario: total costs	145.05	145.60	146.12	146.65	147.16	730.57
Future scenario: cost of testing[b]	0.05	0.10	0.15	0.20	0.25	0.74

	Budget Impact, $ Million[a]					
	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Budget impact	**−0.90**	**−1.82**	**−2.77**	**−3.73**	**−4.71**	**−13.93**
Scenario 4b: Canadian Sample, up to 35% Uptake of Fetal RhD Genotyping						
Current scenario: total costs	145.96	147.42	148.88	150.37	151.87	744.50
Current scenario: cost of testing[b]	0.00	0.00	0.00	0.00	0.00	0.00
Future scenario: total costs	141.44	141.33	141.19	141.06	140.89	705.91
Future scenario: cost of testing[b]	0.24	0.32	0.41	0.50	0.58	2.06
Budget impact	**−4.52**	**−6.09**	**−7.69**	**−9.31**	**−10.98**	**−38.59**

Abbreviations: RhD, rhesus D blood group; OOC-PA, Out-of-Country Prior Approval Program.
[a]All costs are in 2019 Canadian dollars.
[b]Cost of testing captured the costs of labour and consumables (i.e., blood sample collection, plasma preparation, and extraction of cell-free DNA, test interpretation and reporting, and quality assurance and quality control) and the cost of transporting the samples (details presented in Appendix 6).
Note: Results may appear inexact due to rounding. Negative costs indicate savings.

Discussion

We conducted a model-based budget impact analysis to estimate the range of investment needed to publicly fund noninvasive fetal RhD blood group genotyping for RhD− pregnancies in Ontario.

Assuming full access to noninvasive fetal RhD genotyping by year 5, the total budget impact would be about $15 million for nonalloimmunized pregnancies, and for alloimmunized pregnancies there would be total budget savings of about $52 million. The budget needed solely to support the cost of testing for the next 5 years would be about $24 million for nonalloimmunized and $3 million for alloimmunized pregnancies.

In both populations, the estimated budget impact was sensitive to the cost of noninvasive fetal RhD genotyping. Outsourcing this genetic test to the Bristol, UK, reference laboratory via the Out-of-Country Prior Approval Program (as is currently done for selected alloimmunized pregnancies) would cost two to three times more per test than conducting the test in Ontario; consequently, this scenario led to a much greater budget impact. Since noninvasive fetal RhD genotype screening is not currently available for nonalloimmunized pregnancies in Ontario or Canada, a lower per-sample cost may be achieved by using high-throughput technology at the Canadian Blood Services laboratory. Furthermore, using existing resources at an Ontario laboratory that has already validated this test could facilitate implementation of noninvasive RhD genotyping for alloimmunized pregnancies.

Another important finding of our analysis was that lower uptake rates of up to 35% over the next 5 years would decrease the budget impact of the intervention in both populations. However, adopting this highly accurate and needed technology at less than full uptake could result in missed opportunities to reduce unnecessary care, particularly in alloimmunized pregnancies found to be RhD compatible.

Lastly, including paternal screening in both noninvasive fetal RhD genotyping and usual care strategies had an impact on the overall budget in nonalloimmunized and alloimmunized pregnancies, and decreased the budget incurred by the test solely; however, this strategy comes with concerns mentioned previously regarding revealing non paternity.

Strengths and Limitations

Our analyses are restricted by our modeling assumptions; therefore, our estimate of the budget impact depends on the estimated costs of usual care and on assumed costs of the intervention. Nevertheless, we conducted several scenario analyses to examine changes in the overall budget and the cost of testing.

Conclusions

Publicly funding noninvasive fetal RhD blood group genotyping for guiding the management of nonalloimmunized RhD− pregnancies in Ontario would result in a budget impact of about $2.6 million in year 1 (80% access) to about $3.4 million in year 5 (100% access). The total budget impact would be about $14.8 million over the next 5 years.

Publicly funding noninvasive fetal RhD genotyping for guiding the management of alloimmunized pregnancies in Ontario is associated with cost savings ranging from $9 million in year 1 to $12 million in year 5, with total savings of about $51 million over the next 5 years.

PREFERENCES AND VALUES EVIDENCE

Objective

The objective of this analysis is to explore the underlying values, needs, and preferences of those who have lived experience with RhD blood type incompatibility during pregnancy and the potential impact of noninvasive fetal RhD blood group genotyping.

Background

Exploring the preferences and values of patients and health care professionals provides unique information about people's experiences of a health condition and the health technologies or interventions used to diagnose, manage, or treat the health condition. It includes the effect of the condition and its treatment on the person with the health condition, their family and other caregivers, and the person's personal environment. Engagement also provides insights into how a health condition is managed by the province's health system.

Information shared from lived experience can also identify gaps or limitations in published research (e.g., outcomes important to those with lived experience that are not reflected in the literature).[113-115] Additionally, lived experience can provide information and perspectives on the ethical and social values implications of health technologies or interventions.

For this analysis, we used three ways to examine the perspectives and experiences of those with RhD blood type incompatibility during pregnancy:

- A review by our organization of the quantitative evidence on patient and provider preferences and values
- A review by the Canadian Agency for Drugs and Technologies in Health (CADTH) of the published qualitative literature
- Direct engagement by our organization with people with lived experience, through interviews and written responses

Quantitative Evidence

Research Question

What is the relative preference of patients and providers for noninvasive fetal RhD blood group genotyping in RhD negative (RhD−) pregnancies compared with the following current standards of care?

- For nonalloimmunized pregnancies: treat all with Rh immunoglobulin (RhIG) prophylaxis
- For alloimmunized pregnancies: monitor all for anti-D antibodies and fetal well-being

Methods

We performed a targeted literature search for quantitative preferences evidence on February 28, 2019, for studies published from January 1, 1997, to the search date in MEDLINE. The search was based on the population and intervention of the clinical search strategy with a methodological filter applied to limit retrieval to quantitative preferences evidence.[116] See Appendix 1 for our literature search strategies, including all search terms.

Eligibility Criteria

Studies

Inclusion Criteria

- English-language full-text publications
- Studies published between January 1, 1997, and February 28, 2019
- Studies of patient or provider preferences toward noninvasive fetal RhD genotyping in RhD− pregnancies using quantitative methods, such as:
    - Utility measures
        - Direct techniques: standard gamble, time trade-off, rating scales; conjoint analysis such as discrete choice experiment, contingent valuation and willingness-to-pay, probability trade-off
        - Indirect techniques: prescored multi-attribute instruments (e.g., EQ-5D, SF-36, Health Utilities Index)
    - Nonutility quantitative measures
        - Direct choice techniques: decision aids, surveys, questionnaires
- Mixed-method studies in which the quantitative data are separate and can be extracted
- Systematic reviews, meta-analyses, randomized controlled trials, cohort studies, surveys, and questionnaires

Exclusion Criteria

- Conference abstracts, case studies, case series, letters, editorials
- Studies where results for outcomes of interest cannot be extracted
- Animal and in vitro studies

Participants

- Pregnant people with RhD− blood type

Interventions

- Noninvasive fetal RhD genotyping with cell-free fetal DNA in maternal whole blood, serum, or plasma (laboratory-developed tests or commercial test kits)

Comparators

- Universal RhIG prophylaxis (also called anti-D prophylaxis) or prenatal screening tests for fetal anemia (e.g., Doppler ultrasonography), including invasive tests for blood typing (e.g., amniocentesis, chorionic villus sampling), or cord blood typing
- No comparator

Outcome Measures

Inclusion Criteria

- Patient preferences
- Provider preferences
- Trade-offs (e.g., intervention avoidance vs. risk of false test results)

Exclusion Criteria

- Qualitative data including but not limited to direct quotes and thematic analyses
- Provider perceptions of patient preferences, or patient perceptions of provider preferences

Literature Screening

A single reviewer conducted an initial screening of titles and abstracts using Covidence systematic review management software[26] and then obtained the full texts of studies that appeared eligible for review according to the inclusion criteria. A single reviewer then examined the full-text articles and selected studies eligible for inclusion. The reviewer also screened the reference lists of included studies for any additional relevant studies not identified through the search.

Data Extraction

We extracted data on study design, study population, quantitative results, and author conclusions.

Reporting Findings

We provide a narrative summary of the quantitative results from the included studies.

Results

Literature Search

Figure 10 presents the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram for the quantitative preferences evidence search. The literature search yielded 55 citations published from January 1, 1997, to February 28, 2019, after removing duplicates. We identified one study that met the inclusion criteria.[117] We identified one additional study[118] from the reference list of the included study (see Figure 10, other sources), for a total of two. One study examined patient preferences[117] and one canvassed health care provider attitudes.[118]

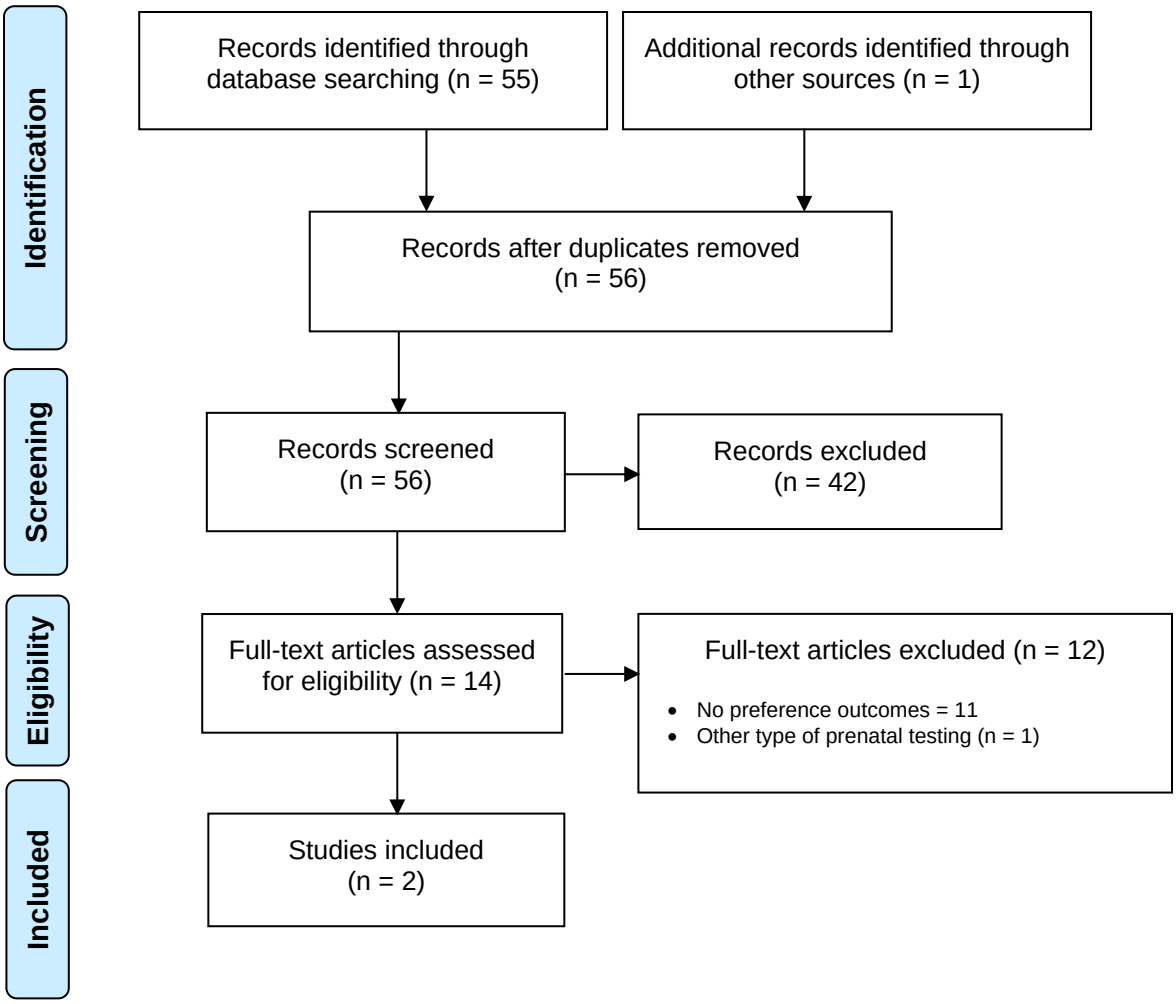

Figure 10: PRISMA Flow Diagram—Quantitative Evidence of Preferences and Values Search Strategy

Abbreviations: PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-analyses.
Source: Adapted from Moher et al, 2009.[27]

Patient Preferences

In the study by Oxenford et al,[117] researchers developed a questionnaire from interviews and focus groups with nonalloimmunized RhD− pregnant people who were offered noninvasive fetal

RhD blood group genotyping and with health professionals providing care for them within a broader research study conducted in the United Kingdom. The survey was distributed to RhD− pregnant people in four National Health Service Trusts who were attending routine prenatal appointments after 12 weeks' gestation. In this context, a midwife is commonly the primary care provider for pregnant people. A study leaflet was provided for patients and included a brief paragraph explaining the test in simple language, and there were 37 questions to answer. Respondents were asked to report on views and preferences about noninvasive fetal RhD genotyping, knowledge of blood group, RhIG prophylaxis and its administration, and their current sources of information.[117] Respondent demographic information was also collected. Table 33 provides an overview of the study.

Table 33: Characteristics of Patient Preference Study

Author, Year	Country	Target Population	N Approached	N Respondents	Response Rate
Oxenford et al, 2013	UK	RhD− patients attending routine prenatal appointments after 12 weeks' gestation	287	270	94%

Abbreviations: RhD, rhesus D blood group; UK, United Kingdom.
Source: Oxenford et al, 2013.[117]

Opinions About Routine Noninvasive Fetal RhD Genotyping

Ninety-two percent (92.1%) of respondents were in favour of routinely offering noninvasive fetal RhD blood group genotyping to all RhD− women. However, 75.9% indicated they were uncertain whether they themselves would accept the test, and this group had less knowledge about RhD blood type and RhIG prophylaxis (lower knowledge score, mean [M] = 7.64, 95% confidence interval [CI]: 6.66–8.63) than those who were accepting of the test (M = 9.49, 95% CI 8.99–9.99, *P* = .002).[117] Knowledge was assessed by asking 15 questions about blood group and anti-D prophylaxis, and knowledge scores were on a scale from 0 to 15.[117]

Reported reasons for declining noninvasive fetal RhD blood group genotyping (patients could indicate more than one reason) included wanting more information (41.7% of responses), wanting RhIG prophylaxis as a precaution (37.5%), and not wanting an additional blood test (20.8%).[117]

Preferences for Being Offered Noninvasive Fetal RhD Genotyping

In being offered noninvasive fetal RhD genotyping, respondents most highly valued the accuracy of the test, having sufficient information, and the opportunity to discuss the test with the midwife.[117] The preferences for timing of the test are in Table 34.

Table 34: Patient Preferences for Timing of Noninvasive Fetal RhD Genotyping

Option for Administration of the Test	% Preferred
Blood test performed at the same time as other routine blood tests	94
Willing to have an extra blood test, if necessary	89
Opportunity to discuss with midwife before having the test	89
Wiling to have an additional appointment to discuss with midwife	79

Abbreviation: RhD, rhesus D blood group.
Source: Oxenford et al, 2013.[117]

Preferences for Receiving Information About Noninvasive Fetal RhD Genotyping

Respondents expressed an overwhelming preference (91%) for receiving information about noninvasive fetal RhD blood group genotyping prior to the day of the test.[117] Nearly half of respondents wanted information during the first appointment with the midwife (46.3%), while others preferred to receive information by mail alongside the initial appointment letter (23.1%) or in the mail along with their initial blood test results (21.6%).

The hospital's website was the least preferred way to receive information about the test (4.6% of respondents), with nearly all respondents preferring to receive information from their midwife (59.7%) or in a booklet with written information (34.5%).[117] The amount of information in the study leaflet was adequate for 47.2% of respondents, with 36.7% wanting more information and the remaining 16.1% unsure. Table 35 lists the type of additional information respondents wanted.

Table 35: Patient-Identified Topics Related to Noninvasive Fetal RhD Genotyping Where More Information Would Be Helpful When Testing Is Offered

Topics
Risks or side effects to mother and baby
Timing of test, whether extra appointment is required
Implications of the results
How the test works
Accuracy and implications if the test result is incorrect
Opportunity to discuss test with a midwife/health professional
General information on blood type, why the test is necessary, risks of RhIG prophylaxis
Whether other information can be found out from the test

Abbreviations: RhD, rhesus D blood group; RhIG, Rh immunoglobulin.
Source: Oxenford et al, 2013.[117]

Provider Preferences

One study reported on the attitudes of obstetrics and gynecologist physicians in the United States regarding cell-free fetal DNA (cffDNA) testing in general.[118] This study was not specific to fetal RhD blood group genotyping but considered all noninvasive prenatal tests for genetic conditions (e.g., chromosome abnormalities, aneuploidies). A total of 180 surveys were distributed and the response rate was 34% (n = 62). Table 36 outlines some views of providers around testing in general; these preferences are not specific to noninvasive fetal RhD blood group genotyping but may be generalizable to this test. These views reflect providers' opinions from the perspective of clinical practice in 2010.

Table 36: Opinions of Obstetrician and Gynecologist Providers About Cell-Free Fetal DNA Testing

Opinion	% Expressed
Have a low level of knowledge about cffDNA testing	85
Genetic counselling[a] and genetic testing are necessary parts of prenatal care	85
Would offer Rh blood group genotyping	56

Abbreviation: cffDNA, cell-free fetal DNA.
[a]Not applicable to fetal RhD testing.
Source: Sayres et al, 2011.[118]

Providers who responded to the survey also were asked to rank six aspects of cffDNA tests from most to least important (Table 37). They viewed clinical utility and risk to the pregnancy as the two most important aspects of the test: 48% of providers believed clinical utility is the most important factor, whereas 43% rated risk to mother or fetus as most important.[118]

Table 37: Ranked Importance of Aspects of Cell-Free Fetal DNA Tests, by Obstetrician and Gynecologist Providers

Test Attribute	Mean Ranking (/6)
Clinical utility	1.98
Risk to fetus or mother	2.41
Test sensitivity	2.93
Ease of use	4.17
Range of conditions	4.43
Cost	5.07

Source: Sayres et al, 2011.[118]

Summary

The quantitative studies on preference found that RhD− pregnant people are supportive of noninvasive fetal RhD blood group genotyping, and that more than half of obstetric providers are supportive of offering the test. Patients would like to engage in conversations with their main pregnancy care provider about the test, including its risks and benefits.

Qualitative Evidence

Ontario Health collaborated with the Canadian Agency for Drugs and Technologies in Health (CADTH) to conduct this health technology assessment. CADTH conducted a review of qualitative literature on patient perspectives.[1] That review identified one qualitative study that examined patient expectations and perspectives on fetal RhD blood group genotyping. Key findings from the evidence were:

- Pregnant people and health care providers find fetal RhD genotyping beneficial and feel it should be offered to all RhD negative pregnant persons
- While fetal RhD genotyping is considered beneficial, there is residual concern around the possibility of false negatives leading some people to prefer to receive anti-D immunoglobulin despite a negative test result
- Pregnant people often experience information overload throughout pregnancy and appreciate when information on fetal RhD genotyping can be taken home in the form of informational pamphlets

Direct Patient Engagement

Methods

Engagement Plan

The engagement plan for this portion of the report focused on consultation to examine the experiences and perspectives of those with lived experience of RhD blood group incompatibility during pregnancy.

We used a variety of approaches, allowing a diverse group of Ontarians to participate. We conducted qualitative interviews (by telephone or email) and an anonymous online survey. Our main task in interviewing is to explore the meaning of central themes in the experiences of those with lived experience of a particular health condition.[119] The online survey made it possible for a greater number and range of people to respond, providing value in the volume of experiences shared. We also consulted with experts in medical ethics to identify potential ethical issues associated with current practice in the care of RhD− pregnancies and with noninvasive fetal RhD genotyping, and to provide additional context for patients' input.

Participant Outreach

We used an approach called purposive sampling,[120-123] which involves actively reaching out to people with direct experience of the health condition and health technology or intervention being reviewed. We approached a variety of clinical experts, support groups, and partner organizations such as the Prenatal Screening Ontario – Advisory Committee, to spread the word about this engagement activity and connect us with those with experience with RhD blood group incompatibility and who may be impacted by the use of noninvasive fetal RhD genotyping.

Inclusion Criteria

We sought to speak with people with lived experience with RhD blood group incompatibility during pregnancy. Participants did not need to have direct experience with noninvasive fetal RhD blood group genotyping to participate.

Exclusion Criteria

We did not set exclusion criteria.

Participants

For this assessment, we conducted direct interviews with six people in Ontario, all of whom had lived experience with RhD incompatibility during pregnancy. We collected another 64 responses through the online survey. Responses came primarily from participants living in the Greater Toronto Area but included respondents from Thunder Bay, Ottawa, and the London, Ontario, areas.

Approach

For the interviews, we explained the role of Ontario Health, the purpose of this report, the risks of participation, and how participants' personal health information would be protected. We gave this information to participants both verbally and in a letter of information if requested (Appendix 10). We then obtained participants' verbal consent before starting the interview. With participants' consent, we audio-recorded and then transcribed the interviews.

Interviews lasted approximately 10 to 20 minutes. The interview was loosely structured and consisted of several open-ended questions. Our list of questions (developed by the Health Technology Assessment International Interest Group on Patient and Citizen Involvement in Health Technology Assessment)[124] focused on participants' experience with RhD blood group incompatibility, their perceptions of using Rh immunoglobin prophylaxis (known as RhIG or by its US trade name, RhoGAM), and the potential impact of noninvasive fetal RhD genotyping. We also inquired about their values in potentially choosing fetal RhD genotyping and any potential benefits or concerns they could perceive. See Appendix 11 for our interview guide.

Data Extraction and Analysis

We used a modified version of a grounded-theory method to analyze interview transcripts and survey results. The grounded-theory approach allowed us to organize and compare information on experiences across participants. This method consists of a repetitive process of obtaining, documenting, and analyzing responses while simultaneously collecting, analyzing, and comparing information.[125,126] We used the qualitative data analysis software program NVivo[127] to identify and interpret patterns in the data.

Results

Awareness of RhD Blood Group Incompatibility

RhD blood group incompatibility between mother and fetus can cause serious health concerns for the fetus. In our interviews and survey, participants indicated they were generally aware of the nature of RhD incompatibility and its potential impact during and after a pregnancy. However, this knowledge was not universal; several participants stated they felt they had very little information on the subject. Among those who were aware of the condition, some said they received information from their family physician or obstetrician-gynecologist (OB-GYN). Others reported learning about RhD blood groups and incompatibility from previous blood donations, researching the topic themselves, or being told by friends or family about the potential for RhD incompatibility during pregnancy:

> *I felt I was well-informed. I had heard that if a woman with Rh negative [like I am] had a baby with Rh positive, there could be issues for the second baby. As my husband is Rh negative, I assumed my babies would be fine.*

> *Before getting the RhoGAM shot I was vaguely familiar with the concept of it.... I knew it had to do with blood types but I didn't quite understand how that would affect mother or baby. All of my information came from online or through friends/family; my doctor did not provide any information.*

> *I actually didn't know my blood type before being pregnant and also didn't know of the incompatibility. My OB-GYN at [the hospital] was very thorough and informative.*

> *I knew from donating blood that I was Rh negative (B−), and from researching stats about blood types I learned about Rh incompatibility.*

Rh Immunoglobin Prophylaxis

To prevent the development of anti-D antibodies in RhD incompatible pregnancies, standard care in Ontario is for RhD− pregnant people to receive a RhIG prophylaxis injection (RhoGAM) during the pregnancy and, if needed, after the delivery.

Given this has been the standard of care in Ontario for several decades, respondents were generally familiar and comfortable with RhIG. Some participants said they wanted more information about the injection, its safety and potential side effects. Additionally, several participants expressed concern about its safety and overall use, since RhIG is a blood product:

> *I wanted to know what was in it and what would happen if I didn't receive it. I wanted to know about side effects and/or complications.*
>
> *I really don't understand if there are any risks of having the RhoGAM shot.*
>
> *As RhoGAM is a blood product, I wondered about how it was made, where the product came from and any side effects it may cause for me after taking it.*

This finding differed from that reported in the CADTH qualitative review which reported that "as a blood product, anti-D immunoglobin is often noted as a potential health risk, but neither health professionals nor women expressed concern and considered the benefits of anti-D to outweigh any associated risks." (CADTH report,[1] p. 7)

In our interviews and survey, some participants commented on their desire to minimize interventions during their pregnancy and, therefore, to know the exact nature of RhIG and the potential impact of deciding not to receive the injection:

> *I'm generally against unnecessary interventions as well so I wanted to really understand the consequences if we chose not to [receive RhoGAM].*

Several participants spoke of the physical process involved in receiving RhIG. Primarily, the process was straightforward and simple; however, a few participants experienced a lengthy delay and had to go through multiple steps to receive the injection:

> *There were no barriers; it was offered at the hospital after the birth of my first baby to prevent a problem with the second baby.*
>
> *Once it was determined I needed the RhoGAM shot, it took quite awhile for it to be ordered (maybe 1.5 hours). Once it was received in the ER department, the nurses were unsure of how it should be delivered and by whom. It didn't seem like it was a regular occurrence.*

Other participants were more hesitant to receive RhIG at all and felt that it was unnecessary if they were certain that there was no RhD incompatibility (for instance, if both mother and father were RhD−):

> *My husband is Rh negative and I knew without a shadow of a doubt that he was the father of my baby. I felt it was completely unnecessary. I did not feel comfortable with agreeing to an injection of a foreign substance in my body when it was completely unnecessary.*

> *I am Rh neg and my partner is Rh neg as well; which means I would not have an incompatibility issue. However, I was given a hard time by providers when I wanted to decline RhoGAM as they said I should receive it regardless of partner status.*

Information on and Impact of Noninvasive Fetal RhD Genotyping

None of the survey or interview participants had direct experience using noninvasive fetal RhD blood group genotyping during pregnancy, and they reported little awareness or knowledge of this test. Therefore, during the interviews we presented background information about noninvasive fetal RhD genotyping and answered questions about its use. The survey included this information in writing.

We then asked participants for their thoughts and perspectives on the potential use and impact of noninvasive fetal RhD genotyping, as it might apply to their own current or future pregnancies. As we found in our review of the quantitative evidence, a majority of participants indicated a desire for this test to be available. Participants also reflected on various types of positive impact the test could have, from reducing resource use to preventing unnecessary testing:

> *This is great; it would save blood products for only those who need it and prevent those who don't need it from being barred from donating blood; only concerns would be risks to developing fetus or mother.*

> *This definitely would have applied to my situation with my second pregnancy. If they'd known my son's blood type, it would have been a definitive decision on whether I needed a RhoGAM shot in the emergency room or not.*

> *I think it's more efficient if it's targeted and maybe more, maybe not cost-effective because you're doing genetic testing in place of it or possibly to prevent the RhoGAM. But no, I'm not open to unnecessary injections. So if they can tell me that I don't need it, then I don't want it.*

> *If I were pregnant today and my husband was Rh positive, and there was a blood test which could be done to determine with 100% accuracy my baby's blood type, then I would prefer to have this test done and only have the Rh immunoglobin if my baby was Rh positive.*

When it came to acting on the results of fetal RhD genotyping, however, some participants expressed a preference to receive RhIG even if the test showed they did not need it. this was similar to the findings of the CADTH qualitative evidence review which found that: "Even when testing predicts an RhD negative phenotype in the fetus, some pregnant people may still prefer to receive anti-D immunoglobin prophylaxis to alleviate their concerns of false negatives." (CADTH report,[1] p. 9)

It is anticipated that noninvasive fetal RhD genotyping would also benefit alloimmunized people who, during a subsequent pregnancy, may undergo intensive fetal monitoring and interventions to prevent and treat complications from RhD incompatibility. In our expert consultations, clinical experts suggested that the burden of intensive fetal monitoring would be more severe in northern and remote Ontario where patients may have to travel long distances for bloodwork,

ultrasounds, or other medical interventions. While we did hear from several participants who live in northern Ontario, no one emphasized this particular impact of fetal RhD genotyping.

However, several nonalloimmunized participants reflected on the potential savings in time and resources that noninvasive fetal RhD genotyping could provide, especially when travel is involved:

> *In my experience, to get RhoGAM treatment I had to go to the hospital, got though admitting, have blood work done at the lab, wait for the blood work [result and for] the pharmacy to get the shot ready, and then get RhoGAM at labour and delivery. Given the number of different employees at the hospital that were involved in the process, I assume it was fairly costly to the system and fetal genotyping might save some of these costs if there is not Rh incompatibility. As well I had to take a day off work for travel for this process which may not have been necessary.*

> *I think this test would benefit higher risk groups such as sensitized [alloimmunized] patients…who may not be able to be accurate historians regarding pregnancy losses and baby blood types.*

Finally, several participants highlighted a potential benefit of noninvasive fetal RhD genotyping, based on their own experiences involving a miscarriage. They expressed frustration with having to receive a RhIG injection at that traumatic time. They welcomed the possibility that noninvasive fetal RhD genotyping could reduce the need for an unnecessary intervention:

> *Could it help avoid the need for RhoGAM shots during a miscarriages? If so I think that could be amazing. One of the worse parts of my miscarriages was needing to go to the ER and sit and wait for hours just to get the shot, when I would have much rather been at home.*

> *I think this would be very nice. I personally had a miscarriage and needed to go to the hospital and wait for hours for RhoGAM. It was hard and very annoying to have to wait for a simple needle that perhaps wasn't even necessary.*

Concerns About Fetal RhD Genotyping

Survey and interview respondents also expressed some concerns about noninvasive fetal RhD genotyping. These concerns were coupled with a desire for more information about the test and particulars about its use: how it is done and at what point during the pregnancy. Our survey of the quantitative evidence also reflected this desire for greater information.

Primarily, participants' concerns centred around the safety and accuracy of the test. There was near universal sentiment that administering the test should not pose any risk to the fetus or mother, and participants wanted assurance that the test is noninvasive:

> *What are the risks of testing the fetus's blood? What are the risks of treating the mother (i.e. all pregnancies the same) now? These are some things I would be interested in knowing.*

> *I'd be interested in knowing the risks to testing the fetus for their blood type as compared to just treating all Rh negative mothers.*
>
> *My thoughts are about the fetus's safety and how the fetus's blood gets tested. If there is a risk to the fetus or the pregnancy in general to find out the fetus's blood type, my preference would always be to defer the risk to the mother – even if that involves additional tests, accepting blood products, risks, etc.*

Additionally, interview participants reported that they would expect the test to be highly accurate to ensure that, if they declined the RhIG injection, it would not result in harm to the fetus:

> *If there was an error in reporting the test results and my baby suffered harm or death, then I would be very upset, so I think a way to ensure accuracy of the test results is essential.*

This concern was also reflected in the study included in CADTH's qualitative review: "[b]y and large, concern with test accuracy seemed situated around the possibility of false negatives as this could put a fetus at risk of developing HDFN [hemolytic disease of the fetus and newborn]." (CADTH report,[1] p. 7) Further, participants in that study placed a priority on the accuracy of the genotyping test, rather than the timing of the results: "Even if testing was postponed until a bit later in pregnancy, women indicated that test accuracy was valued over earlier availability of results. This does not mean women would not want testing earlier, they simply wanted it when it was most likely to be accurate." (CADTH report,[1] p. 8)

Ethical Considerations

In our expert consultations, experts in medical ethics highlighted potential ethical issues associated with current practice in the care of RhD− pregnancies and with noninvasive fetal RhD blood group genotyping. Similarly, a number of interview and survey participants commented on various ethical aspects of both current practice and the noninvasive fetal RhD genotyping test. These ethical considerations can be grouped into five categories: health harms and benefits, resource stewardship, autonomy, equity, and opportunity cost.

In terms of current practice, the universal use of RhIG in RhD incompatible pregnancies carries no known risk to the fetus. For the pregnant person, receiving RhIG may involve unnecessary stress and inconvenience, as well as the concern about blood product–related infection. However, in three decades of use in Canada, there are no recorded cases of RhIG transmitting infection In alloimmunized pregnancies, current practice typically involves intensive fetal monitoring that may lead to invasive tests. Comments from both the online survey and interviews demonstrated participants' awareness of this increased burden. Participants also recognized the potential for noninvasive fetal RhD genotyping to decrease potential health harms by preventing unnecessary intensive monitoring and invasive interventions. However, a false negative result, leading to a decision not to use RhIG, may result in a missed opportunity to prevent alloimmunization of the pregnant person, while a false positive may result in unnecessary use of RhIG.

Considering resource stewardship, participants and experts both noted that fetal RhD genotyping could prevent the unnecessary use of RhIG, a limited resource. The test could also help the health system avoid the cost of unnecessary intensive fetal monitoring in the alloimmunized population by revealing fetal RhD status earlier in the pregnancy than is currently possible.

In current practice, a pregnant person cannot make a truly informed decision about RhIG without knowing the fetus's RhD status, and this impacts their autonomy in decision-making. Thus, noninvasive fetal RhD genotyping could increase patients' autonomy in decision-making about their care. On the other hand, expert consultation as well as patient interviews highlighted the potential for noninvasive fetal RhD genotyping to reveal nonpaternity (meaning the presumed father is not the biological father), a potential ethical issue requiring thoughtful implementation. Currently, with the universal prophylaxis use of RhIG in RhD− pregnancies, revealing paternity does not need to be a factor in the pregnant person's decision-making about RhIG.

Equity issues can arise in alloimmunized pregnancies for people who need intensive fetal monitoring and live in rural or remote locations. They have the added burden of travel and may find it more difficult to access these highly specialized services. Another equity issue is that, currently in Ontario, noninvasive fetal RhD genotyping is available only through the Out-of-Country Prior Approval Program for alloimmunized pregnancies, and therefore access to the test for this population may differ by region or practice, depending on local services and awareness of the test process.

Discussion

We sought to understand preferences and values about noninvasive fetal RhD blood group genotyping through an analysis of the quantitative and qualitative literature, as well as through direct patient engagement. Each method found that patients would like this test to be available and feel positively about the potential benefits it could provide.

More specifically, in quantitative studies RhD− pregnant people expressed support for routine offering of noninvasive fetal RhD genotyping as part of pregnancy care, with a preference to be well informed about the test process, its attributes, timing, and risks in advance of the test, ideally in a dialogue with their health care provider. Obstetric health care providers were generally supportive of offering noninvasive fetal RhD genotyping to RhD− patients.

Through interviews and surveys, participants consistently expressed a desire for more information about noninvasive fetal RhD genotyping and assurance that the test is safe and causes no risk to the fetus. If they could be confident in its safety, patients reacted positively to the potential use of this test. Participants consistently mentioned the management of resources and prevention of unnecessary testing as potential benefits.

Clinical experts and a small number of responses from patients indicated that, if the test were available, noninvasive fetal RhD genotyping would positively impact pregnant people who live in rural or remote areas. This test could potentially alleviate the burden of travel for unnecessary testing and monitoring in pregnancies found to be RhD compatible. However, the low response rate from participants in northern Ontario or who indicated they live rurally makes this a tenuous conclusion based solely on direct patient engagement.

Conclusions

Patient and provider preferences and values, understood through interviews, a survey, and published qualitative and quantitative studies, indicate positive support for the potential use and impact of noninvasive fetal RhD blood group genotyping in RhD− pregnancies. Participants acknowledge a desire for additional information and assurances about the test's safety for both the pregnant person and the fetus. Additionally, patients and clinical experts raised some ethical considerations concerning the widespread use and potential benefits of the RhD genotyping test. .

CONCLUSIONS OF THE HEALTH TECHNOLOGY ASSESSMENT

Noninvasive fetal RhD blood group genotyping using cell-free fetal DNA from maternal blood in RhD− pregnancies was found to be an accurate test from our overview of published systematic reviews. Our systematic review found low to very low quality of evidence, according to the GRADE framework, that noninvasive fetal RhD genotyping to guide care of RhD− pregnancies may lead to avoidance of unnecessary RhIG prophylaxis, high compliance with targeted RhIG prophylaxis programs, and high uptake of genotyping. The risk of alloimmunization may not increase when noninvasive fetal RhD genotyping is used to target RhIG prophylaxis, and testing may allow the avoidance of unnecessary monitoring and invasive procedures in alloimmunized pregnancies.

Our economic evidence review found inconsistent results on the cost-effectiveness of noninvasive fetal RhD genotyping for nonalloimmunized or alloimmunized RhD− pregnancies, and the findings were not directly applicable to Ontario. Therefore, we conducted a full economic evaluation. For the management of nonalloimmunized RhD− pregnancies, noninvasive fetal RhD genotyping may not be cost-effective compared with usual care, unless the cost of testing is much lower than currently proposed. For the management of alloimmunized RhD− pregnancies, noninvasive fetal RhD blood group genotyping is cost saving (i.e., less costly and more effective than usual care).

Publicly funding noninvasive fetal RhD genotyping for guiding the management of RhD− nonalloimmunized pregnancies in Ontario would result in a budget impact of about $2.6 million in year 1 (assuming 80% access) to about $3.4 million in year 5 (100% access). The total budget impact would be about $14.8 million over the next 5 years. For guiding the management of alloimmunized pregnancies, publicly funding noninvasive fetal RhD genotyping in Ontario would result in cost savings ranging from $9 million in year 1 to $12 million in year 5, with total savings of about $51 million over the next 5 years.

Patient and provider preferences and values, obtained through interviews, a survey, and a review of published qualitative and quantitative studies, indicate support for the potential use and impact of noninvasive fetal RhD genotyping in RhD− pregnancies. Published evidence indicated that RhD− pregnant people support routine offering of noninvasive fetal RhD blood group genotyping as part of pregnancy care, with a preference to be sufficiently informed about testing and have a dialogue with their health care provider. The evidence also found that obstetric health care providers were supportive of offering the test. Interview and survey participants in Ontario acknowledged a desire for additional information and assurances about the test's safety for both the pregnant person and the fetus. Additionally, patients and clinical experts raised several ethical considerations concerning the widespread use and potential benefits of this genetic test.

ABBREVIATIONS

CADTH	Canadian Agency for Drugs and Technologies in Health
CBS	Canadian Blood Services
cffDNA	Cell-free fetal DNA
CI	Confidence interval
CVS	Chorionic villus sampling
DNA	Deoxyribonucleic acid
GRADE	Grading of Recommendations Assessment, Development, and Evaluation
HDFN	Hemolytic disease of the fetus and newborn
ICER	Incremental cost-effectiveness ratio
IUT	Intrauterine transfusion
MCA	Middle cerebral artery
NICU	Neonatal intensive care unit
NICE	National Institute for Health and Care Excellence
PCR	Polymerase chain reaction
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analyses
QALY	Quality-adjusted life-year
RhD−	Rhesus D negative blood type
RhD+	Rhesus D positive blood type
RhIG	Rh immunoglobulin
RoBANS	Risk of Bias Assessment tool for Nonrandomized Studies
ROBIS	Risk of Bias in Systematic Reviews tool
RT-PCR	Real-time polymerase chain reaction
SD	Standard deviation

GLOSSARY

Alloimmunization	The development of maternal antibodies as an immune response in reaction to fetal blood carrying a foreign antigen entering the mother's blood stream; a precondition for HDFN.
Antibody titre	A measure of the concentration of antibodies that indicates the level of immune system response. The titre is expressed as a ratio (e.g., 1:16) where the second numeric value represents the number of times the concentration can be diluted and still yield a positive agglutination result on an antibody screening test.
Cell-free fetal DNA	During a pregnancy, blood from the fetus that circulates in the mother's blood stream.
Cost-effectiveness analysis	Used broadly, "cost-effectiveness analysis" may refer to an economic evaluation used to compare the benefits of two or more health care interventions with their costs. It may encompass several types of analysis (e.g., cost-effectiveness analysis, cost–utility analysis). Used more specifically, "cost-effectiveness analysis" may refer to a type of economic evaluation in which the main outcome measure is the incremental cost per natural unit of health (e.g., life-year, symptom-free day) gained.
Cost–utility analysis	A cost–utility analysis is a type of economic evaluation used to compare the benefits of two or more health care interventions with their costs. The benefits are measured using quality-adjusted life-years, which capture both the quality and quantity of life. In a cost–utility analysis, the main outcome measure is the incremental cost per quality-adjusted life-year gained.
Exchange transfusion	A type of potentially life-saving blood transfusion to treat HDFN; the baby's blood is removed and replaced with donor blood to counteract severe anemia (lack of healthy red blood cells) and hyperbilirubinemia (too much bilirubin in the blood).
Fetomaternal hemorrhage	Fetal blood entering the maternal circulation by crossing the placenta during pregnancy in a volume large enough to trigger an immune response.
Genotyping	Testing to determine the genetic variant (genotype) that underlies an observable characteristic (phenotype) of a person by looking at their DNA. Genotype is typically inherited as one gene from each parent, mostly in pairs.
Hemolytic disease of the fetus and newborn (HDFN)	A disease occurring in incompatible pregnancies, including RhD incompatibility, where maternal antibodies cross the placenta and destroy the red blood cells (hemolysis) of the fetus, which can lead to fetal anemia of varying severity. Consequences range from neonatal jaundice to serious

	conditions such as brain damage or fetal death. Also called erythroblastosis fetalis.
Heterozygous	Having two different alleles at a gene locus, typically one dominant and one recessive, that can be passed on to offspring. For instance, with one RhD+ allele and one RhD− allele each inherited from one parent, the offspring will have a phenotype of RhD+ blood type on serology.
Incremental cost-effectiveness ratio (ICER)	The incremental cost-effectiveness ratio (ICER) is a summary measure that indicates, for a given health care intervention, how much more a health care consumer must pay to get an additional unit of benefit relative to an alternative intervention. It is obtained by dividing the incremental cost by the incremental effectiveness. Incremental cost-effectiveness ratios are typically presented as the cost per life-year gained or the cost per quality-adjusted life-year gained.
Intrauterine transfusion	Blood transfusion for the fetus (in utero) delivered via the umbilical cord, to increase the number of red blood cells to treat fetal anemia and prevent hemolytic disease of the fetus and newborn.
Kell (K); Duffy; Kidd; Rh c, C, d, e, E	Common red blood cell antigens, in addition to RhD, each a distinct blood group or blood group antigen that can have maternal-fetal incompatibility during pregnancy, cause alloimmunization, and lead to HDFN.
Markov model	A Markov model is a type of decision-analytic model used in economic evaluations to estimate the costs and health outcomes (e.g., quality-adjusted life-years gained) associated with using a particular health care intervention. Markov models are useful for clinical problems that involve events of interest that may recur over time (e.g., stroke). A Markov model consists of mutually exclusive, exhaustive health states. Patients remain in a given health state for a certain period of time before moving to another health state based on transition probabilities. The health states and events modelled may be associated with specific costs and health outcomes.
Monte Carlo simulation	Monte Carlo simulation is an economic modelling method that derives parameter values from distributions rather than fixed values. The model is run several times, and in each iteration, parameter values are drawn from specified distributions. This method is used in microsimulation models and probabilistic sensitivity analysis.
Nonpaternity	A genetics term meaning the presumed father is not the biological father.
Phenotype	An observable characteristic of a person, such as blood type. Phenotype includes physical and/or biochemical characteristics resulting from the expression of a genotype and its interaction with the environment.

Probabilistic sensitivity analysis (PSA)	A probabilistic sensitivity analysis (PSA) is used in economic models to explore uncertainty in several parameters simultaneously and is done using Monte Carlo simulation. Model inputs are defined as a distribution of possible values. In each iteration, model inputs are obtained by randomly sampling from each distribution, and a single estimate of cost and effectiveness is generated. This process is repeated many times (e.g., 10,000 times) to estimate the number of times (i.e., the probability) that the health care intervention of interest is cost-effective.
Quality-adjusted life-year (QALY)	The quality-adjusted life-year (QALY) is a generic health outcome measure commonly used in cost–utility analyses to reflect the quantity and quality of life-years lived. The life-years lived are adjusted for quality of life using individual or societal preferences (i.e., utility values) for being in a particular health state. One year of perfect health is represented by one quality-adjusted life-year.
RhD incompatibility	A condition during pregnancy when the pregnant person's blood type is RhD− and the fetus's blood type is RhD+, which can cause maternal alloimmunization and hemolytic disease of the fetus and newborn.
RhIG prophylaxis	Rh immunoglobulin, a treatment given at key times during pregnancy and after birth to prevent alloimmunization. It is a blood product derived from pooled human plasma and administered to the mother via intramuscular injection.
Scenario analysis	A scenario analysis is used to explore uncertainty in the results of an economic evaluation. It is done by observing the potential impact of different scenarios on the cost-effectiveness of a health care intervention. Scenario analyses include varying structural assumptions from the reference case.
Sensitivity	The ability of a test to correctly identify true-positives: people *with* the condition being tested for.
Sensitivity analysis	Every economic evaluation contains some degree of uncertainty, and results can vary depending on the values taken by key parameters and the assumptions made. Sensitivity analysis allows these factors to be varied and shows the impact of these variations on the results of the evaluation. There are various types of sensitivity analysis, including deterministic, probabilistic, and scenario.
Serology	Determining an individual's blood type by analyzing blood serum in a laboratory. This test can show a person's RhD phenotype but not genotype which could be underpinned by a number of genotypes for people who are RhD+ on serology.
Specificity	The ability of a test to correctly identify true-negatives: people *without* the condition being tested for.

Utility	A utility is a value that represents a person's preference for various health states. Typically, utility values are anchored at 0 (death) and 1 (perfect health). In some scoring systems, a negative utility value indicates a state of health valued as being worse than death. Utility values can be aggregated over time to derive quality-adjusted life-years, a common outcome measure in economic evaluations.
Willingness-to-pay value	A willingness-to-pay value is the monetary value a health care consumer is willing to pay for added health benefits. When conducting a cost–utility analysis, the willingness-to-pay value represents the cost a consumer is willing to pay for an additional quality-adjusted life-year. If the incremental cost-effectiveness ratio is less than the willingness-to-pay value, the health care intervention of interest is considered cost-effective. If the incremental cost-effectiveness ratio is more than the willingness-to-pay value, the intervention is considered not to be cost-effective.

APPENDICES

Appendix 1: Literature Search Strategies

Clinical Evidence Search

Search Date: February 25, 2019
Databases: EBM Reviews - Cochrane Central Register of Controlled Trials <January 2019>, EBM Reviews - Cochrane Database of Systematic Reviews <2005 to February 21, 2019>, EBM Reviews - Health Technology Assessment <4th Quarter 2016>, EBM Reviews - NHS Economic Evaluation Database <1st Quarter 2016>, Embase <1980 to 2019 Week 08>, Ovid MEDLINE(R) ALL <1946 to February 22, 2019>
Search Strategy:
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1 Rh-Hr Blood-Group System/ (13637)
2 (RhD* or rhesus D* or Rh D*).ti,ab,kf. (17158)
3 (Rh-negativ* or Rh-positiv*).ti,ab,kf. (2078)
4 (Rhesus negativ* or Rhesus positiv*).ti,ab,kf. (574)
5 ((rh or rhesus) adj2 (factor$1 or antigen* or system* or group* or type* or typing or status)).ti,ab,kf. (12981)
6 Rh Isoimmunization/ (2746)
7 ((isoimmun* or iso-immun* or alloimmun* or allo-immun* or unsensiti#ed or un-sensiti#ed or non-sensiti#ed or nonsensiti#ed or sensiti#ation* or sensiti#ed or sensibili#ation) adj6 (rh or rhesus or maternal or mother* or pregnan*)).ti,ab,kf. (7082)
8 ((f?etomaternal or f?eto-maternal) adj2 immuni#ation).ti,ab,kf. (111)
9 ((rh or rhesus) adj2 (immuni* or autoimmuni*)).ti,ab,kf. (1856)
10 Erythroblastosis, Fetal/ (10462)
11 ((h?emolytic adj2 (disease* or disorder*)) or HDFN).ti,ab,kf. (9201)
12 ((rhesus or rh) adj2 (disease* or disorder* or incompatib* or antagonism)).ti,ab,kf. (2698)
13 ((erythroblastos#s or erythroblastotic) adj2 (f?etal* or f?etus*)).ti,ab,kf. (3310)
14 or/1-13 (52366)
15 Prenatal Diagnosis/ (88480)
16 Maternal Serum Screening Tests/ (659)
17 Hematologic Tests/ (21312)
18 ((prenatal or pre-natal or antenatal or ante-natal or f?etal or f?etus* or noninvasive* or non-invasive*) adj3 (test* or screen* or diagnos* or determin* or detect*)).ti,ab,kf. (192715)
19 (NIPT or NIPD or NIDT or gNIPT or NIPS).ti,ab,kf. (3499)
20 ((maternal or mother*) adj2 (plasm* or serum)).ti,ab,kf. (31575)
21 Genotyping Techniques/ (12290)
22 (genotyp* adj2 (f?etal or f?etus* or prenatal or pre-natal or antenatal or ante-natal or maternal or pregnan* or mother* or noninvasive or non-invasive)).ti,ab,kf. (4131)
23 (((f?etal or f?etus* or free-f?etal or placenta*) adj2 dna) or cell-free dna).ti,ab,kf. (12329)
24 (cff DNA or cffDNA or ccffDNA or ccff DNA or cf DNA or cfDNA or f DNA or fDNA or ff DNA or ffDNA).ti,ab,kf. (5596)
25 or/15-24 (301704)
26 14 and 25 (3781)
27 exp Animals/ not Humans/ (15941943)
28 26 not 27 (3040)
29 Case Reports/ or Comment.pt. or Editorial.pt. or (Letter not (Letter and Randomized Controlled Trial)).pt. or Congresses.pt. (5011000)

30 28 not 29 (2736)
31 limit 30 to english language [Limit not valid in CDSR; records were retained] (2153)
32 31 use medall,coch,cctr,clhta,cleed (1131)
33 limit 32 to yr="1997 -Current" (700)
34 blood group rhesus system/ (3032)
35 rhesus D antigen/ (1180)
36 (RhD* or rhesus D* or Rh D*).tw,kw. (17312)
37 (Rh-negativ* or Rh-positiv*).tw,kw. (2085)
38 (Rhesus negativ* or Rhesus positiv*).tw,kw. (582)
39 ((rh or rhesus) adj2 (factor$1 or antigen* or system* or group* or type* or typing or status)).tw,kw. (11457)
40 rhesus isoimmunization/ (2823)
41 ((isoimmun* or iso-immun* or alloimmun* or allo-immun* or unsensiti#ed or un-sensiti#ed or non-sensiti#ed or nonsensiti#ed or sensiti#ation* or sensiti#ed or sensibili#ation) adj6 (rh or rhesus or maternal or mother* or pregnan*)).tw,kw. (7186)
42 ((f?etomaternal or f?eto-maternal) adj2 immuni#ation).tw,kw. (113)
43 ((rh or rhesus) adj2 (immuni* or autoimmuni*)).tw,kw. (1869)
44 newborn hemolytic disease/ (10842)
45 ((h?emolytic adj2 (disease* or disorder*)) or HDFN).tw,kw. (9351)
46 ((rhesus or rh) adj2 (disease* or disorder* or incompatib* or antagonism)).tw,kw. (3242)
47 ((erythroblastos#s or erythroblastotic) adj2 (f?etal* or f?etus*)).tw,kw. (1201)
48 or/34-47 (49314)
49 prenatal diagnosis/ (88480)
50 prenatal screening/ (43709)
51 maternal serum screening test/ (654)
52 blood examination/ (13020)
53 non invasive procedure/ (25423)
54 ((prenatal or pre-natal or antenatal or ante-natal or f?etal or f?etus* or noninvasive* or non-invasive*) adj3 (test* or screen* or diagnos* or determin* or detect*)).tw,kw,dv. (196349)
55 (NIPT or NIPD or NIDT or gNIPT or NIPS).tw,kw,dv. (3580)
56 ((maternal or mother*) adj2 (plasm* or serum)).tw,kw,dv. (31833)
57 genotyping technique/ (13416)
58 (genotyp* adj2 (f?etal or f?etus* or prenatal or pre-natal or antenatal or ante-natal or maternal or pregnan* or mother* or noninvasive or non-invasive)).tw,kw,dv. (4170)
59 (((f?etal or f?etus* or free-f?etal or placenta*) adj2 dna) or cell-free dna).tw,kw,dv. (12577)
60 (cff DNA or cffDNA or ccffDNA or ccff DNA or cf DNA or cfDNA or f DNA or fDNA or ff DNA or ffDNA).tw,kw,dv. (5665)
61 or/49-60 (320440)
62 48 and 61 (3747)
63 (exp animal/ or nonhuman/) not exp human/ (10171681)
64 62 not 63 (3711)
65 Case Report/ or Comment/ or Editorial/ or (letter.pt. not (letter.pt. and randomized controlled trial/)) or conference abstract.pt. (10177583)
66 64 not 65 (2607)
67 limit 66 to english language [Limit not valid in CDSR; records were retained] (2046)
68 67 use emez (988)
69 limit 68 to yr="1997 -Current" (745)
70 33 or 69 (1445)
71 70 use medall (682)
72 70 use coch (1)
73 70 use cctr (14)

74 70 use clhta (1)
75 70 use cleed (2)
76 70 use emez (745)
77 remove duplicates from 70 (896)

Economic Evidence Search

Search date: February 26, 2019
Database: EBM Reviews - Cochrane Central Register of Controlled Trials <January 2019>, EBM Reviews - Cochrane Database of Systematic Reviews <2005 to February 21, 2019>, EBM Reviews - Health Technology Assessment <4th Quarter 2016>, EBM Reviews - NHS Economic Evaluation Database <1st Quarter 2016>, Embase <1980 to 2019 Week 08>, Ovid MEDLINE(R) ALL <1946 to February 25, 2019>
Search Strategy:
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1 Rh-Hr Blood-Group System/ (13637)
2 (RhD* or rhesus D* or Rh D*).ti,ab,kf. (17161)
3 (Rh-negativ* or Rh-positiv*).ti,ab,kf. (2078)
4 (Rhesus negativ* or Rhesus positiv*).ti,ab,kf. (575)
5 ((rh or rhesus) adj2 (factor$1 or antigen* or system* or group* or type* or typing or status)).ti,ab,kf. (12985)
6 Rh Isoimmunization/ (2746)
7 ((isoimmun* or iso-immun* or alloimmun* or allo-immun* or unsensiti#ed or un-sensiti#ed or non-sensiti#ed or nonsensiti#ed or sensiti#ation* or sensiti#ed or sensibili#ation) adj6 (rh or rhesus or maternal or mother* or pregnan*)).ti,ab,kf. (7085)
8 ((f?etomaternal or f?eto-maternal) adj2 immuni#ation).ti,ab,kf. (111)
9 ((rh or rhesus) adj2 (immuni* or autoimmuni*)).ti,ab,kf. (1856)
10 Erythroblastosis, Fetal/ (10462)
11 ((h?emolytic adj2 (disease* or disorder*)) or HDFN).ti,ab,kf. (9203)
12 ((rhesus or rh) adj2 (disease* or disorder* or incompatib* or antagonism)).ti,ab,kf. (2698)
13 ((erythroblastos#s or erythroblastotic) adj2 (f?etal* or f?etus*)).ti,ab,kf. (3310)
14 or/1-13 (52376)
15 Prenatal Diagnosis/ (88482)
16 Maternal Serum Screening Tests/ (659)
17 Hematologic Tests/ (21312)
18 ((prenatal or pre-natal or antenatal or ante-natal or f?etal or f?etus* or noninvasive* or non-invasive*) adj3 (test* or screen* or diagnos* or determin* or detect*)).ti,ab,kf. (192763)
19 (NIPT or NIPD or NIDT or gNIPT or NIPS).ti,ab,kf. (3499)
20 ((maternal or mother*) adj2 (plasm* or serum)).ti,ab,kf. (31582)
21 Genotyping Techniques/ (12292)
22 (genotyp* adj2 (f?etal or f?etus* or prenatal or pre-natal or antenatal or ante-natal or maternal or pregnan* or mother* or noninvasive or non-invasive)).ti,ab,kf. (4132)
23 (((f?etal or f?etus* or free-f?etal or placenta*) adj2 dna) or cell-free dna).ti,ab,kf. (12339)
24 (cff DNA or cffDNA or ccffDNA or ccff DNA or cf DNA or cfDNA or f DNA or fDNA or ff DNA or ffDNA).ti,ab,kf. (5599)
25 or/15-24 (301767)
26 14 and 25 (3784)
27 economics/ (250819)
28 economics, medical/ or economics, pharmaceutical/ or exp economics, hospital/ or economics, nursing/ or economics, dental/ (805846)
29 economics.fs. (415488)

30 (econom* or price or prices or pricing or priced or discount* or expenditure* or budget* or pharmacoeconomic* or pharmaco-economic*).ti,ab,kf. (846336)
31 exp "costs and cost analysis"/ (565217)
32 (cost or costs or costing or costly).ti. (253946)
33 cost effective*.ti,ab,kf. (307124)
34 (cost* adj2 (util* or efficacy* or benefit* or minimi* or analy* or saving* or estimate* or allocation or control or sharing or instrument* or technolog*)).ab,kf. (201071)
35 models, economic/ (12263)
36 markov chains/ or monte carlo method/ (77608)
37 (decision adj1 (tree* or analy* or model*)).ti,ab,kf. (39853)
38 (markov or markow or monte carlo).ti,ab,kf. (123358)
39 quality-adjusted life years/ (38033)
40 (QOLY or QOLYs or HRQOL or HRQOLs or QALY or QALYs or QALE or QALEs).ti,ab,kf. (67382)
41 ((adjusted adj1 (quality or life)) or (willing* adj2 pay) or sensitivity analys*s).ti,ab,kf. (110263)
42 or/27-41 (2448881)
43 26 and 42 (204)
44 exp Animals/ not Humans/ (15942237)
45 43 not 44 (141)
46 Case Reports/ or Comment.pt. or Editorial.pt. or (Letter not (Letter and Randomized Controlled Trial)).pt. or Congresses.pt. (5012448)
47 45 not 46 (130)
48 limit 47 to english language [Limit not valid in CDSR; records were retained] (112)
49 48 use medall,coch,cctr,clhta (61)
50 26 use cleed (2)
51 49 or 50 (63)
52 limit 51 to yr="1997 -Current" (55)
53 blood group rhesus system/ (3032)
54 rhesus D antigen/ (1180)
55 (RhD* or rhesus D* or Rh D*).tw,kw. (17315)
56 (Rh-negativ* or Rh-positiv*).tw,kw. (2085)
57 (Rhesus negativ* or Rhesus positiv*).tw,kw. (583)
58 ((rh or rhesus) adj2 (factor$1 or antigen* or system* or group* or type* or typing or status)).tw,kw. (11461)
59 rhesus isoimmunization/ (2823)
60 ((isoimmun* or iso-immun* or alloimmun* or allo-immun* or unsensiti#ed or un-sensiti#ed or non-sensiti#ed or nonsensiti#ed or sensiti#ation* or sensiti#ed or sensibili#ation) adj6 (rh or rhesus or maternal or mother* or pregnan*)).tw,kw. (7189)
61 ((f?etomaternal or f?eto-maternal) adj2 immuni#ation).tw,kw. (113)
62 ((rh or rhesus) adj2 (immuni* or autoimmuni*)).tw,kw. (1869)
63 newborn hemolytic disease/ (10842)
64 ((h?emolytic adj2 (disease* or disorder*)) or HDFN).tw,kw. (9352)
65 ((rhesus or rh) adj2 (disease* or disorder* or incompatib* or antagonism)).tw,kw. (3242)
66 ((erythroblastos#s or erythroblastotic) adj2 (f?etal* or f?etus*)).tw,kw. (1201)
67 or/53-66 (49324)
68 prenatal diagnosis/ (88482)
69 prenatal screening/ (43711)
70 maternal serum screening test/ (654)
71 blood examination/ (13020)
72 non invasive procedure/ (25423)

73 ((prenatal or pre-natal or antenatal or ante-natal or f?etal or f?etus* or noninvasive* or non-invasive*) adj3 (test* or screen* or diagnos* or determin* or detect*)).tw,kw,dv. (196395)
74 (NIPT or NIPD or NIDT or gNIPT or NIPS).tw,kw,dv. (3580)
75 ((maternal or mother*) adj2 (plasm* or serum)).tw,kw,dv. (31840)
76 genotyping technique/ (13418)
77 (genotyp* adj2 (f?etal or f?etus* or prenatal or pre-natal or antenatal or ante-natal or maternal or pregnan* or mother* or noninvasive or non-invasive)).tw,kw,dv. (4171)
78 (((f?etal or f?etus* or free-f?etal or placenta*) adj2 dna) or cell-free dna).tw,kw,dv. (12587)
79 (cff DNA or cffDNA or ccffDNA or ccff DNA or cf DNA or cfDNA or f DNA or fDNA or ff DNA or ffDNA).tw,kw,dv. (5668)
80 or/68-79 (320501)
81 67 and 80 (3750)
82 Economics/ (250819)
83 Health Economics/ or Pharmacoeconomics/ or Drug Cost/ or Drug Formulary/ (126196)
84 Economic Aspect/ or exp Economic Evaluation/ (442477)
85 (econom* or price or prices or pricing or priced or discount* or expenditure* or budget* or pharmacoeconomic* or pharmaco-economic*).tw,kw. (871707)
86 exp "Cost"/ (565217)
87 (cost or costs or costing or costly).ti. (253946)
88 cost effective*.tw,kw. (319640)
89 (cost* adj2 (util* or efficac* or benefit* or minimi* or analy* or saving* or estimate* or allocation or control or sharing or instrument* or technolog*)).ab,kw. (211051)
90 Monte Carlo Method/ (61972)
91 (decision adj1 (tree* or analy* or model*)).tw,kw. (43655)
92 (markov or markow or monte carlo).tw,kw. (128469)
93 Quality-Adjusted Life Years/ (38033)
94 (QOLY or QOLYs or HRQOL or HRQOLs or QALY or QALYs or QALE or QALEs).tw,kw. (71197)
95 ((adjusted adj1 (quality or life)) or (willing* adj2 pay) or sensitivity analys*s).tw,kw. (131092)
96 or/82-95 (2094943)
97 81 and 96 (213)
98 (exp animal/ or nonhuman/) not exp human/ (10171975)
99 97 not 98 (212)
100 Case Report/ or Comment/ or Editorial/ or (letter.pt. not (letter.pt. and randomized controlled trial/)) or conference abstract.pt. (10179031)
101 99 not 100 (155)
102 limit 101 to english language [Limit not valid in CDSR; records were retained] (137)
103 102 use emez (66)
104 limit 103 to yr="1997 -Current" (59)
105 52 or 104 (114)
106 105 use medall (50)
107 105 use coch (0)
108 105 use cctr (3)
109 105 use cleed (2)
110 105 use clhta (0)
111 105 use emez (59)
112 remove duplicates from 105 (76)

Grey Literature Search

Search date: March 06-08 and 12, 2019; Repeated on July 26, 2019

Websites searched: Pan Canadian HTA Collaborative, Alberta Health and Wellness, BC Health Technology Assessments, Canadian Agency for Drugs and Technologies in Health (CADTH), Institut national d'excellence en santé et en services sociaux (INESSS), Institute of Health Economics (IHE), McGill University Health Centre Health Technology Assessment Unit, Laval University, HTA Database (York University, UK), Epistemonikos, National Institute for Health and Care Excellence (NICE), Agency for Healthcare Research and Quality (AHRQ) Australian Government Medical Services Advisory Committee (MSAC), Council of Australian Governments Health Technologies, Centers for Medicare & Medicaid Services Technology Assessments, Institute for Clinical and Economic Review, Ireland Health Information and Quality Authority, Washington State Health Care Authority Health Technology Assessment Findings Healthcare Improvement Scotland, Tufts Registry, SickKids PEDE Database, ClinicalTrials.gov, PROSPERO, EUnetHTA

Keywords used: Genotyping, non-invasive prenatal, noninvasive prenatal, nipt, nipd, nidt, nips, cell-free dna, (cell-free AND dna), cc dna, cf dna, cff dna, ccff dna, rhd, rhesus, rh d, alloimmunized, allo- immunized, alloimmunization, Isoimmunized, iso-immunized, iso-immunization, sensitized, unsensitized, un-sensitized, hemolytic, haemolytic, HDFN

Clinical results (included in PRISMA): 1
Economic results (included in PRISMA): 4
Ongoing health technology assessments (PROSPERO/EUnetHTA/MSAC): 1
Ongoing randomized controlled trials: (ClinicalTrials.gov): 4

Search for Intervention-Related Health State Utilities

Search date: March 12, 2019Ovid MEDLINE(R) ALL <1946 to March 11, 2019

#	Searches	Results
1	Rh-Hr Blood-Group System/	10644
2	(RhD* or rhesus D* or Rh D*).ti,ab,kf.	6877
3	(Rh-negativ* or Rh-positiv*).ti,ab,kf.	965
4	(Rhesus negativ* or Rhesus positiv*).ti,ab,kf.	252
5	((rh or rhesus) adj2 (factor$1 or antigen* or system* or group* or type* or typing or status)).ti,ab,kf.	7173
6	Rh Isoimmunization/	1690
7	((isoimmun* or iso-immun* or alloimmun* or allo-immun* or unsensiti#ed or un-sensiti#ed or non-sensiti#ed or nonsensiti#ed or sensiti#ation* or sensiti#ed or sensibili#ation) adj6 (rh or rhesus or maternal or mother* or pregnan*)).ti,ab,kf.	3491
8	((f?etomaternal or f?eto-maternal) adj2 immuni#ation).ti,ab,kf.	78
9	((rh or rhesus) adj2 (immuni* or autoimmuni*)).ti,ab,kf.	1087
10	Erythroblastosis, Fetal/	8516
11	((h?emolytic adj2 (disease* or disorder*)) or HDFN).ti,ab,kf.	4905
12	((rhesus or rh) adj2 (disease* or disorder* or incompatib* or antagonism)).ti,ab,kf.	1480
13	((erythroblastos#s or erythroblastotic) adj2 (f?etal* or f?etus*)).ti,ab,kf.	3078
14	or/1-13	28900
15	Quality-Adjusted Life Years/	10769
16	(quality adjusted or adjusted life year*).tw.	14454

17 (qaly* or qald* or qale* or qtime*).tw. 9258
18 (illness state$1 or health state$1).tw. 5826
19 (hui or hui1 or hui2 or hui3).tw. 1349
20 (multiattribute* or multi attribute*).tw. 797
21 (utility adj3 (score$1 or valu* or health* or cost* or measure* or disease* or mean or gain or gains or index*)).tw. 12738
22 utilities.tw. 6343
23 (eq-5d or eq5d or eq-5 or eq5 or euro qual or euroqual or euro qual5d or euroqual5d or euro qol or euroqol or euro qol5d or euroqol5d or euro quol or euroquol or euro quol5d or euroquol5d or eur qol or eurqol or eur qol5d or eurqol5d or euro?qul or eur?qul5d or euro* quality of life or European qol).tw. 9557
24 (euro* adj3 (5 d or 5d or 5 dimension* or 5dimension* or 5 domain* or 5domain*)).tw. 3309
25 (sf36* or sf 36* or sf thirtysix or sf thirty six).tw. 20180
26 (time trade off$1 or time tradeoff$1 or tto or timetradeoff$1).tw. 1735
27 ((qol or hrqol or quality of life).ti. or *quality of life/) and ((qol or hrqol* or quality of life) adj2 (increas* or decreas* or improve* or declin* or reduc* or high* or low* or effect or effects of worse or score or scores or change$1 or impact$1 or impacted or deteriorate$)).ab. 27974
28 Cost-Benefit Analysis/ and (cost effectiveness ratio* and (perspective* or life expectanc*)).tw. 2983
29 *quality of life/ and (quality of life or qol).ti. 48711
30 quality of life/ and ((quality of life or qol) adj3 (improve* or chang*)).tw. 21728
31 quality of life/ and ((quality of life or qol) adj (score$1 or measure$1)).tw. 10542
32 quality of life/ and health-related quality of life.tw. 27527
33 quality of life/ and ec.fs. 9292
34 quality of life/ and (health adj3 status).tw. 8031
35 (quality of life or qol).tw. and cost-benefit analysis/ 11101
36 models, economic/ 9218
37 or/15-36 143097
38 14 and 37 47
39 limit 38 to english language 45
40 limit 39 to yr="1997 -Current" 39

Quantitative Evidence of Preferences and Values Search

Database: Ovid MEDLINE(R) ALL <1946 to February 28, 2019>
Search Date: February 28, 2019
Search Strategy:

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1 Rh-Hr Blood-Group System/ (10642)
2 (RhD* or rhesus D* or Rh D*).ti,ab,kf. (6859)
3 (Rh-negativ* or Rh-positiv*).ti,ab,kf. (965)
4 (Rhesus negativ* or Rhesus positiv*).ti,ab,kf. (252)
5 ((rh or rhesus) adj2 (factor$1 or antigen* or system* or group* or type* or typing or status)).ti,ab,kf. (7169)
6 Rh Isoimmunization/ (1688)
7 ((isoimmun* or iso-immun* or alloimmun* or allo-immun* or unsensiti#ed or un-sensiti#ed or non-sensiti#ed or nonsensiti#ed or sensiti#ation* or sensiti#ed or sensibili#ation) adj6 (rh or rhesus or maternal or mother* or pregnan*)).ti,ab,kf. (3488)
8 ((f?etomaternal or f?eto-maternal) adj2 immuni#ation).ti,ab,kf. (78)

9 ((rh or rhesus) adj2 (immuni* or autoimmuni*)).ti,ab,kf. (1087)
10 Erythroblastosis, Fetal/ (8516)
11 ((h?emolytic adj2 (disease* or disorder*)) or HDFN).ti,ab,kf. (4900)
12 ((rhesus or rh) adj2 (disease* or disorder* or incompatib* or antagonism)).ti,ab,kf. (1479)
13 ((erythroblastos#s or erythroblastotic) adj2 (f?etal* or f?etus*)).ti,ab,kf. (3077)
14 or/1-13 (28871)
15 Prenatal Diagnosis/ (35583)
16 Maternal Serum Screening Tests/ (402)
17 Hematologic Tests/ (8873)
18 ((prenatal or pre-natal or antenatal or ante-natal or f?etal or f?etus* or noninvasive* or non-invasive*) adj3 (test* or screen* or diagnos* or determin* or detect*)).ti,ab,kf. (83194)
19 (NIPT or NIPD or NIDT or gNIPT or NIPS).ti,ab,kf. (1306)
20 ((maternal or mother*) adj2 (plasm* or serum)).ti,ab,kf. (14142)
21 Genotyping Techniques/ (5951)
22 (genotyp* adj2 (f?etal or f?etus* or prenatal or pre-natal or antenatal or ante-natal or maternal or pregnan* or mother* or noninvasive or non-invasive)).ti,ab,kf. (1776)
23 (((f?etal or f?etus* or free-f?etal or placenta*) adj2 dna) or cell-free dna).ti,ab,kf. (4742)
24 (cff DNA or cffDNA or ccffDNA or ccff DNA or cf DNA or cfDNA or f DNA or fDNA or ff DNA or ffDNA).ti,ab,kf. (1810)
25 or/15-24 (130357)
26 14 and 25 (1761)
27 Attitude to Health/ (81200)
28 Health Knowledge, Attitudes, Practice/ (101181)
29 Patient Participation/ (23499)
30 Patient Preference/ (6926)
31 Attitude of Health Personnel/ (114360)
32 *Professional-Patient Relations/ (10972)
33 *Physician-Patient Relations/ (33829)
34 Choice Behavior/ (30418)
35 (choice or choices or value* or valuation*).ti. (187251)
36 (preference* or expectation* or attitude* or acceptab* or knowledge or point of view).ti,ab. (1087608)
37 ((patient*1 or user*1 or men or women or personal or provider* or practitioner* or professional*1 or (health* adj2 worker*) or clinician* or physician* or doctor*) adj2 (participation or perspective* or perception* or misperception* or perceiv* or view* or understand* or misunderstand* or value*1)).ti,ab. (109545)
38 health perception*.ti,ab. (2497)
39 *Decision Making/ (38327)
40 (patient*1 or user*1 or men or women or personal or provider* or practitioner* or professional*1 or (health* adj2 worker*) or clinician* or physician* or doctor*).ti. (2257196)
41 39 and 40 (6979)
42 (decision* and mak*).ti. (25824)
43 (decision mak* or decisions mak*).ti,ab. (122727)
44 42 or 43 (124174)
45 (patient*1 or user*1 or men or women or personal or provider* or practitioner* or professional*1 or (health* adj2 worker*) or clinician* or physician* or doctor*).ti,ab. (7451523)
46 44 and 45 (77026)
47 (discrete choice* or decision board* or decision analy* or decision-support or decision tool* or decision aid* or latent class* or decision* conflict* or decision* regret*).ti,ab. (29520)
48 Decision Support Techniques/ (18435)
49 (health and utilit*).ti. (1309)

50 (gamble* or prospect theory or health utilit* or utility value* or utility score* or utility estimate* or health state or feeling thermometer* or best-worst scaling or time trade-off or TTO or probability trade-off).ti,ab. (11842)
51 (preference based or preference score* or preference elicitation or multiattribute or multi attribute).ti,ab. (2467)
52 or/27-38,41,46-51 (1641601)
53 26 and 52 (110)
54 limit 53 to english language (83)
55 limit 54 to yr="1997 -Current" (55)

Appendix 2: Critical Appraisal of Clinical Evidence

Table A1: Risk of Bias[a] Among Systematic Reviews (ROBIS Tool)

	Phase 2				Phase 3
Author, Year	**Study Eligibility Criteria**	**Identification and Selection of Studies**	**Data Collection and Study Appraisal**	**Synthesis and Findings**	**Risk of Bias in the Review**
Yang et al, 2019[39]	Low	Low	Low	Low	**Low**
Mackie et al, 2017[36]	Low	Low	Low	Low	**Low**
Zhu et al, 2014[20]	High	High	High	Low	**High**
Wright and Burton, 2009[38]	High	High	High	Unclear	**High**
Legler et al, 2009[35]	High	High	High	High	**High**
Geifman-Holtzman et al, 2006[34]	Low	Low	High	Low	**Low**

Abbreviation: ROBIS, Risk of Bias in Systematic Reviews.
[a]Possible risk of bias levels: low concerns, high concerns, unclear concerns.

Table A2: Risk of Bias[a] Among Nonrandomized Studies

Author, Year	Selection of Participants	Confounding Variables	Measurement of Exposure	Blinding of Outcome Assessments	Incomplete Outcome Data	Selective Outcome Reporting
Darlington et al, 2018[43]	High	Low	Low	Low	Low	Unclear
Haimila et al, 2017[46]	Low	Low	Low	Low	Low	Low
Papasavva et al, 2016[47]	Low	Unclear	Low	Low	High	Low
de Haas et al, 2016[44]	Low	Low	Low	Low	Low	Low
Soothill et al, 2015[49]	Low	Low	Low	Low	Low	Low
Clausen et al, 2014[41]	Low	Unclear	Low	Low	Low	Low
Tiblad et al, 2013[50]	High	Unclear	Low	Low	Low	Low
Grande et al, 2013[45]	Low	Low	Unclear	Low	High	Low
Damkjær et al, 2012[42]	Low	Low	Low	High	High	Low
Rijnders et al, 2004[48]	Unclear	Unclear	Unclear	Low	Low	Low

[a]Possible risk of bias judgments: low, high, and unclear, based on the Risk of Bias Assessment Tool for Nonrandomized Studies (RoBANS).

Table A3: GRADE Evidence Profile for Outcomes of Nonalloimmunized RhD− Pregnancies With Noninvasive Fetal RhD Blood Group Genotyping

Number of Studies (Design)	Risk of Bias	Inconsistency	Indirectness	Imprecision	Publication Bias	Upgrade Considerations	Quality
Unnecessary RhIG Prophylaxis Avoided							
8 (observational)[41-43,45-47,49,50]	No serious limitations[a]	No serious limitations	No serious limitations	No serious limitations[a]	Undetected	None	⊕⊕ Low
Compliance With Targeted RhIG Prophylaxis							
5 (observational)[41-43,49,50]	Serious limitations(−1)[b]	No serious limitations	No serious limitations	No serious limitations[a]	Undetected	None	⊕ Very low
Uptake of RhD Genotyping							
4 (observational)[44-46,50]	No serious limitations[a]	No serious limitations	No serious limitations	No serious limitations[a]	Undetected	None	⊕⊕ Low
Alloimmunization							
1 (observational)[50]	Serious limitations(−1)[c]	No serious limitations	Serious limitations(−1)[d]	No serious limitations[a]	Undetected	None	⊕ Very low

Abbreviations: GRADE, Grading of Recommendations Assessment, Development, and Evaluation; RhD, rhesus D blood group.

[a]Some potential limitations with unclear risk of bias or some degree of imprecision, but not judged to be serious to warrant down grading of the body of evidence.

[b]Two studies were at high risk of bias for participant selection, mainly selection of control group from a different time period (Tiblad[50]) or both different time period and centres (Darlington[43]) compared with the RhD genotyping group.

[c]High risk of bias for participant selection because the control group was selected from a different time period than the intervention group, and unclear risk of bias for handling of confounding variables (see Table A2 for details).

[d]Universal prophylaxis in Swedish historical cohort differs from Canadian guidelines as it does not include routine prenatal RhIG prophylaxis and may lead to higher rates of alloimmunization due to administering prophylaxis only at birth or following any event that may increase fetomaternal hemorrhage and risk of alloimmunization during pregnancy.

Table A4: GRADE Evidence Profile for Outcomes of Alloimmunized RhD− Pregnancies With Noninvasive Fetal RhD Blood Group Genotyping

Number of Studies (Design)	Risk of Bias	Inconsistency	Indirectness	Imprecision	Publication Bias	Upgrade Considerations	Quality
Invasive Procedures Avoided							
1 (Observational)[48]	Serious limitations(−1)[a]	No serious limitations[b]	No serious limitations	No serious limitations[b]	Undetected	None	⊕ Very low

Abbreviations: GRADE, Grading of Recommendations Assessment, Development, and Evaluation; RhD, rhesus D blood group.

[a]Unclear risk of bias introduced by participant selection, confounding variables and measurement of intervention.

[b]Not evaluable because there is only a single study.

Appendix 3: Selected Excluded Studies—Clinical Evidence

For transparency, we provide a list of studies that readers might have expected to see but that did not meet our inclusion criteria, along with the primary reason for exclusion. This list includes studies considered for either or both the diagnostic accuracy overview of reviews or the systematic review of clinical utility.

Citation	Primary Reason for Exclusion
Arentz-Hansen H, Brurberg KG, Kvamme MK, Stoinska-Schneider A, Hofmann B, Ormstad SS, et al. [Determination of fetal rhesus D status from maternal plasma of rhesus negative women.] NIPH Systematic Reviews: Executive Summaries. Oslo: Knowledge Centre for the Health Services at The Norwegian Institute of Public Health (NIPH); 2014 Dec. Report No. 25-2014. Norwegian.	Full text not available in English
Demirel E, Kelekci S, Ekmekci E, Sengul M, Iri R, Atasever M. Is the management of Rh-Rh incompatibility with noninvasive fetal Rh genotyping for targeted prophylaxis cost-effective in the Turkish population? Turk J Med Sci. 2018;48(1):1-4.	Outcomes (hypothetical RhIG avoided)
Chitty LS, Finning K, Wade A, Soothill P, Martin B, Oxenford K, et al. Diagnostic accuracy of routine antenatal determination of fetal RHD status across gestation: population based cohort study. BMJ. 2014;349:g5243.	Study type/outcomes (primary study of diagnostic accuracy[a])
Gowri V. Non-invasive antenatal diagnosis of fetal rhesus status in an alloimmunised patient. BMJ Case Rep. 2009.	Study type (case report)
Sorensen K, Kjeldsen-Kragh J, Husby H, Akkok CA. Determination of fetal RHD type in plasma of RhD negative pregnant women. Scand J Clin Lab Invest. 2018;78(5):411-6.	Outcomes (hypothetical RhIG avoided)
Swedish Council on Health Technology Assessment (SBU). [Analysis of fetal DNA in maternal blood: noninvasive fetal diagnostic tests for blood group and sex determination. Summary and conclusions.] SBU. 2011;07(11):16. Swedish.	Full text not available in English

Abbreviation: RhIG, Rh immunoglobulin.

[a]51 citations were excluded because they were not eligible for either the overview of reviews on accuracy nor clinical utility systematic review (i.e., excluded primary studies on diagnostic test accuracy).

Appendix 4: Selected Excluded Studies—Economic Evidence

For transparency, we provide a list of studies that readers might have expected to see in the economic evidence review but that did not meet the inclusion criteria, along with the primary reason for exclusion.

Citation	Primary Reason for Exclusion
Abbey R and Dunsmoor-Su RI. Cost-benefit analysis of indirect antiglobulin screening in RhD-negative women at 28 weeks of gestation. Obstet Gynecol. 2014;123:5.	Not intervention of interest
Allard S, Massey E. Fetal RHD genotyping is a cost-effective option for supporting targeted anti-D prophylaxis in D-negative pregnancies. BJOG. 2018;125(11):1423.	Commentary, not economic evaluation
Arentz-Hansen H, Brurberg KG, Kvamme MK, Stoinska-Schneider A, Hofmann B, Ormstad SS, et al. [Determination of fetal rhesus D status from maternal plasma of rhesus negative women.] NIPH Systematic Reviews: Executive Summaries. Oslo: Knowledge Centre for the Health Services at The Norwegian Institute of Public Health (NIPH); 2014 Dec. Report No. 25-2014. Norwegian.	Full text not available in English
Benachi A, Delahaye S, Leticee N, Jouannic JM, Ville Y, Costa JM. Impact of non-invasive fetal RhD genotyping on management costs of rhesus-D negative patients: results of a French pilot study. Eur J Obstet Gynecol Reprod Biol. 2012;162(1):28-32.	Feasibility costing study, no ICER reported
Centre for Reviews and Dissemination. Costs and clinical outcomes of noninvasive fetal RhD typing for targeted prophylaxis (Provisional abstract). NHS Economic Evaluation Database. 2015.	Commentary (summary of HTA)
Centre for Reviews and Dissemination. Standardization non-invasive fetal RHD and SRY determination into clinical routine using a new multiplex RT-PCR assay for fetal cell-free DNA in pregnant women plasma: results in clinical benefits and cost saving (provisional abstract). NHS Economic Evaluation Database. 2015.	Commentary (summary of HTA)
Demirel E, Kelekci S, Ekmekci E, Sengul M, Iri R, Atasever M. Is the management of Rh-Rh incompatibility with noninvasive fetal Rh genotyping for targeted prophylaxis cost-effective in the Turkish population? Turk J Med Sci. 2018;48:1-4.	Costing analysis, noncomparative retrospective study, no ICER reported
Kacker S, Vassallo R, Keller MA, Westhoff CM, Frick KD, Sandler SG et al. Financial implications of RHD genotyping of pregnant women with a serologic weak D phenotype. Transfusion. 2015;55(9):2095-2103.	Not intervention of interest
Laget L, Izard C, Durieux-Roussel E, Gouvitsos J, Dettori I, Chiaroni J, Ferrera-Tourenc V. Relevance and costs of RHD genotyping in women with a weak D phenotype. Transfus Clin Biol. 2019;26(1):27-31.	Costing analysis, no ICER calculated
Le Ray I, Lee B, Wikman A, Reilly M. Evaluation of a decision tree for efficient antenatal red blood cell antibody screening. Epidemiology. 2018;29(3):453-7.	Not economic evaluation
Ma KK, Rodriguez MI, Cheng YW, Norton ME, Caughey AB. Should cell-free DNA testing be used to target antenatal rhesus immune globulin administration? J Matern Fetal Neonatal Med. 2016;29(11):1866-70.	No ICER reported
Saramago P, Yang H, Llewellyn A, Walker R, Harden M, Palmer S et al. High-throughput non-invasive prenatal testing for fetal rhesus D status in RhD-negative women not known to be sensitised to the RhD antigen: a systematic review and economic evaluation. NICE Health Technology Assessment. 2018;22(13).	Duplicate findings of 2018 NICE HTA report
Swedish Council on Health Technology Assessment (SBU). [Analysis of fetal DNA in maternal blood: noninvasive fetal diagnostic tests for blood group and sex determination. Summary and conclusions.] SBU. 2011;07(11):16. Swedish.	Full text not available in English
Szczepura A, Osipenko L, Freeman K. A new fetal RHD genotyping test: costs and benefits of mass testing to target antenatal anti-D prophylaxis in England and Wales. BMC Pregnancy Childbirth. 2011;11:5.	Costing analysis, no ICER reported

Abbreviations: HTA, health technology assessment; ICER, incremental cost-effectiveness ratio; NHS, National Health Service; NICE, National Institute for Health and Care Excellence.

Appendix 5: Results of Applicability and Limitation Checklists for Studies Included in the Economic Literature Review

Table A5: Assessment of the Applicability of Studies Evaluating the Cost-Effectiveness of Noninvasive RhD Genotyping

Author, Year, Country of Publication	Is the study population similar to the question?	Are the interventions similar to the question?	Is the health care system studied sufficiently similar to Ontario?	Were the perspectives clearly stated? If yes, what were they?	Are all direct effects included? Are all other effects included where they are material?	Are all future costs and outcomes discounted? If yes, at what rate?	Is the value of health effects expressed in terms of quality-adjusted life-years?	Are costs and outcomes from other sectors fully and appropriately measured and valued?	Overall Judgment[a]
Moise et al, 2019,[57] US	Yes	Partially	No	Yes, health care sector	Unclear	NA	No	Partially	Partially applicable
Darlington et al, 2018,[43] France	Partially	Partially	Unclear	Yes, health care sector	No	NA	No	Partially	Partially applicable
Saramago et al, 2018,[56] UK	Partially	Partially	Partially	Yes, health care sector	Yes	Yes, 3.5%	Yes	Yes	Partially applicable
Gordon et al, 2017,[59] Australia	Partially	Partially	No	Yes, health care sector	Yes	NA	No	Unclear	Partially applicable
Neovius et al, 2015,[60] Sweden	Yes	Yes	Partially	Yes, health care sector	Unclear	Partially, 3%	No	Partially	Partially applicable
Teitelbaum et al, 2015,[62] Canada	Partially	Yes	Yes	Unclear, health care sector/payer	No	NA	No	Partially	Partially applicable
Hawk et al, 2013,[58] US	Partially	Yes	No	Yes, health care sector	Unclear	NA	No	No	Partially applicable
Duplantie et al, 2013,[61] Canada	Partially	Yes	Yes	Yes, health care sector/ payer	Unclear	NA	No	Unclear	Partially applicable

Abbreviations: RhD, rhesus D blood group; UK, United Kingdom; US, United States.
Note: Response options for all items were “yes,” “partially,” “no,” “unclear,” and “NA” (not applicable).
[a]Overall judgment may be “directly applicable,” “partially applicable,” or “not applicable.”

Table A6: Assessment of the Limitations of Studies Evaluating the Cost-Effectiveness of Noninvasive Fetal RhD Genotyping

Author, Year, Country of Publication	Does the model structure adequately reflect the nature of the health condition under evaluation?	Is the time horizon sufficiently long to reflect all important differences in costs and outcomes?	Are all important and relevant health outcomes included?	Are the clinical inputs[a] obtained from the best available sources?	Do the clinical inputs[a] match the estimates contained in the clinical sources?	Are all important and relevant (direct) costs included in the analysis?	Are the estimates of resource use obtained from the best available sources?	Are the unit costs of resources obtained from the best available sources?	Is an appropriate incremental analysis presented, or can it be calculated from the reported data?	Are all important and uncertain parameters subjected to appropriate sensitivity analysis?	Is there a potential conflict of interest?	Overall Judgment[b]
Moise et al, 2019,[57] US	Partially	Partially	No	Unclear	Unclear	Yes	Yes	Unclear	No		Unclear	Potentially serious limitations
Darlington et al, 2018,[43] France	Partially	No	No	No	Unclear	Yes	Yes	Unclear	Yes	No	Unclear	Potentially serious limitations
Saramago et al, 2018,[56] UK	Partially	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Yes	Unclear	Minor limitations
Gordon et al, 2017,[59] Australia	Partially	No	Partially	Unclear	Unclear	Yes	Yes	Unclear	No	No	Unclear	Very serious limitations
Neovius et al, 2015,[60] Sweden	Partially	Partially	No	Unclear	Unclear	Yes	Yes	Yes	Yes	Unclear	Unclear	Potentially serious limitations
Teitelbaum et al, 2015,[62] Canada	Partially	Partially	Partially	Unclear	Unclear	Partially	Unclear	Unclear	No	No	Unclear	Very serious limitations
Hawk et al, 2013,[58] US	Partially	Partially	No	Unclear	Unclear	Partially	Unclear	Unclear	Partially	Partially	Unclear	Potentially serious limitations
Duplantie et al, 2013,[61] Canada	Partially	Partially	Yes	Partially	Unclear	Partially	Yes	Yes	No (average CERs)	Unclear	Unclear	Potentially serious limitations

Abbreviation: CER, average cost-effectiveness ratio; RhD, rhesus D blood group; UK, United Kingdom; US, United States.
Note: Response options for all items were “yes,” “partially,” “no,” “unclear,” and “NA” (not applicable).
[a]Clinical inputs include relative treatment effects, natural history, and utilities.
[b]Overall judgment may be “minor limitations,” “potentially serious limitations,” or very serious limitations.”

Appendix 6: Costing Methodology, Primary Economic Evaluation

Cost of Noninvasive Fetal RhD Blood Group Genotyping: Nonalloimmunized and Alloimmunized Pregnancies

In this appendix, we describe the costing approach, developed by the laboratory at Mount Sinai Hospital, that fully validated the noninvasive fetal Rh genotyping test conducted in the reference laboratory in Bristol, UK (email communication, R. Kandel, MD, and G. Charames, PhD, July 2019).

Four types of noninvasive fetal Rh genotyping tests were proposed for this project: RhD alone, RHCE (c, C, and E alleles), Kell, and RhD Asian variant (RHDc) (email communication, R. Kandel, MD, and G. Charames, PhD, July 2019). All tests will also include *SRY* and a housekeeping DNA control (e.g., *CCR5*). The test for RhD is the one indicated in the majority of nonalloimmunized pregnancies, while all four tests are currently required for alloimmunized pregnancies and are sent to the UK reference laboratory. Ministry of Health coverage for testing is provided via the Out-of-Country Prior Approval Program.

Table A7 presents the cost estimates for the first genotyping test (i.e., RhD) and each additional test. The cost estimate is broken down by categories commonly reimbursed by the Ministry. It also includes the percentage cost associated with quality assurance and quality control. Next, Table A7 (column 4) shows the cost for each additional test if two or more alleles are needed, which is required for alloimmunized pregnancies.

The exons and alleles included in each of the four noninvasive fetal Rh genotyping tests are as follows (email communication, R. Kandel, MD, and G. Charames, PhD, July 2019):

1. RhD:

- *RHD* exon 5 (including the *RHD* pseudogene; representative of a deletion in African Americans)
- *RHD* exon 7 (often involved in hybrid alleles, such as Cde^{s} *RHD-CE-D*)
- *RHD* exon 10 (rarely involved in hybrid alleles; loss of exons 5, 7, and 10 are indicative of entire gene deletion)

2. RHCE:

- *RHCE**c allele; exon 2 (c.307C>T; p.Pro103Ser)
- *RHCE**C allele; exon 2 (109bp insertion)
- *RHCE**E allele; exon 5 (c.676G>C; p.Ala226Pro)

3. Kell:

- *KEL**01 ‘K allele’; (c.698C>T; p.Thr193Met)

4. RhD Asian variant:

- *RHD* c.1227G>A (exon 9; a deleterious Asian variant)

Table A7: Costing of Noninvasive Fetal Rh Genotyping, Four Types of Tests[a,b]

Costing Item	FTE	Cost per Sample, 1st Test, $	Cost per Additional Test, $
Labour			
Blood draw (phlebotomist)		25.00	
Sample accessioning (technician)	0.25	9.34	
Plasma separation and DNA extraction (technician)	0.45	16.81	
Testing procedure (technologist)	1.00	52.48	
Analysis (technologist)	0.17	8.75	4.37
Review and signout (professional)		50.00	10.00
Reagents and Supplies			
DNA extraction (from FFPE)		17.50	
qPCR reagents and Consumables		6.36	6.36
Total (Labour, Reagents, Supplies)		**186.22**	**20.73**
Repeats, QA/QC (30%)		**55.87**	**6.22**
Total cost per sample		**242.09**	**26.95**

Abbreviations: FFPE, formalin-fixed, paraffin-embedded (tissues); FTE, full-time equivalent; QA/QC, quality assurance/quality control.

[a]Estimates generated by clinical experts (email communication, R. Kandel, MD and G. Charames, PhD, July 2019). All costs are in 2019 Canadian dollars.

[b]Four types of noninvasive fetal Rh genotyping tests: RhD alone, RHCE (c, C, and E alleles), KEL, and RhHD Asian variant (RHDc).

Appendix 7: Resource Use and Costs, Primary Economic Evaluation, Original Data

Cost of Clinical Pathways: Nonalloimmunized and Alloimmunized Pregnancies

In Tables 18 and 19, cost estimates are reported in 2019 Canadian dollars and some inputs are adjusted for inflation using the Consumer Price Index.[109] Here we report the original mean estimates for procedure codes, study samples, and standard deviations that we used to estimate standard errors of gamma distributions for our model parameters.

Nonalloimmunized Pregnancies

Table A8: Cost Parameters: Nonalloimmunized Pregnancies

Parameter Description	Unit Cost, $, Mean (N; SD, $)	Frequency	Source
RhIG Administration			
Administration of RhIG, ambulatory (procedure)	256.71 (10; 216.74)	1	8ZZ70HABW; 2017/17 OCCI (2017/18)[104]
Administration of RhIG, inpatient after cord blood sampling or invasive procedure (procedure)	181.04 (1,010; 156.11)	1	8ZZ70HABW; ICD: O36013, OCCI (2016/17)[104]
Maternal and Fetal Clinical Care			
Obstetrical ultrasound (procedure)	397.41 (13,742; 230.64)	3	5AB03JA, OCCI (2017/18)[104]
Termination of pregnancy (procedure, day surgery)	1,302.43 (158; 430.00)	1	5CA89GA, OCCI (2017/18)[104]
Delivery, vaginal, occurs 71% of the time[94,95] (procedure)	3,509.04 (1,643; 1,929.72)	1	CGM P562, OCCI (2017/18)[104]
Delivery, Caesarean, occurs 29% of the time[94,95] (procedure)	8971.12 (55; 6,098.66)	1	CGM P559, OCCI (2017/18)[104]
Cord blood sampling (procedure)	752.00 (570; 564.51)	1	5MD11TA, OCCI (2017/18)[104]

Abbreviations: CGM, Case Group Mix grouper code; ICD, International Classification of Diseases code; N, sample size; OCCI, Ontario Case Costing Initiative; RhIG, Rh immunoglobulin; SD, standard deviation.

Alloimmunized Pregnancies

Table A9: Cost Parameters: Alloimmunized Pregnancies

Parameter Description	Unit Cost, $, Mean (N; SD, $)[a,b]	Frequency	Source
Maternal-Fetal Clinical Care			
Obstetrical ultrasound (procedure)	397.41 (13,742; 230.64)	3	5AB03JA, OCCI (2017/18)[104]
Middle cerebral artery Doppler ultrasound screening: monthly if titer is not critical; biweekly if titer is critical (> 16) and twice a week before IUT (procedure)	682.94 (11; 229.72)	1	5AB03GS, OCCI (2017/18)[104]
Maternal care, severe HDFN, 6 hospital days (procedure)	5,953.07 (7; 3,701.17)	1	ICD: O36231, OCCI (2015/16)[104]
Intrauterine transfusion, 3–5 times, severe HDFN (procedure)	2,743.51 (18; 900.68)	1	5FD72HAU1, OCCI (2017/18)[104]
Termination of pregnancy (procedure, day surgery)	1,302.43 (158; 430.00)	1	5CA89GA, OCCI (2017/18)[104]
Delivery			
Delivery, vaginal, occurs 71% of the time[94,95] (procedure)	3,509.04 (1,643; 1,929.72)	1	CGM P562, OCCI (2017/18)[104]
Delivery, Caesarean, occurs 29% of the time[94,95] (procedure)	8971.12 (55; 6,098.66)	1	CGM P559, OCCI (2017/18)[104]
Cord blood sampling (procedure)	752.00 (570; 564.51)	1	5MD11TA, OCCI (2017/18)[104]
Neonatal Clinical Care			
Phototherapy, procedure, 1 day, in ward (procedure, not NICU)	1,780.81 (3,596; 1,612.42)	1	1YZ12JADQ, CGM: P581,P585,P588,P589,P598,P599, OCCI (2015/16)[104]
NICU, level II, inpatient, 7.5 hospital days (procedure)	9183.34 (10; 12,512.12)	1	CGM P590, P598, P599, OCCI (2017/18)[104]
Exchange transfusion (NICU), severe HDFN (procedure)	2482.98 (29; 1023.46)	1	CGM P557 plus multiple codes related to this procedure 1LZ19HHU1A,1LZ19HHU1J, 1LZ19HHU9A, 1LZ19HMU1, 5FD72HAU1, 5FD72HAU4, 5FD72HAU9, OCCI (2017/18)[104]

Abbreviations: CGM, Case Group Mix grouper code; HDFN, hemolytic disease of the fetus and newborn; ICD, International Classification of Diseases code; IUT, intrauterine transfusion; N, sample size; NICU, neonatal intensive care unit; OCCI, Ontario Case Costing Initiative; RhIG, Rh immunoglobulin; SD, standard deviation.

Appendix 8: Results, Primary Economic Evaluation: Probabilistic Scenario Analyses

Table A10: Probabilistic Scenario Analyses: Noninvasive Fetal RhD Genotyping vs. Usual Care[a]

Scenario	ICER ($/QALY): Fetal RhD Genotyping vs. Usual Care; INB > or < 0 ($)[b]	
	Nonalloimmunized Pregnancies	Alloimmunized Pregnancies
Reference case analysis[c]		
Time horizon: 10 years	ICER: Dominated; INB < 0 Δ C = $154; Δ E (mean) = −0.00005 Δ C = $154; Δ E (median) = 0.001	ICER: Dominant; INB > 0 Δ C = –$6,280; Δ E = 0.185
Inclusion of paternal phenotyping in both strategies		
	ICER: Dominant; INB > 0 **Δ C = −$87; Δ E = 0.0016**	ICER: Dominant; INB > 0 Δ C = −$1,891; Δ E = 0.05
Probability of inconclusive results		
Reference case: 7%		
Scenario A: 2%	ICER: 531,034; INB < 0 Δ C = $154; Δ E = 0.00029	ICER: Dominant; INB > 0 Δ C = −$6,616; Δ E = 0.195
Scenario B: 10%	ICER: 855,556; INB < 0 Δ C = $154; Δ E = 0.00018	ICER: Dominant; INB > 0 Δ C = −$6,042; Δ E = 0.178
Accuracy of fetal RhD genotyping		
Reference case: Sn, 99.7%; Sp, 96.1%[39]		
Scenario: Sn, 99.7%; Sp, 98.7%[39]	ICER: 416,216; INB < 0 Δ C = $154; Δ E = 0.00037	ICER: Dominant; INB > 0 Δ C = −$6,211; Δ E = 0.183
Probability of alloimmunization		
Reference case RhIG after 28 weeks and birth: 0.002[62] RhIG after birth only: 0.016[62] RhIG not received: 0.12		
Scenario RhIG after 28 weeks and birth: 0.0031 (95% CI, 0.0021–0.0040)[57] RhIG after birth only: 0.0067 (95% CI, 0.0050–0.0084)[57] RhIG not received: 0.16	ICER: Dominated; INB < 0 Δ C = $154; Δ E = −0.00005	NA
Compliance with fetal RhD genotyping and RhIG		
Reference case: 100%		
Scenario Fetal RhD genotyping: 78% RhIG at 28 weeks and birth: 99% RhIG after bleeding events: 95%	ICER: Dominated; INB < 0 Δ C = $153; Δ E = −0.00134	NA
Analysis perspective		
Reference case: Ministry of Health		
Scenario: Societal perspective	NA	ICER: Dominant; INB > 0 Δ C = −$6,186; Δ E = 0.185
Per-sample cost of testing		
Reference case: Estimated by experts Nonalloimmunized $247.34; alloimmunized $328.19		
Scenarios: Testing provided via OOC-PA Both populations: $510	ICER: Dominated; INB < 0 Δ C = $416; Δ E = −0.00005	ICER: Dominant; INB > 0 Δ C = −$6,098; Δ E = 0.185

Scenario	ICER ($/QALY): Fetal RhD Genotyping vs. Usual Care; INB > or < 0 ($)[b]	
	Nonalloimmunized Pregnancies	Alloimmunized Pregnancies
Both populations: $710	ICER: Dominated; INB < 0 Δ C = $615; Δ E = −0.00005	ICER: Dominant; INB > 0 Δ C = −$5,898; Δ E = 0.185
Testing provided by CBS for a lower cost: Nonalloimmunized: $125	ICER: Dominated; INB < 0 Δ C = $31; Δ E = −0.00005	NA
Threshold analysis (one-way sensitivity analysis)	Threshold cost: < $88	NA
PSA scenario, threshold value of $66 (SE: 25% of the mean)	ICER: 547,589; INB > 0 **Δ C = −$28; Δ E = −0.00005** Δ C = −$28; Δ E (median) = 0.001 **CEAC: 57% at $50,000/QALY**	NA (saving for estimated test costs)
Cost of cord blood sampling		
Reference case: Procedure cost		
Scenario: Lab fee cost solely	ICER: Dominated; INB < 0 Δ C = $154; Δ E = −0.00005	ICER: Dominant; INB > 0 Δ C = −$6,298; Δ E = 0.185
Probability of long-term neurodevelopmental problems (p), HSUs and costs of state with major HDFN complications		
Reference case p = 0.048 (SE: 0.003) HSUs = 0.42/0.30, costs combined, major developmental issues and cerebral palsy		
Scenario p = 0.016 (3 times smaller the reference case); HSUs and costs kept the same	NA	ICER: Dominant; INB > 0 Δ C = −$6,034; Δ E = 0.178
Long-term costs and HSUs of the health states with major HDFN complications		
Reference case: HSU = 0.42/0.30, costs combined, major developmental issues and cerebral palsy	NA	
Scenario A, assumed: • HSUs of major developmental problems in general: 0.42 (SE: 0.03)[37] • Costs of major developmental issues in general (costs of cerebral palsy excluded)	NA	ICER: Dominant; INB > 0 Δ C = −$6,035; Δ E = 0.178
Scenario B, assumed: • HSUs of major developmental problems in general: 0.42 (SE: 0.03)[37] • Twice lower costs of major developmental issues (costs of cerebral palsy excluded)	NA	ICER: Dominant; INB > 0 Δ C = −$6,032; Δ E = 0.178
Scenario C, assumed: • HSUs of major developmental problems in general: 0.42 (SE: 0.03)[37] • 10 times higher costs of major developmental issues (costs of cerebral palsy excluded)	NA	ICER: Dominant; INB > 0 Δ C = −$6,089; Δ E = 0.178
Scenario D, assumed: • HSUs of major developmental problems of 0.67 (SE:0.03)[87], based on upper-end HSUs for people with cerebral palsy • Twice lower the reference case cost estimate (including Ontario's estimates for costs of cerebral palsy)	NA	ICER: Dominant; INB > 0 Δ C = −$6,032; Δ E = 0.178
Discount rate		
Reference case: 1.5%		
Scenario: 5%	ICER: Dominated; INB < 0 Δ C = $154; Δ E = −0.00004	ICER: Dominant; INB > 0 Δ C = −$6,186; Δ E = 0.155

Scenario	ICER ($/QALY): Fetal RhD Genotyping vs. Usual Care; INB > or < 0 ($)[b]	
	Nonalloimmunized Pregnancies	Alloimmunized Pregnancies
Time horizon		
Reference case: 10 years		
Scenarios[d] 1 year	ICER: Dominated; INB < 0 Δ C = $154; Δ E = −0.000003	ICER: Dominant; INB > 0 Δ C = −$6,151; Δ E = 0.003
5 years	ICER: Dominated; INB < 0 Δ C = $154; Δ E = −0.00002	ICER: Dominant; INB > 0 Δ C = −$6,206; Δ E = 0.09
Lifetime[e]	**ICER: 34,050; INB > 0** Δ C = $154; Δ E = 0.0045	ICER: Dominant; INB > 0 Δ C = −$6,130; Δ E = 0.948
Multiple pregnancies: 2 pregnancies, first nonalloimmunized followed by either non- or alloimmunized pregnancy; assumed the same mean incremental costs and effects as those in the two reference case analyses (see Tables 23b and 24b) and 10-year time horizon		
Scenarios Alloimmunization rate: 0.003, 10-year time horizon	ICER: 164,847; INB > 0 Δ C = $237; Δ E = 0.0014	NA
Alloimmunization rate: 0.016, 10-year time horizon	ICER: 169,215; INB > 0 Δ C = $186; Δ E = 0.0011	NA
Threshold cost: $66 (SE: 25% of the mean), 10-year time horizon and alloimmunization rate: 0.003	ICER: Dominant; NB > 0 Δ C = $–56; Δ E = 0.0014	NA
Alloimmunization rate: 0.003, cost of fetal RhD genotyping same as in reference case, lifetime time horizon	ICER: 32,459; INB > 0 Δ C = $238; Δ E = 0.0073	NA

Abbreviations: Δ E, incremental effects; Δ C, incremental costs; CBS, Canadian Blood Services; HDFN, hemolytic disease of fetus and newborn; HSU, health state utility; ICER, incremental cost-effectiveness ratio; INB, incremental net benefit; MOH, Ontario Ministry of Health; OOC-PA, Out-of-Country Prior Approval Program (MOH); QALY, quality-adjusted life-year; RhIG, Rh immunoglobulin; Sn, sensitivity; Sp, specificity.

[a]All costs are in 2019 Canadian dollars.

[b]ICER = Δ C ÷ Δ E and INB = Δ E X 50,000 − Δ C; if INB($) > 0, then the strategy is cost-effective at a willingness-to-pay of $50,000/QALY gained; otherwise, the strategy (fetal RhD genotyping) is not cost-effective. Dominant or cost-saving strategy means that the intervention is associated with lower costs and greater QALYs. Negative incremental costs indicate savings.

[c]Probabilistic sensitivity analyses included 5,000,000 simulations with 50,000 trials (individual patients); in the reference case, the mean of the distribution shifted due to random sampling error in distribution coverage; thus, we provided both estimates.

[d]All costs and effects were discounted at 1.5% in the reference case and all scenarios, except a last scenario related to 1-year time horizon.

[e]For lifetime time horizon, we also ran analyses on 100,000 trials (10 million simulations).

Appendix 9: Patient Volumes for Budget Impact Scenario of Smaller Rates of Uptake of Fetal Genotyping

Table A11 and Table A12 describe patient volumes of nonalloimmunized and alloimmunized pregnancies for our Ontario-based scenarios using a smaller uptake rate increasing to 15% over the next 5 years.

Table A11: Total Volumes—Nonalloimmunized Pregnancies: Intervention Uptake up to 15%[a]

	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Current Scenario: Nonalloimmunized Pregnancies						
Usual care	21,000	21,210	21,422	21,636	21,853	107,120
Fetal RhD genotyping	0	0	0	0	0	0
Total volume	**21,000**	**21,210**	**21,422**	**21,636**	**21,853**	**107,120**
Future Scenario: Nonalloimmunized Pregnancies						
Usual care	20,370	19,937	19,494	19,040	18,575	97,416
Fetal RhD genotyping	630	1,273	1,928	2,596	3,278	9705
Total volume	**21,000**	**21,210**	**21,422**	**21,636**	**21,853**	**107,120**

[a]Uptake increased from 3% in year 1, by 5% each year to reach 15% in year 5 (future scenario).
Note: Results may appear inexact due to rounding.

Table A12: Total Volumes—Alloimmunized Pregnancies: Intervention Uptake up to 15%[a]

	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Current Scenario: Alloimmunized Pregnancies						
Usual care	1,820	1,838	1,857	1,875	1,894	9,284
Fetal RhD genotyping	0	0	0	0	0	0
Total volume	**1,820**	**1,838**	**1,857**	**1,875**	**1,894**	**9,284**
Future Scenario: Alloimmunized Pregnancies						
Usual care	1,765	1,728	1,690	1,650	1,610	8,443
Fetal RhD genotyping	55	110	167	225	284	841
Total volume	**1,820**	**1,838**	**1,857**	**1,875**	**1,894**	**9,284**

[a]Uptake increased from 3% in year 1, by 5% each year to reach 15% in year 5 (future scenario).
Note: Results may appear inexact due to rounding.

Table A13 and Table A14 describe patient volumes of nonalloimmunized and alloimmunized pregnancies for our Ontario-based scenarios using a smaller uptake rate increasing to 35% over next 5 years.

Table A13: Total Volumes—Nonalloimmunized Pregnancies: Intervention Uptake up to 35%[a]

	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Current Scenario: Nonalloimmunized Pregnancies						
Usual care	21,000	21,210	21,422	21,636	21,853	107,120
Fetal RhD genotyping	0	0	0	0	0	0
Total volume	**21,000**	**21,210**	**21,422**	**21,636**	**21,853**	**107,120**
Future Scenario: Nonalloimmunized Pregnancies						
Usual care	17,850	16,968	16,066	15,145	14,204	80,234
Fetal RhD genotyping	3,150	4,242	5,355	6,491	7,648	26,887
Total volume	**21,000**	**21,210**	**21,422**	**21,636**	**21,853**	**107,120**

[a]Uptake increased from 15% in year 1, by 5% each year to reach 35% in year 5 (future scenario).
Note: Results may appear inexact due to rounding.

Table A14: Total Volumes—Alloimmunized Pregnancies: Intervention Uptake up to 35%[a]

	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Future Scenario: Alloimmunized Pregnancies						
Usual care	1,820	1,838	1,857	1,875	1,894	9,284
Fetal RhD genotyping	0	0	0	0	0	0
Total volume	**1,820**	**1,838**	**1,857**	**1,875**	**1,894**	**9,284**
Future Scenario: Alloimmunized Pregnancies						
Usual care	1,547	1,470	1,393	1,313	1,231	6,953
Fetal RhD genotyping	273	368	464	563	663	2,331
Total volume	**1,820**	**1,838**	**1,857**	**1,875**	**1,894**	**9284**

[a]Uptake increased from 15% in year 1, by 5% each year to reach 35% in year 5 (future scenario).
Note: Results may appear inexact due to rounding.

Appendix 10: Letter of Information[a]

LETTER OF INFORMATION

Health Quality Ontario is conducting a review of **noninvasive fetal RhD blood group genotyping test** for those who are have Rh negative blood type during pregnancy. The purpose is to understand whether this test should be more broadly funded in Ontario.

An important part of this review involves speaking to Rh negative patients who have experience with Rh incompatibility and using Rh immunoglobin (RhIG, sometimes known as RhoGAM) treatment. Our goal is to make sure the experiences of individuals and families are considered in the funding recommendations for this test.

WHAT DO YOU NEED FROM ME?

- ✓ 10 to 20 minutes of your time for a phone or in-person interview to share your story
- ✓ Permission to audio- (not video-) record the interview

WHAT YOUR PARTICIPATION INVOLVES

If you agree to share your experiences, you will be asked to have an interview with Health Quality Ontario staff. The interview will likely last 10 to 20 minutes. It will be held in a private location or over the telephone. With your consent, the interview will be audio-recorded. The interviewer will ask you questions about you perspectives of Rh incompatibility, RhoGAM treatment, and your thoughts about potential fetal RhD genotyping in Ontario.

Participation is voluntary. You may refuse to participate, refuse to answer any questions or withdraw before your interview. Withdrawal will in no way affect the care you receive.

CONFIDENTIALITY

All information collected for the review will be kept confidential and privacy will be protected except as required by law. The results of this review will be published, however no identifying information will be released or published. Any records containing information from your interview will be stored securely.

RISKS TO PARTICIPATION:

There are no known physical risks to participating. Some participants may experience discomfort or anxiety after speaking about their lived experience. If this is the case, please speak to our staff.

If you are interested in participating, please contact Health Quality Ontario staff:

[a]Health Quality Ontario is now a part of Ontario Health.

Appendix 11: Interview Guide

Interview Questions for Noninvasive Fetal RhD Genotyping

Intro
Explain the purpose of Health Quality Ontario[a], the health technology assessment (HTA) process, and the purpose of the interview

Lived Experience
Background of pregnancy(ies) and RhD incompatibility
Understanding/information around RhD incompatibility and its potential risks

Therapies
Information surrounding RhIG (Rh immunoglobulin) and its use
Impact/barriers to using or receiving RhIG
Any concerns or hesitations to using RhIG?

*If alloimmunized, experiences receiving intensive fetal monitoring?
(any barriers/challenges to receiving intensive fetal monitoring?)

Noninvasive Fetal RhD Genotyping
Any previous information surrounding these genetic tests?
General thoughts on potential impact of fetal RhD genotyping?
Any concerns with withdrawing of RhIG prophylaxis based on genetic test?

Other??

[a]Health Quality Ontario is now a part of Ontario Health.

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