

Approach to red blood cell antibody testing during pregnancy

Answers to commonly asked questions

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Abstract

Objective To provide family physicians with an understanding of blood bank tests performed during pregnancy. The value of routine blood type and antibody tests, as well as the follow-up required when a patient develops a red blood cell antibody or experiences a fetal-maternal hemorrhage (FMH) will be reviewed.

Sources of information The approach described is based on the authors' clinical expertise and peer-reviewed literature from 1967 to 2020.

Main message An ABO and RhD group and antibody screen test is performed on every pregnant patient during the first trimester. Although antibodies to red blood cell antigens occur infrequently, some can lead to substantial adverse fetal or neonatal consequences including hemolytic disease of the fetus and newborn. Early identification and quantification of important antibodies ensures that at-risk mothers are referred to and followed by obstetricians experienced with high-risk care. Another valuable and related test is the FMH test. For RhD-negative women, these tests are performed at every delivery and following antepartum events that could contribute to FMH. This test determines the number of fetal red blood cells in the maternal circulation and is used to determine the dose of Rh immune globulin an RhD-negative mother requires to prevent alloimmunization to fetal RhD.

Conclusion An understanding of blood bank tests performed during pregnancy and their role and limitations is vital to optimal practice and aids clinicians in their decision making. When there is doubt or confusion regarding antenatal testing or immunoprophylaxis, consult the regional laboratory or transfusion medicine specialists for additional guidance.

Approche de dépistage des anticorps anti-érythrocytaires durant la grossesse

Réponses aux questions souvent posées

Résumé

Objectif Permettre aux médecins de famille de mieux comprendre les analyses de banque de sang effectuées durant la grossesse. On parlera de la valeur des tests systématiques de détermination du type sanguin et de dépistage des anticorps, de même que du suivi nécessaire lorsqu'une patiente développe des anticorps anti-érythrocytaires ou subit une hémorragie fœto-maternelle (HFM).

Sources d'information L'approche décrite s'appuie sur l'expertise clinique des auteurs et sur des articles révisés par les pairs publiés entre 1967 et 2020.

Editor's key points

- ▶ Maternal ABO, RhD, and red blood cell antibody screening should be performed at the initial prenatal visit. Antibodies most commonly associated with severe hemolytic disease of the fetus and newborn include anti-D, anti-c, and anti-Kell antibodies. Clinically significant antibodies should be monitored by titration testing every 2 to 4 weeks. A critical titre should not be used to predict neonatal outcome; they alert clinicians that follow-up by a high-risk obstetric team is needed.
- ▶ All pregnant women with weak RhD expression should have *RHD* genotyping performed to determine whether Rh immune globulin (RhIg) is required.
- ▶ Fetal-maternal hemorrhage (FMH) tests assess the maternal blood for the number of fetal cells present. If FMH has occurred and the pregnant patient is RhD negative, a standard dose of RhIg (300 µg) should be administered. Supplemental doses are required for larger FMHs.
- ▶ For an RhD-positive mother, a group and screen is not required after delivery. At delivery, an FMH test and RhIg is required for RhD-negative women with an RhD-positive fetus.

Points de repère du rédacteur

- ▶ La mère doit subir le typage du groupe ABO et du gène RhD, et le dépistage des anticorps anti-érythrocytaires dès la première visite prénatale. Les anticorps le plus souvent associés à une maladie hémolytique grave du fœtus et du nouveau-né sont les anticorps anti-D, anti-C et anti-Kell. Il faut réaliser le titrage des anticorps cliniquement significatifs toutes les 2 à 4 semaines. Un titre critique ne doit pas servir à prédire les issues néonatales; il prévient plutôt le clinicien qu'un suivi en obstétrique auprès des patientes à risque élevé est nécessaire.
- ▶ Toutes les femmes enceintes avec faible expression du gène RhD doivent subir le génotypage *RHD* afin de déterminer s'il est nécessaire d'administrer l'immunoglobuline anti-Rh (RhIg).
- ▶ Le test d'hémorragie fœto-maternelle (HFM) évalue le nombre d'hématies fœtales présentes dans le sang de la mère. Si une HFM est survenue et que la patiente enceinte est RhD négatif, une dose standard de RhIg (300 µg) doit être administrée. Des doses supplémentaires sont nécessaires dans les cas de HFM plus importante.
- ▶ Chez une mère du groupe RhD positif, la détermination du groupe sanguin et le dépistage ne sont pas nécessaires après l'accouchement. À l'accouchement, un test d'HFM et le dépistage de RhIg sont nécessaires chez les femmes RhD négatif dont le fœtus est RhD positif.

Message principal Le typage du groupe ABO et du gène RhD et test de dépistage des anticorps est effectué au premier trimestre chez toutes les femmes enceintes. Même si les anticorps anti-érythrocytaires sont peu fréquents, certains entraînent des conséquences considérables pour le fœtus ou le nouveau-né, y compris la maladie hémolytique du fœtus et du nouveau-né. La détermination et la quantification précoces des anticorps importants veillent à ce que les mères à risque soient recommandées et suivies en obstétrique par des médecins expérimentés en soins des femmes à risque élevé. Le test d'HFM est un autre test connexe utile. Chez les femmes RhD négatif, ces tests sont effectués à chaque accouchement et après la survenue, durant l'accouchement, d'événements pouvant contribuer à l'HFM. Ce test détermine le nombre d'hématies fœtales présentes dans la circulation maternelle et est utilisé pour déterminer la dose d'immunoglobuline Rh dont une mère RhD négatif a besoin pour prévenir l'allo-immunisation au gène RhD fœtal.

Conclusion Pour une pratique optimale, il est crucial que les médecins comprennent les analyses de banque de sang réalisées durant la grossesse ainsi que leur rôle et leurs limites afin d'éclairer leurs décisions. En cas de doute ou de confusion concernant les tests prénataux ou l'immunoprophylaxie, consulter le laboratoire régional ou les spécialistes de médecine transfusionnelle pour plus de renseignements.

Prenatal testing is essential to ensure the safety of the mother and fetus by identifying any need for intervention at an early stage. Pregnant patients require a variety of screening tests during pregnancy, including blood group and antibody screening, known as the *group and screen* or *type and screen*.¹ The group and screen is performed routinely during the first prenatal visit. Its main role is to identify the patient's need for Rh immune globulin (RhIg) and to identify maternal red blood cell (RBC) antibodies.² Numerous guidelines offer approaches to testing and monitoring these patients; however, these guidelines are from a variety of jurisdictions and medical specialties and do not offer a unified approach to prenatal tests. This makes it challenging for clinicians to navigate how and when to test. This article explains the value of serology tests during pregnancy, reviews which RBC antibodies are clinically significant, and describes an approach to first-trimester and second-trimester screening. A summary box of the main points of this article is available from **CFPlus**.*

*A summary box of the article's main points is available at www.cfp.ca. Go to the full text of the article online and click on the **CFPlus** tab.

Case description

Mrs T is a 32-year-old woman (2 pregnancies, 1 birth) who is 13 weeks pregnant. Group and screen results confirm her blood group is O RhD negative. At 22 weeks' gestation, the patient is involved in a car accident. An FMH test indicates a 5-mL bleed and a 300-µg dose of RhIg is administered. At 28 weeks, a repeat group is performed and passive anti-D is noted. You wonder if this antibody is truly passive or a new antibody. You are not sure if another dose of RhIg is needed.

Sources of information

The approach described is based on the authors' clinical practice along with research and clinical review articles from 1967 to 2020.

Main message

Prenatal blood bank testing

A 32-year-old woman (2 pregnancies, 1 birth) is 13 weeks pregnant. Group and screen results confirm her blood group is O RhD negative. Why do we perform group and screen tests during pregnancy and how often are antibodies identified? The group and screen test has 2 parts: the blood group test determines the ABO and RhD type, and the antibody screen looks for non-ABO antibodies such as anti-Kell, anti-c, anti-E, anti-Jk^a, anti-Jk^b, anti-Fy^a, and anti-Fy^b. This testing helps to determine the potential for ABO incompatibility between the mother and fetus; identifies antibodies that might result in hemolytic disease of the fetus and newborn (HDFN); and determines eligibility for RhIg.³

Hemolytic disease of the fetus and newborn is a rare condition that occurs when maternal RBC antibodies cross the placenta and cause fetal RBC destruction or fetal bone marrow suppression of RBC progenitors. Presentation of HDFN ranges from asymptomatic maternal antibodies to severe edema, ascites, hydrops, heart failure, and death in the fetus or neonate.⁴ The risks of severe disease are mitigated by early recognition of maternal antibodies and close monitoring and treatment of the fetus.

ABO incompatibility between the mother and fetus is common and occurs in approximately 20% of pregnancies.² Fortunately, ABO incompatibility is rarely associated with HDFN-related morbidity (<0.05%), as A and B antigens are only weakly expressed on fetal RBCs.² Weak expression of A and B on a variety of fetal tissues lowers the amount of anti-A or anti-B IgG antibodies in the fetal circulation, leaving less available to bind to the fetal RBCs. Group O mothers with group A, B, or AB fetuses are most at risk, but even in these cases the risk of anemia or substantial hyperbilirubinemia is low and most neonates are asymptomatic or experience mild jaundice.⁵

Red blood cell antibodies (non-ABO antibodies) are rarely detected in the first trimester, with prevalence rates estimated at approximately 1% to 2%.^{2,6} The most common non-ABO RBC antibodies implicated in HDFN include anti-D, anti-Kell, anti-E, and anti-c.^{7,8} These

antibodies can develop after blood transfusion, pregnancy, or fetal-maternal hemorrhage (FMH) events in a process known as *RBC alloimmunization*. A list of events that might result in FMH, with the potential for alloimmunization during pregnancy, is presented in **Box 1**.⁹

Pregnancy-related alloimmunization occurs when the fetus inherits paternal RBC antigens that differ from the maternal RBC antigen profile. The mother might produce antibodies to the fetal antigens that differ from her own.² As little as 0.1 mL of fetal RBCs can result in sensitization¹⁰ and, therefore, alloimmunization can occur after an uncomplicated pregnancy with routine delivery or after an FMH event.

How often should I perform a group and screen test during pregnancy? Current guidelines suggest that initial ABO blood typing, RhD typing, and antibody screening should be performed during the first trimester for each pregnancy at the first visit (at around 12 weeks' gestation).^{2,11,12}

- If the antibody screening result is negative, consider the following:
 - For an RhD-positive patient, no further testing is needed.
 - RhD-negative patients might be tested at 28 weeks and before RhIg administration to detect new alloimmunization.
 - Repeat testing should be done if a potentially sensitizing event (**Box 1**)⁹ occurs (even in RhD-positive patients) and before any RhIg administration.
- If the antibody screening result is positive, further testing to identify the antibody type should follow. Once a clinically significant antibody is detected, the antibody is quantified; the laboratory test used for quantification is called a *titration test*.¹³

Table 1 highlights the antibodies that are considered clinically significant with respect to the risk of HDFN.¹⁴ Most antibodies deemed clinically significant are regularly monitored with quantification tests (titrations) every 2 to 4 weeks.¹³ Antibodies that are known to be associated with severe HDFN include anti-D, anti-c, and anti-Kell.¹⁵⁻¹⁹

Box 1. Causes of FMH

The following are common causes of FMH, with the potential for alloimmunization during pregnancy:

- Delivery
- Antepartum hemorrhage
- Spontaneous or therapeutic abortion
- Amniocentesis
- Cordocentesis
- Chorionic villus sampling
- Ectopic pregnancy
- Fetal death
- Abdominal trauma
- External cephalic version

FMH—fetal-maternal hemorrhage.

How do we monitor clinically significant antibodies? Once a clinically significant antibody has been identified, antibody levels are assessed and monitored every 2 to 4 weeks. The level, or titre, is used as a marker of the “strength” of the antibody within the maternal circulation. There are several laboratory techniques used to assess the titre: conventional tube testing is the most commonly used test in North America.^{20,21} When there is a concern regarding a rise in titre, ensure that both the baseline and follow-up tests were performed using the same method.

The titration level does not correspond to nor predict the clinical outcome for the fetus or neonate; rather, the titre can be considered a screening test that shows whether HDFN is possible. If a cutoff titre is reached (1:16 to 1:64), or the titre increases rapidly between measurements, the patient should be referred to a high-risk obstetric team for ongoing monitoring, including a Doppler ultrasound scan of the middle cerebral artery. This technique allows direct assessment of fetal anemia and risk stratification of the fetus for HDFN.^{2,22}

An antibody that requires special consideration is anti-Kell. This antibody is associated with suppression of erythropoiesis and has been associated with fetal demise as early as 12 weeks. Controversy as to whether titres should be performed at all for Kell antibodies exists. Hemolytic disease of the fetus and newborn has been shown to occur with maternal anti-Kell antibodies even at low titres.²³⁻²⁵ Therefore, referral to a high-risk obstetrics team for Doppler ultrasound monitoring whenever anti-Kell antibodies are detected, even at low titre, is recommended.

What is the role of paternal testing? If clinically significant maternal antibodies have been detected on routine screening, fetal risk can be partly addressed by determining the father's RBC antigen type (if paternity is assured) (**Box 2**).²⁶

Table 1. Severity of HDFN and the relative frequency of association with various antibodies

ASSOCIATION WITH HDFN	SEVERITY OF POTENTIAL HDFN		
	MILD	MODERATE	SEVERE
Common	ABO (A, B) Rh (E)		Rh (D, C, c) Kell (K, k)
Not common	MNS (M, S)*	Duffy (Fy ^a , Fy ^b) Kidd (Jk ^a , Jk ^b) Rh (C)	
No association with HDFN	Lewis (Le ^a , Le ^b), Ii (I), P (P ₁)		

HDFN—hemolytic disease of the fetus and newborn.

*Severity of HDFN related to any antibody can range from mild to severe.

Data from Clarke and Hannon.¹⁴

Although many other antibodies are associated with HDFN, only the most common or important antibodies are listed here.

Box 2. Paternity testing in HDFN

Fetal risk can be partly addressed by determining the father's RBC antigen type:

- When there is no paternal antigen expression, the fetus is not at risk of HDFN
- When the father's test result is positive for homozygous expression of the antigen, the fetus is at risk of HDFN
- When the father's test result shows heterozygous expression of the antigen, the fetus has a 50% chance of being at risk of HDFN

HDFN—hemolytic disease of the fetus and newborn, RBC—red blood cell.

Data from the American College of Obstetricians and Gynecologists.²⁶

Is there a noninvasive test available to assess fetal antigen status? With an accurate means of predicting a negative RBC antigen phenotype of the fetus, follow-up monitoring for HDFN in mothers with antibodies could be averted. In the past, amniocentesis, chorionic villus sampling, and umbilical cord blood sampling have been used to predict or determine the RBC phenotype.^{27,28} These tests are costly and invasive, and pose a risk to the fetus, including a 0.5% to 1% risk of pregnancy loss in all procedures and a 17% risk of transplacental hemorrhage in amniocentesis, which can cause an increase in maternal antibodies.²⁹

In 1997, a procedure to assess the fetal genotype for prediction of RBC and other fetal antigen typing from maternal plasma was developed. This noninvasive procedure is based on the presence of cell-free fetal DNA circulating in maternal plasma. Sampling maternal plasma and amplifying fetal gene sequences using polymerase chain reaction and probes specific to the target of interest allows prediction of the fetal RBC antigen type.³⁰

Currently, testing is available to predict the D, Kell, C, c, and E antigen status of the fetus. It should be performed in patients in which the antibody has reached the critical cutoff level, and for which the father's test result is heterozygous for the antigen, leading to the possibility of an antigen-positive or antigen-negative fetus. The test has become the standard of care in many countries owing to high sensitivity, with very few false-negative results.^{31,32} These tests are not yet widely available in North America, but samples can be sent to reference laboratories by the transfusion service or local laboratory.³²

What is RhIg? When is RhIg indicated during pregnancy? Rh immune globulin is a blood product prepared from pooled plasma containing anti-D antibody.¹⁴ Initially developed in the 1960s and shown to prevent HDFN, it is a form of passive anti-D antibody immunoprophylaxis against maternal alloimmunization. Rh immune globulin has been licensed for use in Canada since 1968 and has been a part of a larger routine antenatal alloimmunization program since 1976.²³ Before the development

and widespread use of RhIg, the risk of RhD alloimmunization in pregnancy was 13.2%.³³ Following routine RhIg administration to RhD-negative women, alloimmunization rates for anti-D antibody in pregnancy have decreased 100-fold to 0.4 in 1000 births.³⁴

RhD incompatibility is common in pregnancy; approximately 60% of RhD-negative women carry an RhD-positive fetus, although the fetal blood type is often not known until delivery. All women who are RhD negative without evidence of immune anti-D antibody on initial screening are eligible to receive antenatal RhIg, as fetal RhD status is usually unknown.³³ The routine dose of RhIg is 300 µg and is typically given at approximately 28 weeks' gestation. An alternate dosing regimen of 120 µg given at 28 weeks and at 34 weeks has equivalent efficacy.¹⁴ Before 28 weeks, the risk of alloimmunization is very low; one study found the risk of alloimmunization during the first trimester in pregnancy to be only 0.24%, which is believed to be an overestimate.³⁵

Rh immune globulin (300 µg) should also be administered to all RhD-negative women at the time of delivery, as the risk of alloimmunization during delivery is 17.3%.¹⁰ As little as 0.1 mL of fetal RBCs might result in sensitization.¹⁰ Additional RhIg is required in the setting of any potentially sensitizing event. This will be discussed in a later section.

The RhIg preparation used in Canada has never been associated with any blood-borne infections, including HIV, hepatitis B, or hepatitis C.³⁴ As RhIg is a blood product, informed consent must always be obtained before its administration. While complications of RhIg injection are rare, some women might develop rash, pain, and swelling at the injection site.³³

Unfortunately, to date, immunoprophylaxis for other antibodies (non-ABO antibodies) is not available.

My patient's results were reported as positive for "weak D." What does this mean? Does she still require immunoprophylaxis with RhIg? When a weak D phenotype is reported, it can cause some uncertainty as to whether RhIg prophylaxis is required. A weak D phenotype often reflects decreased numbers of RhD antigens on the RBC surface, causing the laboratory test result for RhD to appear weak. Most patients with weak D RBCs are functionally RhD positive and do not develop anti-D antibodies. It is estimated that 0.6% to 1.0% of white individuals have a serologic expression of the weak D phenotype.¹¹ Most of these weak D types in the white population correspond to genetic variants known as *weak D type 1*, *weak D type 2*, and *weak D type 3*, and patients with these types can be treated serologically as RhD positive.³⁶ If weakened D expression is noted, *RHD* genotyping will be performed (or referred out) by the transfusion laboratory to determine the genetic *RHD* variant.³⁷ Current recommendations suggest *RHD* genotyping when the weak D phenotype is detected or in the setting of RhD typing discrepancies between laboratories.³⁶

If a weak D result is not tested or recognized as RhD positive, RhIg might be given unnecessarily. The laboratory should be consulted if results are unclear.

At 22 weeks' gestation, the patient is involved in a car accident. An FMH test indicates a 5-mL bleed and a 300- μ g dose of RhIg is administered. What is an FMH, how is testing performed, and why should I worry about these events? Fetal-maternal hemorrhage can occur naturally during pregnancy and at delivery and is defined as the transfer of fetal RBCs to the maternal circulation. Fetal-maternal hemorrhage can also occur through a sensitizing event (**Box 1**).^{9,38} Rates and volumes of spontaneous FMH increase as pregnancy progresses, with detection of fetal cells in maternal circulation rising from 6.7% to 15.9% and 28.9% in the first, second, and third trimesters, respectively.¹⁰ These rates represent natural FMH due to pregnancy alone. As mothers and fetuses might have different RBC antigens, this can potentially lead to maternal alloimmunization to the paternally derived fetal antigens, and thus might put future pregnancies at risk of HDFN.³⁹ All pregnant RhD-negative women have FMH testing performed by the laboratory at the time of delivery. These tests are used to assess the size of the FMH and to determine RhIg dosing to prevent alloimmunization to anti-D antibody.

In RhD-negative or RhD-positive women, these tests might also help to predict fetal anemia and guide management after a pregnancy complication or obstetric manipulation.

After an FMH event, what dose of RhIg should be administered? Typically, a standard 300- μ g dose of RhIg is administered after an FMH. The amount of bleeding can be determined through a Kleihauer-Betke test or flow cytometry test on a maternal blood sample. Results of this testing determine whether the standard 300- μ g dose or an additional dose of RhIg is required for RhD-negative women. The typical dose of 300 μ g prevents alloimmunization from 30 mL of fetal whole blood (15 mL of fetal RBCs). The incidence of FMHs greater than 30 mL in uneventful pregnancies is very low (0.3%).⁴⁰ The incidence of large-volume FMH is substantially higher in pregnancies with perinatal death, placental abruption, manual removal of the placenta, third-trimester abdominal or multiple trauma, third-trimester amniocentesis, and multiple births.⁴⁰

Testing for FMH before 20 weeks' gestation is not required, as the total fetal blood volume is insufficient to exceed the 30 mL covered by the standard RhIg dose.³⁹ If miscarriage, ectopic pregnancy, threatened abortion, or induced abortion occurs during the first 12 weeks of gestation, women might be given a 120- μ g (600-IU) dose of RhIg to cover this smaller fetal blood volume.³⁸ Some guidelines suggest that RhIg is unnecessary when gestational age is certain or is less than 6 weeks, and when the hemorrhage is spontaneous (ie, not initiated by a surgical instrument or medication).^{12,41} Generally, it is recommended that 300 μ g

(1500 IU) be given after 12 weeks' gestation, with any additional RhIg doses in women at more than 20 weeks' gestation determined by FMH testing.

Following the FMH, should an antibody screening test be performed? Does an additional antibody screening test need to be performed? Is the 28-week dose of RhIg still required? If a potential sensitizing event occurs before 28 weeks' gestation, the maternal blood group and antibody screening should be performed again before giving RhIg. This baseline assessment will determine if additional antibodies have developed since the previous screening. This information is important, because after RhIg is given, it is challenging to discern if the anti-D antibody present in the screening results is passive anti-D antibody from RhIg, or a new antibody.²² This distinction is important, as passive anti-D antibody will not cause fetal issues, while immune-related anti-D antibody can cause HDFN.

Despite the use of RhIg before 28 weeks owing to antenatal FMH, routine antepartum anti-D antibody immunoprophylaxis should be given at 28 weeks. This dose will cover the remainder of the pregnancy, preventing third-trimester alloimmunization due to any additional FMH.³⁹

If the mother has recurrent vaginal bleeding or spotting, how frequently should FMH testing be performed and how often should the RhIg dose be repeated? While guidelines vary, expert opinion that informs practice in many Canadian centres suggests that repeat FMH testing is not required in the setting of recurrent antepartum bleeding in an RhD-negative woman. Typically, FMH would be determined once after 20 weeks and an appropriate dose of RhIg would be given at the onset of bleeding. If bleeding continues, additional RhIg doses can be given at 3-week intervals after the initial dose. The half-life of RhIg is 17 to 22 days and it persists for approximately 12 weeks after administration (for the remainder of a term pregnancy).^{42,43}

RhIg was administered to our patient at 27 weeks' gestation. A group and screen test was performed thereafter, and the results are positive for anti-D antibody. Does this indicate alloimmunization to anti-D antibody? When a new result of anti-D antibody appears during pregnancy after an RhIg dose has been given, it should be assumed to be passive anti-D antibody from RhIg until proven otherwise. There is no laboratory test that can clearly distinguish immune from passive anti-D antibody. Thorough history taking and record searching should be performed to evaluate whether RhIg has been administered within the past several weeks.³ While many laboratory techniques have been recommended to distinguish immune from passive anti-D antibody, the only way to be sure is to wait; the passive antibody will weaken and disappear over time. Passive anti-D antibody can remain until at least 3 months after administration. One study risk-stratified individuals by strength

of reaction and time after RhIg injection using various methods; this might be useful for tracking the level of anti-D antibody over time and predicting alloimmunization status.⁴⁴

Postpartum blood bank testing

How much RhIg needs to be given at delivery? All RhD-negative women who give birth to an RhD-positive neonate should receive at least the standard 300- μ g dose of RhIg within 72 hours of delivery.²² If the blood type of the neonate remains unidentified in this 72-hour window (eg, site is in a rural area, laboratory closures), this RhIg dose should be given while awaiting neonatal blood group confirmation.

How long after delivery can the FMH screening be performed? It is recommended that a sample for FMH testing be taken from the mother at least 30 minutes after delivery and within 2 hours postpartum for optimal results.⁹ This allows for adequate mixing of fetal cells within the mother's circulation.³⁹

Is a maternal group and screen test required at the time of delivery? What testing should be done on the cord blood? Routine maternal group and screen testing at delivery is not necessary unless antenatal testing has not been documented or the mother requires testing for a planned transfusion.¹² It is important to have at least one blood group on file to determine the Rh status of the mother and to determine whether RhIg is required.³⁴ While some suggest that a peripartum group and screen test is needed in case blood is required at the time of the delivery,⁴⁴ the rate of transfusion during normal vaginal delivery and cesarean section is very low (approximately 0.5% to 1%).⁴⁵ If these patients require a blood transfusion, O Rh-negative uncross-matched blood could be provided, or an urgent cross-match could be requested before transfusion.¹³ Given the low likelihood of requiring a blood transfusion during labour and delivery, performing routine group and screen testing for all women is not warranted.

If the mother is RhD negative, or found to have clinically significant antibodies, cord-blood testing is required. For an RhD-negative mother, cord typing for Rh is needed to determine the need for maternal RhIg.^{45,46} ABO blood typing is not required, although it is often done with the RhD type. Cord blood typing (or neonatal antigen typing if cord blood typing is not possible) is also performed when the mother is known to have an antibody, along with a direct antiglobulin test (DAT). These latter tests help determine whether the neonate is likely to be affected by the maternal antibody.

Postpartum maternal antibody titrations are not required. Titrations inform antepartum monitoring and are not diagnostic tests for HDFN.

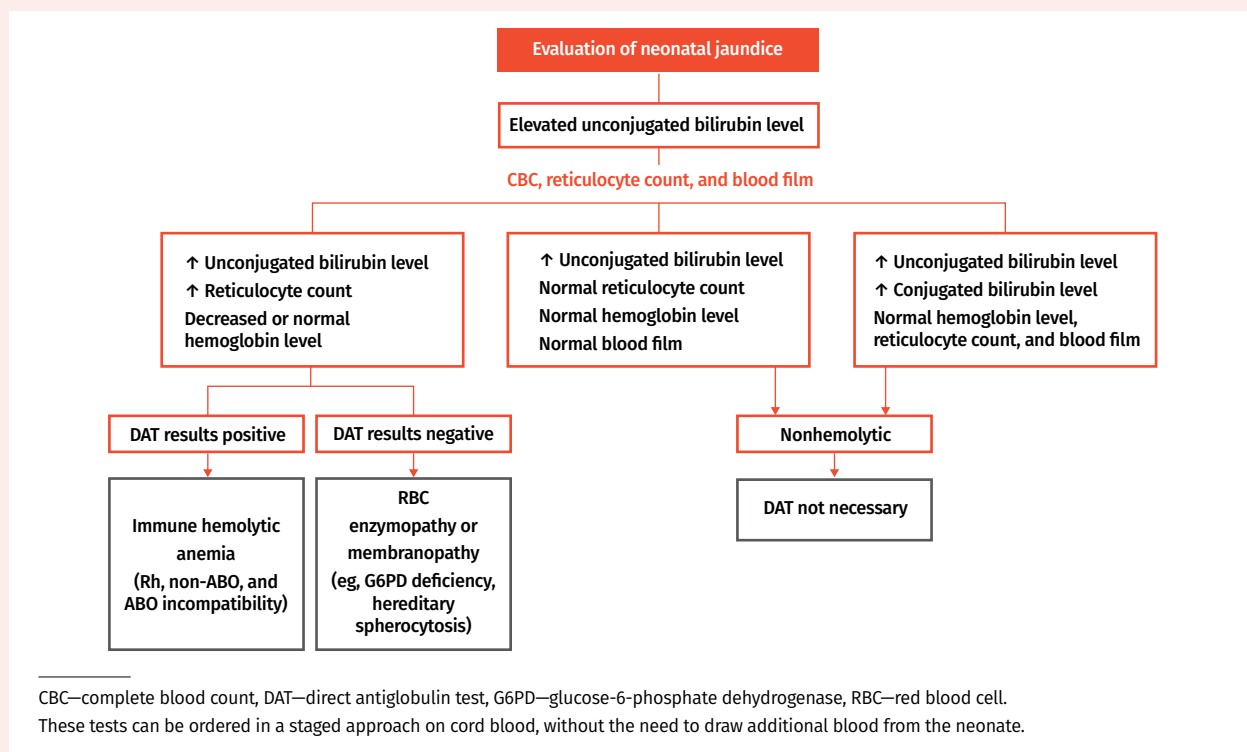
How do I manage an RhD-negative mother with a postpartum group and screen result that is positive for anti-D antibody? Passive anti-D antibody can also be seen on the postpartum group and screen result. The

group and screen test can be repeated 6 months postpartum to assess if the antibody is passive (ie, from RhIg) or immune related, as passive anti-D antibody typically disappears within 3 to 6 months.¹² Alternatively, re-assessment in the first trimester of the next pregnancy will generally confirm whether a postpartum positive anti-D antibody result was passive or immune. Generally, if there is a history of receiving RhIg, it should be assumed that a screening result for anti-D antibody represents passive immunity; women with these results should be treated as though they are not alloimmunized.

Should I order a DAT for all newborns with jaundice? The DAT, also known as the *direct Coombs test*, is not a screening test for hyperbilirubinemia. If there are clinical concerns about immune-mediated jaundice, tests for hemolysis including a complete blood count, reticulocyte count, unconjugated bilirubin test, and blood film examination should be performed on a peripheral sample from the neonate.⁴⁷ Direct antiglobulin testing should be performed only if immune-mediated causes of jaundice are suspected; it should not be ordered routinely.⁴⁸

Mothers with group O Rh-negative blood type are more likely to have anti-A and anti-B IgG, and some guidelines suggest infants of group O mothers are at a higher risk of ABO HDFN.¹³ Although ABO-incompatible neonates have an increased risk of jaundice compared with group O neonates born to group O mothers, their rates of more severe jaundice are low. The DAT has not been found to predict jaundice owing to a poor positive predictive value, and hence, this test should not be used as a screening test.⁴⁹ Instead, clinical evaluation and assessment of anemia is vital for these patients.^{46,50} Hemolysis should be confirmed. A recommended diagnostic strategy for infants with jaundice is provided in **Figure 1**.

After delivery, how should I follow a neonate whose mother had a clinically significant antibody present during pregnancy? If a mother has a clinically significant antibody during pregnancy, the neonate should be assessed for the corresponding antigen at birth through cord-blood testing.^{22,46} For example, if the mother has anti-Kell antibodies, the cord blood should be tested (phenotyped) for the Kell antigen. This is performed routinely in most hospitals. Results of the neonate's cord antigen status should be available before discharge. If the neonate does not have the corresponding antigen, there is no risk of hemolysis and no further testing or monitoring is required.⁴⁹ If the neonate does have the antigen corresponding to the maternal antibody, hemolytic tests, including a neonatal complete blood count, reticulocyte count, DAT, and blood film examination, should be performed to assess the risk of immune-mediated HDFN with jaundice.⁴⁶ Close follow-up of the neonate for anemia is recommended for any neonate born to a mother with clinically significant antibodies following delivery.

Figure 1. Suggested approach for the evaluation of immune-mediated neonatal jaundice

Case resolution

Mrs. T delivers a term baby boy without complications. Neonatal RBC antigen phenotyping reveals that the neonate is RhD positive. Mrs. T receives a 300- μ g dose of RhIg to prevent alloimmunization. The newborn experiences mild jaundice on day 1 of life; no phototherapy is needed. No additional cord testing is performed.

Conclusion

Testing for alloimmunization in pregnancy is an important part of determining the risk of HDFN, which is a rare but potentially severe event. Where there is doubt or confusion regarding antenatal testing or immunoprophylaxis, contact the laboratory or a transfusion medicine specialist in your region for additional guidance.

Future testing options in pregnancy will likely include more widespread availability of fetal DNA testing from maternal blood to identify fetuses at risk of HDFN and to determine the need for antenatal RhIg. This would also circumvent the need for unnecessary titres and aggressive monitoring in patients with clinically significant antibodies and an antigen-negative fetus.

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Contributors

All authors contributed to the literature review and interpretation, and to preparing the manuscript for submission.

Competing interests

None declared

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