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GUIDELINES

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Guideline for the investigation and management of red cell antibodies in pregnancy: A British Society for Haematology guideline

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KEYWORDS: anti-D, erythroblastosis fetalis, haemolytic disease of the foetus, haemolytic disease of the newborn, immunoglobulin, maternal, pregnancy, red cell antibody, RhD

1 | METHODOLOGY

This guideline was compiled according to the British Society for Haematology (BSH) process at [https://b-s-h.org.uk/media/lvdbh3p5/2023-11-14-bsh-guidelines-development-process-updated-nov-2023-1.pdf?cf=638356449664970000]. Grading of Recommendations Assessment, Development and Evaluation (GRADE) nomenclature was used to evaluate levels of evidence and to assess the strength of recommendations. The GRADE criteria can be found at http://www.gradeworkinggroup.org and are also summarised in an appendix of the full BSH process guidance above.

1.1 | Literature review details

A search of published literature was undertaken using the Cochrane Library, Pubmed, MedLine and internet searches using the following key words and relevant MeSH terms: anti-D, anti-D Ig immune globulin, pregnancy, red blood cell antibodies in pregnancy, maternal red blood cell antibodies, antenatal anti-D prophylaxis, rhesus, RhD, haemolytic disease of newborn, erythroblastosis fetalis. This search covered the period 1999 to March 2022 and was restricted to manuscripts written in English, describing studies carried out in humans, and only including article types: clinical studies, clinical trials, comparative studies, evaluation studies, guidelines, meta-analysis, observational studies, systematic reviews and validation studies.

1.2 | Review of the manuscript

Review of the manuscript was performed by the BSH Guidelines Committee Transfusion Task Force, the BSH Guidelines Committee

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and the Transfusion sounding board of BSH. It was also on the members section of the BSH website for comment. It has also been reviewed by Royal College of Obstetricians and Gynaecologists; these organisations do not necessarily approve or endorse the contents.

2 | INTRODUCTION

The purpose of the guideline is to make evidence-based recommendations for the application of blood grouping and red cell antibody testing in pregnancy. The aim is to predict the potential for, and where possible, prevent, haemolytic disease of the foetus and newborn (HDFN). The blood group and antibody status of a pregnant woman should be tested at booking and at 28 weeks' gestation to identify the ABO and D group and to detect red cell antibodies that have the potential to be clinically significant. Some antibodies (including anti-D, anti-K and anti-c) are associated with significant foetal and neonatal risks, such as anaemia, jaundice and perinatal loss. There are antibodies that are unlikely to significantly affect the foetus but that can cause neonatal anaemia and hyperbilirubinaemia, and others that may cause problems for the screening and timely provision of appropriate blood for the woman or baby. This guideline updates the previous British Society for Haematology (BSH) guidance published (2016) and is intended to incorporate necessary scope from the prior Royal College of Obstetricians and Gynaecologists (RCOG) Green-top Guidelines (2014) for the management of women with red cell antibodies during pregnancy. The testing protocols recommended here are designed to provide clarity for practice in order to protect pregnant women and their babies.

3 | RECOMMENDATIONS FOR CONSENT, REQUEST FORMS AND SAMPLES

Information about the implications of a blood test should be provided and informed consent obtained prior to samples being taken.[1]

3.1 | Completion of request form and identification of samples

It is essential that request forms are accurately completed and samples from pregnant women are correctly identified. Misidentification at the time of sampling could lead to an incorrect blood group being assigned to the transfusion record. This could result in errors in anti-D immunoglobulin (Ig) prophylaxis (missed or inappropriate administration of prophylactic anti-D Ig) and errors in the selection of blood components.[2,3]

It is essential that the sample and request form conform to the requirements described in the BSH guidelines on the administration of blood components.[4]

In addition, it is essential that any previous administration of prophylactic anti-D Ig in the current pregnancy, including date and dose, is recorded on the laboratory request form. A clinical history, particularly of HDFN, miscarriages, previous transfusions and historical antibodies, is essential information and should where known be stated on the request form.

Pre-printed labels should not be used to label pre-transfusion blood sample tubes for compatibility testing or antenatal screening. Samples should be hand labelled or identified with labels that are printed "on demand" (printed at the patient's bedside) and that are acceptable as an alternative to handwritten labels.

> Recommendation
> It is essential that the sample and request form conform to the requirements described in the guidelines on the administration of blood components (GRADE 1C).[4]

> Recommendation
> Samples should be dated, labelled and signed by the person taking them, in the presence of the pregnant woman who should, whenever possible, be asked to state their full name and date of birth. Sample labels pre-printed away from the phlebotomy procedure or taken from the notes for example, 'addressograph' labels should not be used (GRADE 1C).[4]

4 | LABORATORY TESTS

All laboratory testing procedures must be validated in compliance with published guidelines.[5] Wherever possible, testing should be performed on automated equipment that ensures positive sample identification, with electronic transfer of results to the Laboratory Information Management System (LIMS).

> Recommendation
> All laboratory testing procedures should be validated in compliance with published guidelines (GRADE 1C).[5]

4.1 | ABO/D typing

A record of the pregnant woman's ABO and D type performed at booking is useful as confirmation of any subsequent testing performed on another sample taken at the point of need, should the woman or her baby require blood transfusion at a later date.

Maternal D typing is also undertaken to identify D-negative women who require anti-D Ig prophylaxis.

> Recommendation
> ABO and D grouping should be performed in accordance with the guidelines for compatibility procedures in blood transfusion laboratories (GRADE 1C).[5]

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Recommendation

If clear-cut positive results are not obtained in D typing, the woman should be classified as D negative until the D status is confirmed (GRADE 1C).[5]

Recommendation

All pregnant women confirmed to be D negative should be given written information about their D-negative status and the importance of anti-D Ig prophylaxis if the foetus is predicted to be D positive by cell free foetal DNA (cffDNA), or if the foetal D type is unknown. Their D status should be clearly recorded in the notes to inform those responsible for their care of the need to offer prophylactic anti-D Ig[2] (GRADE 1C).

4.2 | Screening for red cell antibodies

Maternal antibody screening is undertaken to detect clinically significant antibodies, which might affect the foetus and/or newborn, and to detect antibodies that may cause problems with the provision of compatible blood components for the woman and for the foetus/newborn.

Approximately 1% of pregnant women are found to have clinically significant red cell antibodies.[6–8] Of these, the most common specificity is still anti-D. Although the universal introduction of routine antenatal anti-D Ig prophylaxis (RAADP) has reduced the sensitisation rate, there has been an increase in the number of positive antibody screens as a result of passive (administered) anti-D Ig.

Recommendation

The screening cells and methods used for red cell antibody screening should comply with the guidelines for compatibility procedures in blood transfusion laboratories[5]; (GRADE 1A).

Due to a lack of reproducibility and a relatively low incidence of severe ABO haemolytic disease in a UK population, testing for a high concentration of immune anti-A and/or anti-B in pregnant women is not recommended.[9]

There is no additional value in using an enzyme technique in routine antibody screening, because additional clinically insignificant antibodies might be detected, resulting in unnecessary follow-up testing.[10] Antibodies only reactive by enzyme technique, are not considered clinically significant in terms of HDFN.

4.3 | Antibody identification

When a positive antibody screen is obtained and red cell antibodies are detected, further testing of maternal blood should be undertaken to determine the specificity(ies) present, to exclude other clinically significant specificities, to determine the concentration / strength of antibodies (using titration or a method of quantification) and the likelihood of HDFN.

Recommendation

The procedures used for identification and exclusion of red cell antibodies should comply with the guidelines for compatibility procedures in blood transfusion laboratories[5]; (GRADE 1A).

Once red cell antibodies have been identified in pregnancy, additional clinically significant maternal alloantibodies should be excluded whenever a repeat sample is tested.

This will ensure that all antibodies that have potential to cause HDFN are monitored and will facilitate timely provision of compatible blood if required for the woman and/or for the baby. The frequency of repeat tests for antibody screening and identification will be determined by the specificity and strength of the antibody and whether an intrauterine transfusion (IUT) has been administered.

4.4 | Measurement of antibody concentration

The concentration of each clinically significant red cell antibody is measured initially to guide the need for referral to a foetal medicine specialist and subsequently to guide management of the pregnancy including investigations and intervention.[11]

Recommendation

The concentration of each antibody capable of causing HDFN should be assessed independently. For specificities where there is a national standard preparation, quantification should be undertaken. Other antibody specificities should be measured using titration (GRADE 1C).

4.4.1 | Antibody quantification

Quantification requires specific equipment and measures antibody concentration against a national standard (National Institute for Biological Standards and Control [NIBSC]). Anti-D and anti-c are the only antibodies that are currently quantified, and they are reported as IU/mL.

Where possible, each sample should be tested in parallel with the stored previous sample and the results compared to identify significant changes in antibody concentration.

4.4.2 | Antibody titration

Titration is used to assess the concentration of clinically significant red cell antibodies other than anti-D and anti-c.

Doubling dilutions (1 in 2, 1 in 4, etc.) of plasma prepared in phosphate buffered saline are tested by an indirect antiglobulin test (IAT) using reagent red cells with heterozygous expression of the corresponding antigen(s); reagent cells designed for use in titration should be used where other reagent cells are unsuitable. Care must be taken

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in selecting cells for titration where more than one antibody specificity is present (including prophylactic anti-D Ig and clinically non-significant antibodies) to ensure that the concentration of the antibody of each specificity is assessed independently, for example, where anti-K and Fy^a are present titrate against K−, Fy(a + b+) and K + k+, Fy(a−) cells.

Careful attention to technique is necessary to minimise the variables in the methodology employed, and it is recommended that the NIBSC anti-D standard[12] be titrated in parallel, as an internal control, to ensure reproducibility of results in-house. The reported titre is the reciprocal of the highest dilution that gives a positive reaction, and the grade of reaction taken as the end point for this should be defined in the standard operating procedure, for example, the last dilution giving a 1+ reaction. An increase in titre of more than one dilution (e.g. a previous titre of 2 rising to a subsequent titre of 8) is considered to be a significant rise, and the titration of the previous sample in parallel is recommended wherever possible, to verify that the change in titre is not due to variability in the method. Use of the NIBSC anti-D standard does not control for variability in antigen density in the cell used for testing when investigating antibodies other than anti-D.

4.5 | Paternal testing

Where a clinically significant antibody capable of causing HDFN is present in a maternal sample, determining the father's phenotype including whether heterozygous or homozygous, can provide useful information to predict the likelihood of the foetus expressing the relevant red cell antigen and for counselling the couple regarding future pregnancies. It should be recognised that in any pregnancy the partner may not be the biological father. Furthermore, in cases where the pregnancy has been facilitated by assisted conception with sperm donation from a donor panel, the pregnant woman's partner will not be the biological father; similarly, with egg donation, the foetus will express antigens from the egg donor. It is reasonable to omit paternal testing and proceed directly to foetal genotyping using cell-free foetal DNA (cffDNA), where available, in order to avoid issues of non- paternity or donor eggs where indicated.[11]

> Recommendation
> If potentially clinically significant maternal antibodies have been identified, paternal testing including whether heterozygous or homozygous, should be considered to predict the risk to current and future pregnancies. This may be particularly relevant if non-invasive foetal genotyping is not available for the corresponding red cell antigen (GRADE 1B).

4.6 | Fetal genotyping

It is possible to determine foetal *RHD*, *RHCE* and *KEL*01* genotypes using cffDNA from maternal blood samples, thereby avoiding the need for invasive foetal blood sampling.[13] This is useful both in predicting HDFN in individual cases where clinically significant red cell antibodies are present, and as mass screening to guide anti-D Ig prophylaxis for D-negative women with no immune anti-D.

4.6.1 | Fetal genotyping in alloimmunised pregnancies

It is important to recognise that the methods used for non-invasive foetal genotyping in alloimmunised pregnancies differ from those used for high-throughput screening of unalloimmunised D negative women and the tests should not be used interchangeably.

Fetal genotyping is a useful diagnostic tool when either a, b or both are identified in combination with c:

a. A pregnant woman has a clinically significant antibody.
b. A pregnant woman has a history of HDFN and
c. The father's antigen status is unknown, or he expresses the corresponding antigen.

The false negative rate for such tests have been reported to be 0.1–0.3% based on large volume testing.[14,15] For low throughput testing the figure is less well defined. The *KEL*01* assay detecting a single nucleotide polymorphism in the foetus has a very close cut off between positivity and negativity, making distinction difficult. It is therefore important that reference laboratories are provided with feedback on the blood group of the baby at delivery to provide additional quality assurance. Given the low rate of response to requests for feedback on the neonatal blood group there may be additional assay failures that are not known about or investigated by the reference laboratory (E Massey personal communication).

It is important that samples are not sent too early in pregnancy as the levels of cffDNA rise with gestation. False negative results of cffDNA typing have been reported for *KEL*01* (K) at 17 weeks' gestation.[16] The false negative rate for *RHCE* (for c) and *RHD* (for D) genotyping performed at or after 16 weeks' gestation is less than 1% with the reference laboratory in England reporting 2/2514 tests as false negatives for *RHD* between 2001 and 2014 and 0/783 in the years 2019–2021.[14] As stated above this relies on referring centres informing the reference laboratory of errors identified later in pregnancy or on the birth of the baby.

Testing even later than 16 weeks may be advised for certain assays by individual reference laboratories and it is important to ensure that laboratory specific guidance on the earliest reliable gestation for testing is followed. Samples for foetal K genotyping should not be taken before 20 weeks' gestation. Similarly, repeat sampling may need to be undertaken, where this is advised by the reference laboratory,[17,18] to minimise the risk of a false negative result, particularly with foetal K genotyping cffDNA tests should be performed in pregnant women who have a history of HDFN, or where quantification values or titres suggest that the pregnancy is at risk of HDFN. These investigations should be requested by foetal medicine specialists who have the expertise to evaluate and explain the implications of the test results, in the context of clinical history and the outcome of any other investigations. These test results may then be used to guide the frequency and nature of further monitoring.

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Recommendation

Non-invasive foetal blood grouping using cell-free foetal DNA (cffDNA) from maternal plasma in alloimmunised pregnancies can be performed for *RHD* (D), *RHCE* (c and/or E) or *KEL*01* (K) with a false negative rate of <1%. These tests should be requested at the gestation advised by the reference laboratory used, by obstetricians or foetal medicine specialists who can explain the implications of the test findings having undertaken testing at the appropriate gestation (GRADE 1C).

4.6.2 | Fetal genotyping to guide anti-D Ig prophylaxis in non-immunised Rh D negative women

Before the availability of foetal genotyping, approximately 40% of Rh D negative women (40 000 in the UK per annum) would have been given anti-D Ig prophylaxis unnecessarily as they were carrying a D-negative foetus. Routine foetal *RHD* typing for all D-negative pregnant women has been introduced in Denmark, Finland and the Netherlands to allow selective use of anti-D Ig prophylaxis. This helps reduce unnecessary exposure of women and their foetuses to a blood product with reduction in costs related to the provision of anti-D Ig prophylaxis and tests related to fetomaternal haemorrhage.[15,19,20] This service is now provided by NHS Blood and Transplant (NHSBT) in England and the Welsh Blood Service (WBS) in Wales. The use of high- throughput, non-invasive prenatal diagnosis of foetal *RHD* status was recommended by the National Institute for Health and Care Excellence (NICE) in 2016 (http://www.nice.org.uk).

For mass throughput screening of all D-negative pregnant women, cffDNA testing for *RHD* is sufficiently accurate from 11 weeks' gestation with false negative rates of 0.1%–0.3%.[13–15,21] It is however advisable to test the foetal *RHD* status using a more sensitive and specific methodology at a later gestation for alloimmunised pregnant women as described above.

Recommendation

Fetal RHD typing using a high-throughput methodology in pregnant women who have not formed anti-D, as part of a screening program to target anti-D Ig prophylaxis, is sufficiently accurate for implementation from 11 weeks' gestation. The cost effectiveness of such testing is dependent on provision at a point in the antenatal care pathway that does not necessitate an additional visit (GRADE 1C).

5 | ANTENATAL TESTING PROTOCOLS

5.1 | Routine antenatal testing

All pregnant women should have blood samples taken early in pregnancy, at booking, typically by 10 + 0 weeks of pregnancy, for ABO and D group and for screening for the presence of red cell alloantibodies. When an antibody screen is positive, further tests should be carried out to determine the antibody specificity and significance (see Section 3).

All pregnant women, whether D positive or D negative, should have a further blood sample taken at the 28 week appointment for re-checking the ABO and D group and further screening for red cell alloantibodies.[1] D-positive women are just as likely as D-negative women to form antibodies (other than anti-D) late in pregnancy.[7,22]

Local policies must ensure that D-negative women who are eligible for routine antenatal anti-D Ig prophylaxis (RAADP) have the 28 week antibody screening sample taken before the first dose of RAADP is administered. Samples taken after the injection could result in passive anti-D being detected, which may be mistaken for immune anti-D, and conversely, potentially dangerous immune anti-D being mistaken for passive anti-D Ig[23] (see Section 5).

Late developing antibodies first detected after 28 weeks' gestation (i.e.: they were absent in a sample taken at 28 weeks), are less likely to cause clinically significant HDFN.[24,25] The introduction of RAADP has resulted in the detection of anti-D Ig in samples taken after 28 weeks' gestation from D-negative women.[26] Since it is not possible to differentiate between prophylactic anti-D Ig and immune anti-D until the latter has reached a high enough concentration in reference quantification to exclude the former, there is the potential for confusion between the two[23] (see Section 5.1).

Recommendation

All pregnant women should be ABO and D typed and screened for the presence of red cell antibodies early in pregnancy (at booking) and at 28 weeks' gestation.[1] In D negative women the 28-week sample should be taken before the administration of RAADP (GRADE 1B).

Recommendation

In pregnant women with no clinically significant red cell antibodies capable of causing HDFN present at 28 weeks' gestation, no further routine antenatal blood grouping or antibody screening is necessary (GRADE 1B) (Figures 1 and 2).

6 | RED CELL ANTIBODIES DETECTED IN PREGNANCY

Anti-D, anti-c and anti-K are the antibodies most often implicated in causing haemolytic disease severe enough to warrant antenatal intervention.[7] Pregnancies in women with a previous history of confirmed significant HDFN should however be considered at risk, and referral as early as possible to a foetal medicine specialist made regardless of the antibody specificity(ies) identified. In all other cases, follow-up testing protocols are dictated by the specificity and concentration of the antibodies identified. Regardless of antenatal follow-up regimens, antibody identification should always be performed to check for the

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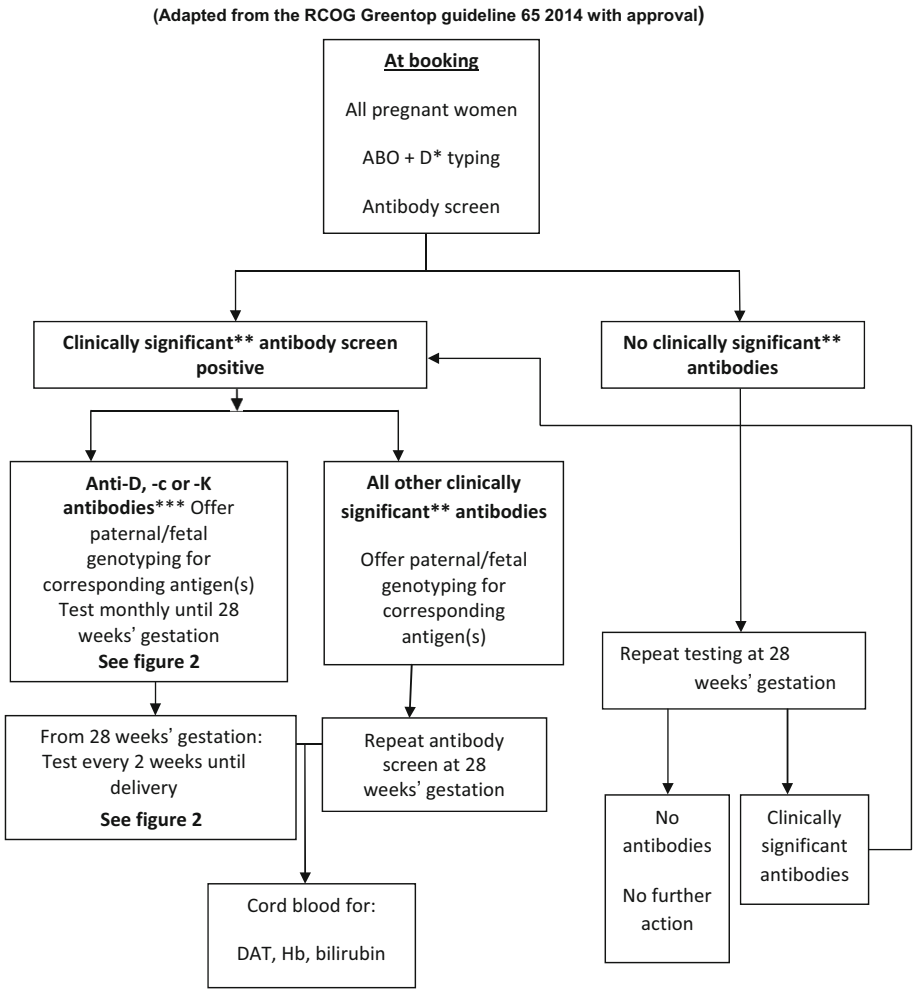

FIGURE 1 **Timing and frequency of antibody screening in pregnancy (Adapted from the RCOG Greentop guideline 652 014 with approval).** *If the woman is D negative with no immune anti-D, then advise anti-D Ig prophylaxis for any potentially sensitising events in pregnancy and give routine antenatal anti-D Ig prophylaxis (RAADP) either as a single dose or as two doses (see BSH guidelines for the use of anti-D Ig guidelines to prevent HDFN); after delivery check cord sample for D type and maternal sample for an FMH test to check if further anti-D Ig is needed, in addition to the standard dose which should be given in the first instance after delivery. **Clinically significant in an antenatal setting that is, with potential to cause HDFN. ***Pregnancies with immune anti-D, -K or -c are at particular risk of severe foetal HDFN, so further early assessment and referral to a foetal medicine specialist is indicated (see Section 5).

appearance of additional specificities, prior to maternal or foetal transfusion.

> Recommendation
> All pregnant women who have previously had a baby affected by HDFN should be referred as early as possible ahead of 20 weeks' gestation to a foetal medicine specialist for assessment and advice, irrespective of antibody concentration or specificity (GRADE 1B).

6.1 | Pregnant women with detectable anti-D

Prophylactic anti-D Ig has been very successful in reducing the number of sensitisations to D but it has created difficulties in determining whether anti-D detected in pregnancy is passive (due to anti-D Ig prophylaxis given as RAADP or for a potentially sensitising event) or immune. The risks associated with the misinterpretation of the nature of anti-D are clear: if passive anti-D Ig is misinterpreted as immune anti-D, then further anti-D Ig prophylaxis may be omitted leaving the women unprotected from sensitisation. If immune anti-D is misinterpreted as passive anti-D Ig, appropriate follow-up of the antibody concentration during pregnancy may be curtailed, and interventions that might be required to manage HDFN, not instigated. The Serious Hazards of Transfusion (SHOT) annual report 2011 included a 'learning point' highlighting the difficulties in differentiating between immune anti-D and passive anti-D Ig, as there were seven cases reported where women with immune anti-D were not followed up as closely as they should have been because the anti-D detected was wrongly assumed to be passive anti-D Ig, and in six of these cases the babies were born with some degree of HDFN.[27]

6.1.1 | Distinguishing between passive and immune anti-D

Passive anti-D Ig and immune anti-D cannot be qualitatively distinguished serologically. While the concentration of passive anti-D Ig will fall with time, the concentration of immune anti-D will usually remain stable, or rise if there is re-stimulation (Figure 3).

The concentration of passive anti-D Ig in maternal samples post-prophylaxis rarely exceeds 0.4 IU/mL unless anti-D Ig exceeding 1500 IU has been administered. The peak concentration of anti-D Ig detected after 1500 IU of intravenous anti-D Ig in a pharmacokinetic study in pregnant women was equivalent to 0.4 IU/mL and following intramuscular anti-D Ig 0.2 IU/mL.[28] These values are approximate

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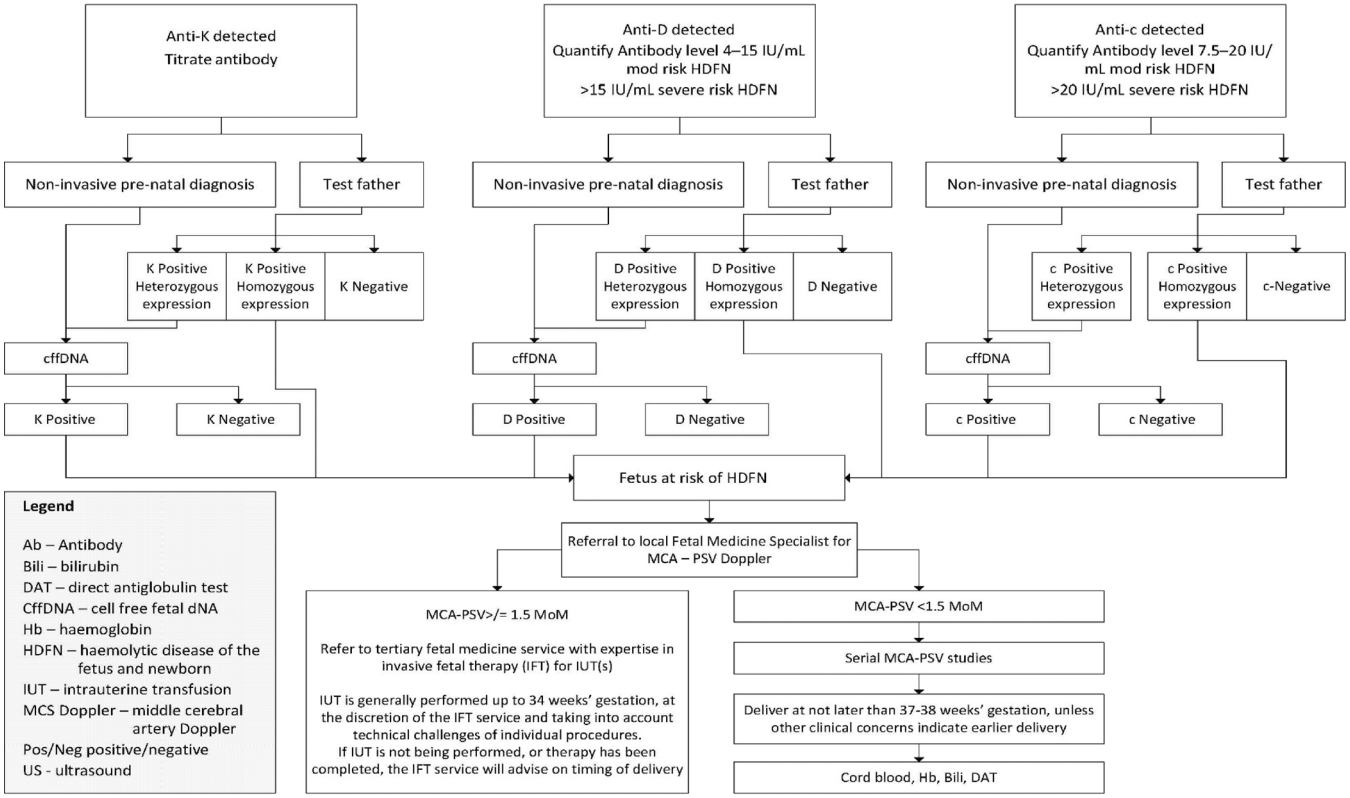

FIGURE 2 Management algorithm for pregnancies complicated by anti-D, anti-K or anti-C alloimmunisation (adapted from RCOG Greentop guideline 652 014 with approval[11]).

conversions based upon the concentrations stated in the publication. Following administration of an intramuscular injection of anti-D Ig, a serologically detectable concentration of anti-D Ig is potentially present within minutes and the peak blood concentration is reached within three to 7 days. The half-life of passive anti-D Ig is approximately 3 weeks.[29] Passive anti-D Ig can be detected by serological tests for several weeks: by an IAT at 8 weeks or more following injection of 500 IU, and for more than 12 weeks where more sensitive techniques are used or following higher doses of anti-D Ig. Immune anti-D becomes detectable approximately 4 weeks after primary exposure to D-positive cells, and reaches a peak concentration after 6–8 weeks, if there is no further exposure.[30]

Prediction of the nature of anti-D (immune anti-D or passive anti-D Ig) based on the strength of reaction with D-positive cells is unreliable, as this will vary with the technique and Rh phenotype of the reagent red cells used. Babies have been severely affected by HDFN as a result of assumptions made on the basis of the strength of reactions using methods that have not been validated.[27]

Quantification by continuous flow analyser (CFA) gives an objective measurement of antibody concentration in IU/mL anti-D. All anti-D detected in pregnancy should be quantified by CFA with reference to the NIBSC anti-D standard,[31] or tested by a method that has been extensively validated against CFA and that gives a result that is expressed in or can easily be converted to IU/mL anti-D.

The only exception is where anti-D is detected for the first time immediately prior to or at the time of delivery, for example, in a pre-delivery group and screen sample, in which case the sample need not be sent for quantification, but the baby should be monitored for signs of HDFN as treatment decisions will need to be made on the basis of the severity of any anaemia and/or jaundice. The results of quantification are unlikely to have a major influence on clinical decisions at this stage and are not available as quickly as measures of haemoglobin and bilirubin. Maternal anti-D quantification may be performed at a later stage if deemed necessary (e.g. to help determine advice on clinical management in future pregnancies).

The quantification results should be viewed in the context of the timing and dose of any anti- D Ig given previously, the reason for its administration, the route of administration and the antibody status at the time of administration. The clinical history and knowledge of the results of previous laboratory testing are paramount in clinical decision making where anti-D is detected in pregnancy, and every effort should therefore be made to obtain this information.

Even if anti-D Ig has been administered for a potentially sensitising event prior to 28 weeks it must be assumed that anti-D detectable at or before 28 weeks may be immune as levels can rise dangerously between 28 weeks and term. Therefore, monitoring should be undertaken as if the antibody may be immune (until anti-D is no longer detectable), while continuing to administer anti-D Ig as indicated in the BSH guideline for the use of anti-D Ig to prevent HDFN.[2]

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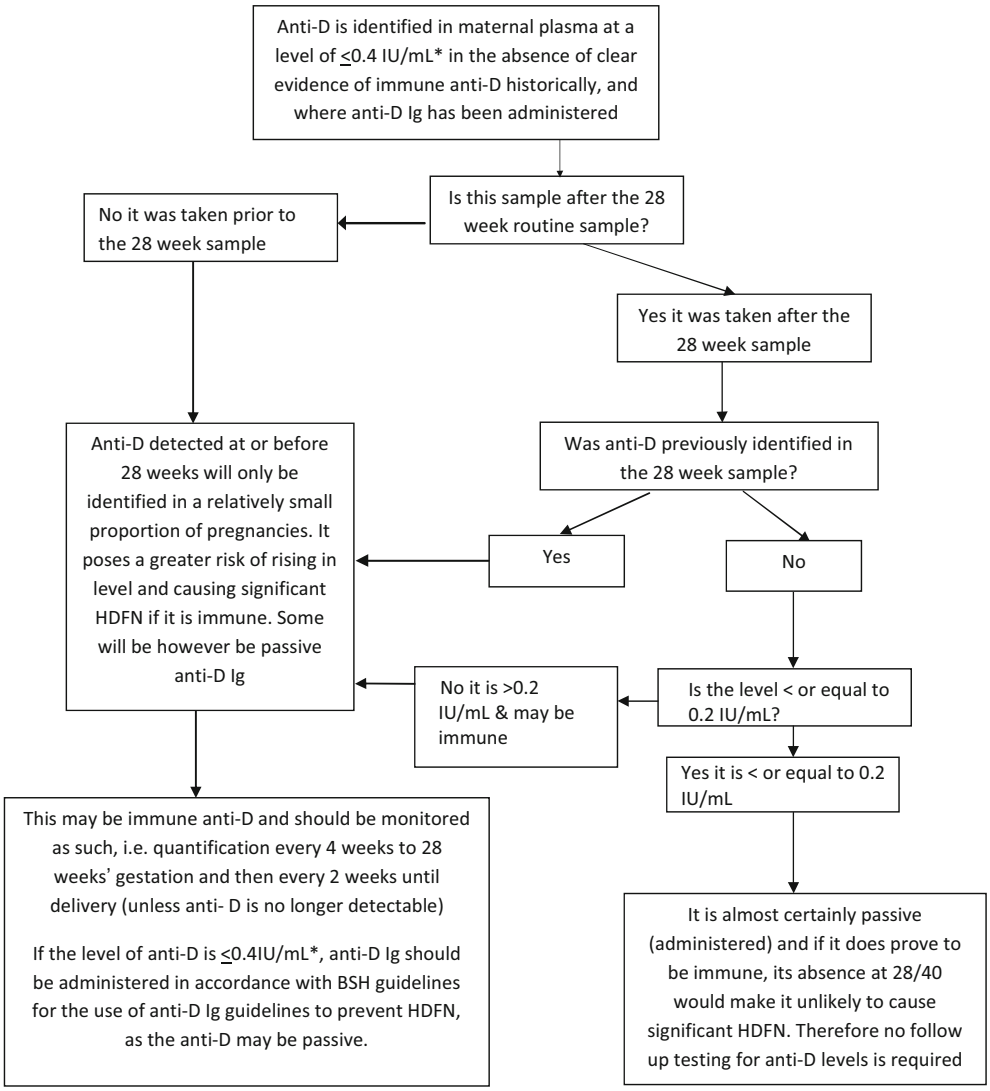

FIGURE 3 **Managing pregnancies when anti-D has been detected in a woman's plasma for the first time after a dose of anti-D Ig prophylaxis.** *Levels of anti-D >0.4 IU/mL are assumed to be immune in origin and managed accordingly unless a large dose of anti-D Ig has been given (>1500 IU) or the sample was taken within hours of an intravenous dose of anti-D immunoglobulin, in which case it could be passive and anti-D Ig prophylaxis should also continue.

6.1.2 | Procedures to follow where anti-D is detected in pregnancy

a. Determine whether anti-D Ig has been administered (not just issued) and its clinical indication that is, whether as RAADP or following a potentially sensitising event, by asking the woman and seeking written confirmation in the notes.
b. Undertake quantification (unless immediately prior to delivery, in which case the baby should be monitored for signs of HDFN).
c. If *any* of the following apply, then the antibody should be monitored as for immunised women, that is, at 4 weekly intervals to 28 weeks gestation, and at 2 weekly intervals after 28 weeks, until delivery (or until serial monitoring by foetal middle cerebral artery (MCA) peak systolic velocity Doppler has been instituted and serial testing deemed unnecessary, or anti-D is no longer detectable):
   - Anti-D was detected at or before the 28 week gestation RAADP administration (even if anti-D Ig was administered earlier in pregnancy as the risk of falsely labelling immune anti-D as passive anti-D Ig when detected at or prior to 28 weeks gestation is significant)
   - There is no definite record of prior anti-D Ig administration.
   - Anti-D was present before the first administration of anti-D Ig at any gestation.
   - The level of anti-D is greater than 0.2 IU/mL.
d. If the anti-D level is ≤0.4 IU/mL after up to 1500 IU of anti-D Ig have been administered, then prophylactic anti-D Ig should continue to be offered for routine prophylaxis and potentially sensitising events in accordance with the BSH guideline for the use of anti-D Ig to prevent HDFN[2] unless it is established beyond doubt that the anti-D is immune, in which case anti-D Ig prophylaxis is no longer indicated.
e. If doses in excess of 1500 IU anti-D Ig have been administered, then a level of >0.4 IU/mL may be achieved as a result of passive anti-D Ig. Therefore, prophylactic anti-D Ig should continue to be offered for routine prophylaxis and potentially sensitising events in accordance with the BSH guideline for the use of anti-D Ig to prevent HDFN[2] unless it is established beyond doubt that the anti-D

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is immune, in which case anti-D Ig prophylaxis is no longer indicated.

f. After 28 weeks' gestation, where *all* of the following apply, testing should continue as for non-sensitised women that is, there is no requirement for further antibody testing after 28 weeks' gestation, and anti-D Ig prophylaxis should continue to be offered in accordance with the BSH guideline for the use of anti-D Ig to prevent HDFN.[2]
   - Anti-D was not detectable in a sample taken immediately prior to RAADP administration (the timing of RAADP should be at 28 weeks gestation but will sometimes be slightly earlier or later).
   - There is a written record of administration of anti-D Ig in the preceding 8 weeks
   - The concentration of anti-D is less than or equal to 0.2 IU/mL

Women with anti-D should not be issued with an antibody card documenting the finding of anti-D until it is conclusively established that the anti-D is immune (Figure 3).

6.1.3 | Pregnant women with immune anti-D

Anti-D is the most frequent cause of serious HDFN. Blood samples from pregnant women with immune anti-D should be tested at least monthly until 28 weeks gestation and every 2 weeks thereafter until delivery, to monitor the concentration of anti-D (with reference to the NIBSC anti-D standard[31]) and to identify any additional antibodies that may develop.

Where anti-D has been shown to be immune (see Section 5.1), an increase in concentration of 50% or greater, compared with the previous concentration, suggests a significant increase, irrespective of the period of gestation as this would be a greater than expected variation in a validated test.[32]

When account has been taken of previous history of HDFN, the concentrations of anti-D shown in Table 1 have been used to guide the management of pregnancies.[33]

In addition, the D status of the foetus of pregnant women with a significant level of anti-D should be determined using cffDNA from a maternal peripheral blood sample. This technique currently has a false negative rate of <1%.[34] The result of this test can be used as a guide to the frequency and nature of ongoing monitoring (see Section 3).

TABLE 1 Significance of anti-D antibody concentration.

Anti-D concentration	Predicted clinical outcome
Less than 4 IU/mL	HDFN unlikely, continue to monitor
4–15 IU/mL	Moderate risk of HDFN, requiring referral to a foetal medicine specialist
More than 15 IU/mL	High risk of HDFN requiring urgent referral to a foetal medicine specialist

Non-invasive middle cerebral artery (MCA) Doppler, measuring peak systolic velocity (MCA-PSV) is used to monitor for, and diagnose, foetal anaemia.[35] An MCA-PSV >1.5 MoM strongly correlates with moderate to severe foetal anaemia. Pregnant women with an anti-D concentration of 4 IU/mL or greater and/or a rising anti-D concentration and/or a history of previous offspring affected by HDFN should be referred to a foetal medicine specialist for further assessment including MCA Doppler. When the indication for MCA-PSV is a critical and/or rising anti-D concentration the foetal medicine assessment is generally performed at weekly intervals. When the indication for MCA-PSV is previous history of HDFN the frequency of MCA-PSV assessment will take into account this history, trend in anti-D concentration and trend of MCA-PSV measurements. The minimum frequency of monitoring is likely to be once a fortnight, and will increase as dictated by rising trends in these parameters. The technique for MCA-PSV measurement is described in International Society of Ultrasound in Obstetrics and Gynaecology (ISUOG) Practice guidelines: use of Doppler velocimetry in obstetrics.[36] It is recognised that MCA Doppler, for assessment of all foetal anaemia, becomes less accurate after 35 weeks' gestation.[37]

It should be noted that HDFN has rarely also been reported with anti-D at less than 4 IU/mL.[38,39] Where the anti-D concentration is greater than that attributable to the presence of passive anti-D Ig, but <4 IU/mL, anti-D monitoring is required.

Once referral to a foetal medicine specialist has been made and serial MCA Doppler is performed, the value of anti-D quantification on subsequent samples is doubtful, especially if the concentration is already in the high risk range.[11] As a minimum however a sample should still be tested for the presence of further red cell antibodies at 28 weeks' gestation, and whenever blood is required for transfusion, for example, for IUT. The multidisciplinary team including the laboratory should be informed of the testing plan to prevent confusion and unnecessary interventions, for example, the laboratory requesting follow up samples in cases where the clinical team have decided that they are not required.

Foetuses at risk of HDFN should be delivered at not later than 37–38 weeks' gestation to minimise the duration of exposure to maternal blood group antibodies,[11] unless earlier delivery is indicated due to rising antibody levels or titres or intra-uterine transfusions have been required.

Intrauterine transfusion may be indicated if the MCA-PSV is consistently >1.5 MoM. The procedure is performed in a tertiary centre with expertise in invasive foetal therapy. Fetal blood sampling (FBS) is carried out to assess the foetal haematocrit with the necessary preparation and set up in place to proceed seamlessly to the anticipated IUT. Depending on the gestational age at diagnosis of foetal anaemia, serial IUTs may be required. Most clinicians do not perform IUT beyond 34 weeks' gestation. Following IUT(s) the invasive foetal medicine team will advise on the timing of delivery.

If the sample in which anti-D is detected is for routine antenatal antibody screening or is pre- transfusion, D-negative screening cells, selected to provide all red cell antigens as specified for screening panels in the BSH compatibility guidelines,[5] can be useful to detect or

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exclude the presence of alloantibodies of other specificities. However, if this approach is taken, the risk of using such a screening panel for other patients in error and potentially missing an immune anti-D should be considered. A proportion of antibodies with apparent anti-C + D specificity, (usually, but not always, with disproportionately high anti-C titres) can be demonstrated, by advanced serological techniques, to be anti-G or anti-C + G, rather than straightforward anti-C + D.[40] Women who have produced anti-G or anti-C + G, but not anti-D, remain at risk of producing immune anti-D and should be offered RAADP and post-delivery anti-D Ig prophylaxis. It is important to identify these cases to ensure appropriate follow-up and access to anti-D Ig prophylaxis, and therefore, a reference centre should confirm examples of apparent anti-C + D specificity. Where anti-G is suspected, the Rh phenotype of the partner may be useful as part of confirmatory testing at a reference centre.

Recommendation
A reference centre should confirm antibody specificity in women with apparent anti- C + D (GRADE 2B).

6.2 | Pregnant women with immune anti-c

Pregnant women with anti-c should be re-tested with the same frequency as women with immune anti-D that is, at least 4 weekly to 28 weeks' gestation and every 2 weeks thereafter, until delivery. Quantification of anti-c is useful in monitoring any increase in the antibody concentration. Samples from pregnant women with anti-c should be quantified with reference to the NIBSC anti-c standard[31] and, where possible, the previous sample should be tested in parallel, as for immune anti-D. Antibody identification should be undertaken to exclude or confirm the presence of other clinically significant antibodies.

In conjunction with any previous history of HDFN, the concentrations of anti-c shown in Table 2 are indicative of the requirement to refer to a foetal medicine specialist.[41]

The c status of the foetus of pregnant women with a significant concentration of anti-c should be determined using cffDNA from a maternal peripheral blood sample. This technique currently has a false negative rate of <1%.[18] The result of this test can be used as a guide to the frequency and nature of ongoing monitoring (see Section 3).

TABLE 2 Significance of anti-c antibody concentration.

Anti-c concentration	Predicted clinical outcome
Less than 7.5 IU/mL	HDFN unlikely, continue to monitor
7.5 to 20 IU/mL	Moderate risk of HDFN, requiring referral to a foetal medicine specialist
More than 20 IU/mL	High risk of HDFN requiring urgent referral to a foetal medicine specialist

Pregnant women with an anti-c concentration of 7.5 IU/mL or greater and/or a rising anti-c concentration and/or a history of offspring affected by HDFN should be referred to a foetal medicine specialist for further assessment including MCA Doppler. The frequency of MCA Doppler is as described in Section 5.1. Once referral to a foetal medicine specialist has been made and serial MCA-PSV measurements are being performed, the value of undertaking anti-c quantification on subsequent samples is doubtful, especially if the concentration is already in the high risk range.[11] As a minimum however a sample should still be tested for the presence of further red cell antibodies at 28 weeks' gestation, and whenever blood is required for transfusion purposes for example, for IUT. The multidisciplinary team including the laboratory should be informed of the testing plan to prevent confusion and unnecessary interventions, for example, the laboratory requesting follow up samples in cases where the clinical team have decided that they are not required.

Foetuses at risk of HDFN should be delivered at not later than 37–38 weeks' gestation to minimise the duration of exposure to maternal blood group antibodies,[11] unless earlier delivery is indicated due to rising antibody levels or titres, or intra-uterine transfusions have been required.

6.3 | Pregnant women with immune anti-K, or other Kell blood group system antibodies

Where detected, antibodies to other antigens in the Kell blood group system (e.g. anti-k, -Kp^a, -Kp^b, -Js^a, -Js^b) should be investigated and monitored in the same way as anti-K, as these have the potential to cause HDFN.[42] HDFN due to anti-K is characterised by low haemoglobin concentration, but elevated amniotic and/or cord bilirubin levels are not generally reported. The foetal anaemia associated with anti-K may be due to the inhibition of K-positive erythroid early progenitor cells[43] and/or to promotion of their immune destruction.[44] It has been stated in some texts that the severity of HDFN due to anti-K is not correlated with the titre of the antibody and publications prior to 1995 cited occasions where severe HDFN occurred despite low titres of anti-K. More recent case series of affected pregnancies have however suggested that severe HDFN is associated with antibodies with a titre of 32 or greater,[45,46] though a more recent larger series has suggested a titre of 4.[47] Therefore, samples from women with anti-K should be titrated (see Section 3) when first identified in the pregnancy, as for any clinically significant antibody, and serial titration should then be undertaken every 4 weeks until 28 weeks' gestation, and 2 weekly thereafter, until delivery. Owing to its less predictable nature, all cases of anti-K should be referred to a foetal medicine specialist on first identification of the antibody, to establish a management plan. A titre of ≥1:4 is a recommended cut off point for commencing serial MCA Doppler.[47]

Once serial MCA -PSV measurements are being performed, the value of undertaking anti-K titration on subsequent samples is doubtful, especially if the titres are already in the high risk range.[11] The

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multidisciplinary team including the laboratory should be informed of the testing plan to prevent confusion and unnecessary interventions, for example, the laboratory requesting follow up samples in cases where the clinical team have decided that they are not required.

Foetuses at risk of HDFN should be delivered at not later than 37–38 weeks' gestation to minimise the duration of exposure to maternal blood group antibodies,[11] unless earlier delivery is indicated due to rising antibody levels or titres, or intra-uterine transfusions have been required.

Historically the majority of cases of anti-K in pregnant women were the consequence of previous K-positive transfusions in the UK. The incidence of anti-K can be reduced by selecting K-negative units for transfusion to females with potential for childbearing, unless known to be K-positive themselves.[48] Therefore, K-negative units should be selected for females under the age of 50 years who are themselves K-negative, or whose K type is unknown. However, emergency transfusions should not be delayed if suitable K-negative units are not immediately available.[5]

Testing of the father, discussed in Section 3, may be particularly relevant for women with anti-K as historically approximately 80% of anti-K detected in pregnant women results from previous transfusion and only 9% of the general population are K-positive (although a higher proportion of partners of women with anti-K are K-positive[48,49]). It remains important that the transfusion history of women with anti-K is established. K typing the father of the foetus should be considered to support counselling for the current and any future pregnancies. If the biological father's K type is unknown or he is K-positive the pregnant woman should be referred to a foetal medicine specialist when anti-K is first identified for counselling and monitoring.

If the father is K-negative and a confidential enquiry establishes paternity, no further samples are required until 28 weeks' gestation when further antibodies should be excluded (as for all women and any antibodies detected at 28 weeks). Any clinically significant antibodies identified, in addition to anti-K, should be monitored according to their specificity.

The K (*KEL*01*) status of the foetus of pregnant women with anti-K should be determined using cffDNA from a maternal peripheral blood sample if the father is heterozygous for the K antigen or if his antigen status is unknown. This technique currently has a false negative rate of <1% at or after 20 weeks gestation.[18] The result of this test can be used as a guide to the frequency and nature of ongoing monitoring (see Section 3). If the foetus is predicted to be K negative upon cffDNA testing the cffDNA test should be repeated to confirm the initial results,[18] monitoring should continue in the interim.

Recommendation

Samples from pregnant women with immune anti-D or anti-c should be assessed serologically at 4 weekly intervals to 28 weeks' gestation and at fortnightly intervals thereafter, until delivery. Such cases should be referred to a foetal medicine specialist if the antibody reaches the critical level and/or the level is rising significantly, where assessment of the need for further monitoring will be made (GRADE 1B).

Recommendation

Pregnant women with anti-K or other Kell system antibodies should be referred to a foetal medicine specialist when the antibody is first identified. If the foetus is known or assumed to be at risk (based on biological father or foetal typing) serological assessment at monthly intervals to 28 weeks' gestation and at fortnightly intervals thereafter is required (GRADE 1B).

Recommendation

In all cases where serial Doppler assessment of foetal middle cerebral artery peak systolic velocity (MCA-PSV) is being performed and antibody levels are in the high risk range, ongoing assessment of antibody strength is unlikely to be of value and can be discontinued at the discretion of a foetal medicine specialist[11] (GRADE 1C).

Recommendation

All women who have had an IUT in a previous pregnancy, for alloimmunization, should be alerted to the invasive foetal therapy service in any subsequent at risk pregnancy to facilitate workload planning (GRADE 1D).

Recommendation

Where possible, scheduled MCA-PSV monitoring is best performed at the beginning of the week to facilitate optimal access to the invasive foetal therapy service for IUT, when required, and availability of appropriate blood products (GRADE 2D).

6.4 | Pregnant women with other red cell antibodies

It is only IgG antibodies that are capable of entering the foetal circulation, and red cell antibodies with a significant IgG component are detectable by IAT. 'Cold reactive', IgM and low affinity antibodies to high prevalence antigens (e.g. CR1-related antibodies) have not been implicated in HDFN.

In addition to anti-D, -c and -K, the following specificities are most commonly associated with HDFN: anti-C, -e, -E, -Fy^a, and -Jk^a.[7,50–52] Many other specificities have however been reported as the cause of HDFN (Table 3). Less common antibody specificities may be identified, some of which may be more prevalent in women of a specific ethnic origin, such as anti-Ge3 in the Hispanic population,[53] and anti-M in the Japanese population[54]; the anti-M having caused a form of delayed HDFN, similar to that caused by anti-K, including suppression of erythropoiesis. Anti-Mur and anti-Dia are more prevalent in Chinese populations.[55] In most cases, re-testing at 28 weeks gestation' generally provides sufficient information to determine management of the pregnancy.

The situation is different for women with a previous history of a baby with HDFN and a clinical decision should be made regarding

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TABLE 3 Red cell antibodies showing published clinical significance.

Antibody	HDFN	Haemolytic transfusion reaction	Proposed HDFN management
D	Severe in foetus and neonate	Severe	Fetal Assessment[a]
c	Severe in foetus and neonate	Severe	Fetal Assessment
K	Severe in foetus and neonate	Severe	Fetal Assessment
c + E	Yes in neonate[b]	Yes	Fetal Assessment
E	Yes in neonate[b]	Yes	Fetal Assessment
C	Yes in neonate[b]	Yes	Fetal Assessment
e	Yes in neonate	Yes	Routine Obstetric Care
Ce	Yes in neonate	Yes	Routine Obstetric Care
Hr_o	Yes in neonate[b]	No	Fetal Assessment
Rh29	Yes in neonate[b]	Yes	Fetal Assessment
Fy^a	Yes in neonate[b]	Yes	Fetal Assessment
Fy^b	Yes in neonate	Yes	Routine Obstetric Care
Fy^3	No	Yes	Routine Obstetric Care
Jk^a	Yes in neonate[b]	Yes	Fetal Assessment
Jk^b	No	Yes	Routine Obstetric Care
S	Yes in neonate[b]	Yes	Fetal Assessment
s	Yes in neonate[b]	Yes	Fetal Assessment
U	Yes in neonate[b]	Yes	Fetal Assessment
M	Yes in neonate (occasionally)[b]	Yes (if active at 37°C)	Fetal Assessment
N	Mild (1 case)	Yes (if active at 37°C)	Routine Obstetric Care
Mi^a	Yes in neonate[b]	Yes	Fetal Assessment
Mt^a	Yes in neonate[b]	No	Fetal Assessment
Vw	Yes in neonate[b]	Yes	Fetal Assessment
Mur	Yes in neonate[b]	Yes	Fetal Assessment
H (Bombay)	Yes in neonate[b]	Yes	Fetal Assessment
G	Yes in neonate	Yes	Routine Obstetric Care
k	Yes in neonate[b]	Yes	Fetal Assessment
Kp^a	Yes in neonate occasionally[b]	No	Routine Obstetric Care
C^w	Yes in neonate occasionally[b]	No	Routine Obstetric Care
Lu^a	No	Yes	Routine Obstetric Care
Lu^b	No	Yes	Routine Obstetric Care
Di^a	Yes in neonate[b]	Yes	Fetal Assessment
Di^b	Yes in neonate	Yes	Routine Obstetric Care
Co^a	Yes in neonate[b]	Yes	Fetal Assessment
Co^b	No	Yes	Routine Obstetric Care
Vel	No	Yes	Routine Obstetric Care
En^a	Yes in neonate[b]	Yes	Fetal Assessment
Yt^a	Yes in neonate (occasionally)	Yes	Routine Obstetric Care
Yt^b	Yes in neonate (occasionally)	No	Routine Obstetric Care
Wr^a	Yes in neonate[b]	Yes	Fetal Assessment
Wr^b	No	No	Routine Obstetric Care
Ata	Yes in neonate (occasionally)	Yes	Routine Obstetric Care
Jsa	Mild	Yes	Routine Obstetric Care
Jsb	Yes in neonate	Yes	Routine Obstetric Care

[a]Fetal assessment includes obstetric history, maternal serology, and serial scans and so forth also see Figure 2.
[b]Anti-D, -c and -K are the 3 main antibodies that have been reported to causes severe anaemia, jaundice or death in the foetus or neonate. Many other antibodies can cause anaemia or jaundice predominantly in the neonatal period but there have also been occasional case reports of the foetus being severely affected.

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more frequent testing. The nature of the past history may help define the intensity of monitoring. If there has been a previous baby that suffered hydrops, significant anaemia or jaundice then monitoring to prevent this at an intensity similar to that for anti-D, -c or -K is warranted with regular MCA-PSV Doppler and consideration of delivery at 37–38 weeks' gestation. Similar to anti-D, -c and -K once the titres are above the trigger for MCA Doppler monitoring the value of serial measurement of antibody titre in addition is unclear.

In the absence of a history of HDFN, the likelihood of developing hydrops with antibodies of other specificities is lower than that of anti-D, -c and -K. For example, in a case series of 32 pregnancies at risk of HDFN from anti-E only, 5 (15%) had Hb <100 g/L at delivery, 1 (3%) had hydrops fetalis and there was 1 (3%) perinatal death attributable to anti-E.[56] The likelihood appears to be even lower for other specificities such as anti-Fy^a, -Fy^b, -Fy3, -S, -s or -U, though some case series are selective and do not provide a denominator of the population of pregnant women with antibodies of the defined specificity.[52,57–59]

It is likely that in the absence of a previous history of HDFN pregnant women with antibody titres 32 or greater than 32 for specificities other than anti-D, -c and -K would not need to be monitored as frequently as those with a history of severe HDFN and / or the high risk antibody specificities anti-D, -c or -K. However, it is difficult to precisely quantify the risk for this particular group and if a reduced frequency of monitoring is being considered an individual management plan should be discussed with a foetal medicine specialist, which will be informed by the level and/or rise in antibody titre and the MCA-PSV trend. The decision on the frequency of monitoring and the need for early delivery therefore will primarily be based on the history of previous pregnancies for the affected family and the reports and case series available in the world literature. A balance has to be made between intensive monitoring to the level advised for anti-D, -c and -K with a low yield in terms of intervention being required and a reduced level of monitoring with an attendant risk of missing progressive anaemia and hence delaying intervention. The numbers of reported cases and well-defined case series for antibodies against antigens other than anti-D, -c and -K are low. Research defining the risks and benefits of taking a less intensive approach to monitoring pregnant women with such antibodies would be welcomed. For information about other antibodies and their ability to cause clinically significant HDFN, it is recommended that further advice is sought from a transfusion medicine specialist in a red cell reference department. Reference tables for antibodies that have been associated with HDFN are available in the Scottish National Transfusion Service (SNBTS) guidelines, Royal College of Obstetricians and Gynaecologists (RCOG) guidelines and NHSBT guidelines.[11,17,60]

Where an IgG antibody has been detected, testing of both first appointment and 28-week gestation samples should include titration (see Section 3). In general, there is a risk that an antibody with a titre of 32 or greater will cause some degree of HDFN, although a clear-cut association between titre and HDFN has not been established.

The presence of additional antibodies should be established and any clinically significant antibodies should be titrated or quantified depending on the specificity (see Section 3).

Recommendation

Clinically significant antibodies, other than anti-D, -c or -K, should be excluded or, if present, assessed by titration, at the booking appointment and at 28 weeks' gestation. If deemed necessary based on a high titre (32 or greater) and / or a past history of HDFN, referral to a specialist in foetal medicine should be made for further assessment (GRADE 1C).

7 | REPORTS OF LABORATORY INVESTIGATIONS

In addition to blood group and specificity of any red cell alloantibodies present, reports must inform the clinician[s] responsible for the pregnant woman's antenatal care of the likely significance of the antibodies, with respect to both the development of HDFN and potential difficulties in providing compatible blood for transfusion.[1] Reports should also, where relevant, alert the clinician to the need to refer the woman to a foetal medicine specialist. The woman should be given verbal and written information about the clinical significance of the antibody(ies) and it should be documented that she has been counselled in the patient record.

Details of the timing of further samples required should also be provided.

Recommendation

In addition to blood group and specificity of any red cell alloantibodies present, reports must inform the clinician[s] responsible for the pregnant woman's antenatal care of the likely significance of the antibodies, with respect to both the development of HDFN and transfusion problems[1] (GRADE 1B).

Recommendation

Pregnant women with clinically significant red cell antibodies should be given verbal and written information with details of each antibody specificity and its potential for causing HDFN and delay in providing compatible blood (GRADE 1D).

8 | REQUIREMENTS FOR BLOOD

8.1 | Blood or blood components for the woman

For any woman with an antibody, time for cross-matching and local blood transport should be taken into account when the decision for transfusion is made. Blood can usually be sourced within hospital stocks for women with anti-D, -c, -C, -E or -K antibodies, as all units are labelled with full Rh and K antigen status. For antibodies other than anti-D, -c, -C, -E and -K, blood may need to be obtained from a regional blood centre, entailing additional transport time.

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For women with multiple antibodies or antibodies against high prevalence antigens, close collaboration between maternity, neonatology and haematology staff is essential to prevent delays in obtaining blood. A national search for compatible blood may be required, or rare blood donors may need to be called up to donate if time permits, or frozen blood may need to be obtained from the National Frozen Blood Bank in Liverpool. With advance planning this requires more than 6 h' processing time, plus transportation time to the hospital. The shelf life of frozen units once thawed is 72 h and they cannot be refrozen.

Cytomegalovirus (CMV)-negative blood or platelets are not required for the mother once the baby is delivered, or in an emergency setting.[11]

Recommendation
For women with multiple antibodies or antibodies against high prevalence antigens an assessment of the likelihood of maternal need for blood, and discussion of appropriate measures to reduce the likelihood of transfusion, including correction of any haematinic deficiency, should take place as early in pregnancy as feasible between the maternity and haematology staff (GRADE 1C).

Recommendation
Multidisciplinary planning of delivery should take place to reduce blood loss and intra-operative cell salvage should be available where appropriate. This ensures optimal use of rare blood, taking account of the needs of the patient and the scarcity of the blood. Clear communication is required between teams within a hospital, and also between hospitals if cases are referred to tertiary centres, to ensure appropriate management of transfusion in the mother and the baby (GRADE 1D).

8.2 | Blood for intrauterine transfusion

Blood for intrauterine transfusion (IUT) has the same special requirements as blood for neonatal exchange (see 6.0.3), except that plasma is removed to increase the haematocrit to 0.70–0.85 and it is always irradiated to prevent transfusion-associated graft-versus host disease. Blood for IUT is processed to order as it only has 24 h' shelf life after processing and normally requires a minimum of one working day's notice, unless an emergency.[61]

8.3 | Blood for neonatal exchange

Blood should be ABO compatible with the neonate and mother (to avoid ABO HDFN from maternal anti-A or -B antibodies present), D negative (or the same D group as the neonate), K negative, negative for the corresponding antigen to which the mother has an antibody and cross-match compatible with the mother's blood sample. Blood should be less than 5 days old (to ensure low supernatant potassium levels), CMV-negative and irradiated unless the risk to the baby of delaying exchange transfusion while obtaining irradiated blood outweighs this. It should be plasma reduced (rather than in saline-adenine-glucose-mannitol [SAGM] additive solution), with a haematocrit of 0.50–0.60.[61]

Blood for exchange transfusion is therefore normally obtained from a regional blood centre, which routinely stocks blood suitable for babies whose mothers have anti-D, -c, -K, -C or -E antibodies. If maternal antibodies other than these are present, clinical staff should communicate with the blood transfusion laboratory in advance of delivery to ensure that appropriate blood meeting all the requirements is available. Once irradiated, exchange blood has a shelf life of only 24 h.[61]

Recommendation
For IUT or neonatal exchange transfusion, if maternal antibodies other than anti-D, -c, -C, -E or -K are present, advance warning of at least 24 h should be given where possible to ensure that blood of suitable specification and negative for all relevant antigens, is available (GRADE 1D).

8.4 | Blood for neonatal small volume ('top-up') transfusion

Blood should be ABO compatible with the neonate and mother (to avoid ABO HDFN from maternal anti-A or -B antibodies present), D negative (or the same D group as the neonate), K negative and negative for the corresponding antigen(s) to which the mother has an antibody(ies) and cross-match compatible with the mother's blood sample.

Blood should be CMV negative, but does not need to be irradiated unless the neonate has had a previous IUT. Blood can be stored in SAGM (rather than plasma reduced) and be up to 35 days old.

Recommendation
Clinicians considering transfusion in a neonate must check if the baby has had an IUT, as if so, blood must be irradiated to prevent transfusion-associated graft-versus-host disease.[60] (GRADE 1A).

Recommendation
In view of the less restrictive shelf life of blood for top-up transfusion, blood compatible with maternal antibodies other than anti-D, -c, -C, -E or -K, may be more readily available than blood for exchange or IUT, but advance warning should still be given to transfusion laboratories whenever possible (GRADE 1D).

8.5 | What blood or blood components can be administered in the emergency situation to the mother known to have red cell antibodies?

The decision to use ABO-, D- and K-compatible blood that is not matched for other antibodies (or to use O D negative, K negative, where the mother's ABO and D groups are unknown) should be made on the balance of risks: severe haemorrhage versus a haemolytic

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transfusion reaction vs risk to future pregnancies. Transfusion should not be delayed in the event of life-threatening haemorrhage.[62] Close liaison with the transfusion laboratory is essential.

> Recommendation
> Obstetric, haematology and transfusion laboratory staff should discuss when to give alternative blood, when sufficient compatible blood is not readily available, based on the balance of clinical risks (severe haemorrhage versus a haemolytic transfusion reaction with potential associated complications, including renal failure) (GRADE 1C).

> Recommendation
> If ABO-, D- and K-compatible blood that is not matched for other antibodies is used for resuscitation in the event of life-threatening haemorrhage, consider giving intravenous methylprednisolone 1 g +/− intravenous (IV) immunoglobulin 1 g/kg pre-emptively.[63] (GRADE: 1C).

The presence of maternal red cell antibodies has no implications for other blood components such as platelets, fresh frozen plasma, cryoprecipitate or fractionated products.

9 | ACTION AT TIME OF BIRTH

9.1 | D typing of cord samples from D-negative women with no immune anti-D

A maternal sample and a cord blood sample should be obtained. The cord blood sample should be used to determine the baby's D group if it is not already known, thus identifying pregnant women who must receive post-delivery prophylactic anti-D Ig. There is minimal evidence that foetal red cells expressing the DVI antigen can cause maternal sensitisation. Therefore, the use of different reagents (detecting DVI as D positive) for typing cord samples is not recommended, as the risks of using the wrong reagent for routine testing, outweighs the risk of missing a DVI cord sample. Most examples of weak D antigen can be easily detected by selecting high affinity anti-D reagents.[5]

In the presence of a large scale programme of cffDNA testing a health economic analysis should be considered to assess the merits of omitting the D typing of cord samples.

9.2 | Direct antiglobulin test on cord samples

9.2.1 | Routine direct antiglobulin test on the cord samples of D-positive babies born to D-negative women with no red cell alloantibodies

This is not recommended as a routine investigation. It has been shown that following RAADP, anti-D Ig can cross the placenta, enter the foetal circulation and bind to foetal D antigen sites. Consequently 3–6% of D-positive cord samples have been found to have a positive direct antiglobulin test (DAT)[64] (Dillon et al., 2011) and this may result in unnecessary additional investigations. Prophylactic anti-D Ig does not cause significant haemolysis of foetal/neonatal red cells.[65]

9.2.2 | Testing of the cord blood of babies of women who have IAT-reactive red cell antibodies

When the maternal serum has been found to contain an immune, IAT-reactive red cell antibody(ies), the red cells from the cord should be tested for the corresponding antigen[s], wherever possible. A DAT should be performed on the cord sample, and the haemoglobin concentration and bilirubin levels should be checked[11] in addition, the baby should be observed for clinical signs of jaundice.[66]

A positive DAT is not, in itself, diagnostic of HDFN. Where the DAT is positive and the baby shows signs of HDFN, a red cell eluate may be helpful to confirm the red cell antibody specificity. IgG ABO antibodies occasionally cause severe HDFN, and so, if the baby has a major ABO mismatch with the woman, the eluate should also be tested with A_1 and/or B cells, negative for any other antigen against which the woman has made IgG alloantibodies. Regular assessment of bilirubin and haemoglobin concentrations is necessary and hence early discharge is not advisable.[11]

Babies who have been transfused in utero with D-negative units for the prevention of HDFN due to anti-D may type as D negative for several months after birth.[2] In this case, the baby should have neonatal follow-up and haemoglobin and bilirubin concentrations should still be checked to diagnose and/or exclude further evidence of clinically significant red cell destruction when D-positive cells are released into the circulation. Significant late anaemia can occur up to 6–8 weeks after birth.

> Recommendation
> All babies born to women who have clinically significant antibodies should be closely observed for evidence of HDFN. A DAT should be performed on a cord blood sample and haemoglobin and bilirubin concentrations should be measured (GRADE 1C).

9.3 | Pre- and post-delivery testing of maternal samples

For women with no red cell antibodies at 28 weeks' gestation, routine blood grouping and antibody screening of maternal samples, other than confirmatory D typing, is not necessary unless pre-transfusion compatibility testing is required. Detection of new antibodies immediately pre-delivery in previously non-sensitised pregnancies will not influence management of the current pregnancy, although if testing is undertaken and antibodies detected the woman should be informed as this there may be implications for future pregnancies. In alloimmunised pregnancies a pre- or post-delivery maternal sample is required to predict neonatal transfusion requirements (as additional red cell antibodies potentially formed since the last screen will need to be matched for) and for crossmatching should neonatal transfusion be necessary.

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A test for fetomaternal haemorrhage (FMH) should be performed on the maternal blood sample from D-negative women with no immune anti-D, to detect foetal cells in the maternal circulation, and, if necessary, to measure the volume of FMH, in order to determine any requirement for additional anti-D Ig.[67]

> Recommendation
> D-negative women with no immune anti-D should have an initial FMH test at delivery and follow up testing if required, in accordance with BSH guidelines for estimation of FMH.[67] (GRADE 1B).

10 | OBSTETRIC CONSIDERATIONS

10.1 | Prepregnancy counselling

Women with red cell antibodies, particularly if there is a risk of foetal anaemia or if compatible donor red cells for transfusion may be difficult to obtain, should attend for prepregnancy counselling with a clinician with knowledge and expertise of this condition. This is usually a foetal medicine specialist.

10.2 | Assisted reproductive techniques

There is no evidence that assisted reproductive techniques (ART) increase the risk of red cell alloimmunisation. However, if donor eggs are used for a mother with an alloantibody and the donor red cell antigen is not known, foetal genotyping may be required. Sperm and ovum donor-derived pregnancies may lead to complexities in interpreting antibody results.

10.3 | Invasive prenatal diagnostic testing

Invasive testing is not contraindicated if alloimmunisation has occurred. There is a risk of increasing the degree of alloimmunisation, or causing alloimmunisation to new antigens, as a result of the procedure, and this should be discussed with the woman. If the mother is RhD negative and is not already sensitised, anti-D immunoglobulin should be given following the procedure.

10.4 | Delivery

Timing of delivery is described in Figure 2. Mode of delivery is determined by standard obstetric indications. IUT does not, of itself, mandate delivery by caesarean section. As these are high risk pregnancies continuous electronic foetal monitoring is advised in labour. Decision making regarding place of delivery will include consideration of neonatal services and the potential complexity of transfusion requirements, should this be required, for the mother.

10.5 | Short and long term consequences

The presence of red cell antibodies does not pose a long term health risk for the woman, but an antibody card should be carried in the event transfusion is required.

Owing to passively acquired maternal antibodies some infants may remain anaemic for a few weeks following birth. Some infants will experience delayed anaemia – risk factors for this are anti-c antibodies and multiple IUTs.[11]

The incidence of severe developmental delay in children treated with IUT is comparable to the general population (3.1 v 2.3%). Severe hydrops, a consequence of not treating in utero foetal anaemia with IUT, is independently associated with neurodevelopmental impairment.[68]

10.6 | Other treatments

Non-invasive treatment options are under investigation for the treatment of severe early onset foetal anaemia. This has the aim of avoiding IUT at early gestation (<20 weeks) which is associated with a higher foetal death rate. The available data suggests a potential effect on the clinical course of the disease, and the timing of first IUT, which needs to be confirmed in a multicentre randomised trial.[69]

11 | AUDIT

Audits of clinical and laboratory practice should be undertaken on a continuing basis to ensure compliance with these guidelines and, where identified, variance or concerns in relation to compliance, should be addressed.

Examples of audits relevant to these guidelines (see Table A1, audit tool):

- Sample labelling
- Laboratory audit of testing and procedures vs. BSH guidelines and performance in External Quality Assessment Schemes.
- Appropriate utilisation of non-invasive foetal typing.
- Compliance with the care pathway for blood grouping, antibody screening and administration of anti-D Ig.
- Ongoing monitoring and referral for specialist advice.
- That all women of childbearing potential receive K antigen-negative blood (This could be an audit of the reason for alloimmunisation in women identified to have anti-K, or a laboratory practice audit).

A detailed gap analysis for choosing audit topics is included as Table A1.

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CONFLICT OF INTEREST STATEMENT

The BSH paid the expenses incurred during the writing of this guidance. All authors have made a declaration of interests to the BSH and Task Force Chairs, which may be viewed on request. The following authors Christoph Lees has undertaken Samsung, GE and Canon (speaker fees), Canon (research grant), Samsung & GE (webinars & research grant). The following members of the writing group Susan Robinson, Fiona Robinson, Edwin Massey, Fiona Regan, Janet Brennand, Tim Watts, Kirstin Finning, Katy Veale and Esther Southgate have no conflicts of interest to declare.

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APPENDIX A

TABLE A1 An audit tool to assess compliance with the BSH guidelines for blood grouping and red cell antibody testing in pregnancy.

Section	Criteria	Compliance Y (or) N (or) N/A	Action required	To be completed by
Section 1	*Consent, samples and request forms*			
	The request form and samples for antenatal screening conform to the requirements described in the guidelines on the administration of blood components.			
	Samples are dated, labelled and signed by the person taking them, in the presence of the pregnant woman who should, whenever possible, be asked to state her full name and date of birth. Sample labels pre-printed away from the phlebotomy procedure or taken from the notes, for example, 'addressograph' labels should not be used.			
	Details (including date and dose) of any previous administrations of anti-D Ig given in the current pregnancy are recorded on the laboratory request form			
	The woman's clinical history particularly of HDFN and previous transfusions is recorded on the laboratory request form			
Section 2	*Laboratory tests*			
	All laboratory testing procedures are validated in compliance with published guidelines			
	ABO and D grouping is performed in accordance with the guidelines for compatibility procedures in blood transfusion laboratories.			
	If clear-cut positive results are not obtained in D typing, women are classified as D negative until the D status is confirmed.			
	All pregnant women found to be D negative are given written information about their D-negative status and the importance of anti-D Ig prophylaxis. The D-status is clearly recorded in the notes.			
	The screening cells and methods used for red cell antibody screening comply with the guidelines for compatibility procedures in blood transfusion laboratories.			
	The procedures used for identification and exclusion of red cell antibodies should comply with the guidelines for compatibility procedures in blood transfusion laboratories			
	Once red cells antibodies are identified in pregnancy, the identification process is repeated with each additional sample to exclude any additional clinically significant maternal antibodies.			
	The concentration of each antibody capable of causing HDFN is assessed independently. For specificities where there is a national standard preparation, quantification is undertaken. Other antibody specificities are measured using titration.			
	Where possible each sample for titration is tested in parallel with the previous sample and the results compared to identify significant changes in antibody concentration.			
	If potentially clinically significant maternal antibodies have been identified paternal testing should be considered to predict the risk to current and future pregnancies. This may be particularly relevant if non-invasive foetal genotyping is not available for the corresponding red cell antigen.			
	Pregnant women with anti-D, -c -E and -K antibodies are offered non-invasive foetal blood grouping using cell-free foetal DNA (cffDNA) from maternal plasma.			
	This is performed at a gestation recommended by the reference laboratory used.			
	cffDNA tests are requested by obstetricians or foetal medicine specialists who can explain the implications of the test findings in the context of the full history and investigation findings.			
	Fetal RHD typing is offered using a high-throughput methodology to pregnant women who have not formed anti-D, as part of a screening program to target anti-D Ig prophylaxis, from 11 weeks gestation.			
Section 3	*Antenatal testing protocols*			
	All pregnant women who are D negative and have not formed anti-D are considered for prophylactic anti-D Ig for potentially sensitising events as defined in guidelines for the use of anti-D Ig for the prevention of HDFN, the exception being women in whom			

(Continues)

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TABLE A1 (Continued)

Section	Criteria	Compliance Y (or) N (or) N/A	Action required	To be completed by
	maternal screening for foetal RHD by cffDNA predicts the foetal genotype to be D-negative.			
	All pregnant women are ABO and D typed and screened for the presence of red cell antibodies both early in pregnancy and at 28 weeks' gestation, before the administration of RAADP when appropriate.			
	In women with no red cell antibodies present at 28 weeks' gestation, no further routine antenatal blood grouping or antibody screening is undertaken.			
Section 4	*Red cell antibodies detected in pregnancy*			
	All women who have previously had a baby affected by HDFN are referred before 20 weeks' gestation to a foetal medicine specialist for advice and for assessment of foetal haemolysis, irrespective of antibody concentration or specificity.			
	The laboratory keeps clear records of anti-D Ig administration.			
	All anti-D detected in pregnancy should be quantified by CFA, or tested by a method that has been extensively validated against quantification, and there is a clear procedure to distinguish between immune and passive anti-D Ig, including review of clinical history and previous laboratory results.			
	When there is doubt as to the passive or immune nature of anti-D, concentration is monitored and prophylactic anti-D Ig offered, where indicated, until the nature of anti-D is established.			
	A reference centre confirms antibody specificity in women with apparent anti-C + D.			
	Cases of anti-D and anti-c are referred to a foetal medicine specialist if the antibody concentration reaches the critical level and/or the level is rising significantly. The antibody concentration is assessed serologically at 4-week intervals to 28 weeks' gestation and at fortnightly intervals thereafter until delivery, unless advised otherwise by clinicians undertaking serial MCA Doppler.			
	Cases of anti-K or other Kell system antibody (unless the father is confirmed to be antigen negative) are referred to a foetal medicine specialist when the antibody is first identified. The antibody strength is assessed serologically at monthly intervals to 28 weeks' gestation and at fortnightly intervals thereafter until delivery, unless advised otherwise by clinicians undertaking serial MCA Doppler.			
	Clinically significant antibodies, other than anti-D, -c or -K, are excluded or, if present, assessed by titration, at the booking appointment and at 28 weeks' gestation. Where the titre is >32 and/or there is a past history of HDFN, referral to a specialist in foetal medicine is made for further assessment.			
Section 5	*Reports of laboratory investigations*			
	Reports inform the clinician[s] of the significance of the antibodies, with respect to the development of HDFN and transfusion problems.			
	Pregnant women with clinically significant red cell antibodies should be given verbal and written information with details of the antibody specificity and its potential for causing HDFN and delay in providing compatible blood.			
Section 6	*Requirements for blood*			
	For women with multiple antibodies or antibodies against high prevalence antigens, an assessment of the likelihood of maternal need for blood, and discussion of appropriate measures to reduce the likelihood of transfusion, including correction of any haematinic deficiency, should take place as early in pregnancy as feasible between the maternity and haematology staff.			
	For such women, multidisciplinary planning of delivery should take place to reduce blood loss and intra-operative cell salvage should be available where appropriate.			
Section 7	*Action at time of birth*			
	All babies born to women who have clinically significant antibodies are closely observed for evidence of HDFN. A DAT is performed, and haemoglobin and bilirubin concentrations are measured.			

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TABLE A1 (Continued)

Section	Criteria	Compliance Y (or) N (or) N/A	Action required	To be completed by
	A DAT is not routinely performed on cord samples of D-positive babies born to D-negative women thus avoiding unnecessary additional investigations. D-negative women with no immune anti-D have an initial FMH test at delivery and follow up testing if required, in accordance with BSH guidelines for estimation of FMH.			
Section 8	*Audit* Audits of compliance with the clinical and laboratory aspects of these guidelines are undertaken on a continuing basis and all variations or concerns addressed in a timely manner.			

Note: See text of guidelines for references and the grades of evidence.

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