

GUIDELINE FOR BLOOD GROUPING AND ANTIBODY TESTING IN PREGNANCY

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INTRODUCTION

Purpose of the Guideline

The purpose of the guideline is to define the red cell immunohaematology tests which should be applied in pregnancy. The aim of the testing programme is the prevention of haemolytic disease of the fetus and newborn. Since the majority of publications use the term 'haemolytic disease of the newborn', HDN, to refer to both fetus and newborn, it is used here.

The guideline group was selected to be representative of UK based medical experts and patients' representatives. A search of published literature was

undertaken using Pubmed, Cochrane Library and Ingenta databases. The NICE guidance [NICE, 2002] and Health Technology Assessment [Chilcott et al, 2002] underpinned the evidence base to support the review work. Also, a comprehensive literature search was undertaken to capture information applicable to the review aims. The search was undertaken in 2004 using Medline, for the past 20 years and the key words were anti D, prophylaxis, antibodies in pregnancy, haemolytic disease of the newborn. In addition, broad termed searches were made of the Cochrane Library and Medscape. Appropriate non-published literature, published policy documents and knowledge from experts in the field were incorporated and utilised. A writing group was formed to synthesise and collate the information. This covered the period 1999-2004. The papers included were subjected to critical reading by the authors using the CASP appraisal tool (CASP, 2004) and were also ranked according to the hierarchy of evidence. This approach took account of the NICE systematic review undertaken in 2000 (Chilcott et al, 2003) so as to be contemporary in locating and including the relevant literature. The writing group produced the draft guideline which was subsequently revised by consensus by members of the Transfusion Task Force of the British Committee for Standards in Haematology. The guideline was reviewed by a sounding board of UK haematologists the BCSH (British Committee for Standards in Haematology) and the BSH Committee (British Society for Haematology) and comments incorporated where appropriate. Criteria used to assign levels of evidence and grades of recommendations are as outlined by the Agency for Healthcare Research and Quality (AHRQ) at <http://www.ahrq.gov> (Appendix 1).

Significant Developments

Since the publication of the previous guidelines [BCSH-a,1996] there have been improvements in laboratory practice, particularly the use of monoclonal reagents and automation. The Serious Hazards of Transfusion [SHOT] haemovigilance scheme [Stainsby et al, 2004] has focused attention on blood grouping and red cell serology practice and revised guidelines for compatibility testing in blood transfusion laboratories [BCSH-b, 2004] have been published.

The introduction of non-invasive techniques to monitor fetal anaemia has influenced the management of allo-immunised pregnancies [Mari, 2000] and the concentration of care of these cases in fetal medicine units has resulted in improved outcomes of intra-uterine transfusion.

Most significantly, in May 2002 the National Institute for Clinical Excellence (NICE) endorsed the recommendation that routine antenatal anti-D prophylaxis (RAADP) should be offered to all D negative women in the UK who have no detectable immune anti-D [NICE, 2002]. Injections at 28 weeks and again at 34 weeks gestation were recommended. As the relative incidence of immune anti-D has declined [Mayne et al, 1997; MacKenzie et al 1999] the incidence of positive antibody screens due to prophylactic anti-D has increased [Dalton 2003; Parker 2003] and the two types of antibody cannot be distinguished by laboratory tests.

The risks associated with the misinterpretation of passive and immune anti-D are clear: if passive anti-D is misinterpreted as immune, anti-D prophylaxis may be omitted leaving the women unprotected from sensitisation. If immune anti-D is misinterpreted as passive, appropriate follow-up of the antibody level during pregnancy may be curtailed putting the fetus at risk.

The testing protocols recommended here are designed to provide clarity for practice in order to protect pregnant women including those who are D-negative, and their infants.

Informed consent

Providing information about any blood test and obtaining consent is a clinical responsibility and informed consent should be obtained and documented prior to samples being taken [National Collaborating Centre for Women's and Children's Health, 2003]. A discussion of issues around choice and informed consent is outside the scope of this guideline and will not be addressed here.

1. PURPOSES OF LABORATORY TESTS

1.1 ABO and D typing to identify D negative women who require anti-D prophylaxis.

1.2 Screening and identification of red cell alloantibodies

- to detect clinically significant antibodies which might affect the fetus and/or newborn
- to highlight possible transfusion problems

1.3 Follow-up tests when clinically significant red cell antibodies are present:

- to monitor the strength of antibodies to identify those pregnancies which are at risk of HDN and to predict fetuses/infants who are likely to require treatment for HDN.
- to identify additional maternal alloantibodies. Women who have developed one or more antibodies may go on to form further antibodies of different specificities.

2. RECOMMENDATIONS FOR SAMPLES AND REQUEST FORMS

2.1 Identification of samples

It is essential that samples from pregnant women are correctly identified and that request forms are accurately completed. SHOT reports provide evidence that errors in patient identification and sample labelling may lead to ABO incompatible transfusions [Stainsby et al, 2004]. The record of ABO/D type derived from an antenatal sample may be used as the basis for the provision of suitable blood for transfusion, and the sample could be used for a crossmatch. Misidentification can also lead to failure in, or inappropriate, administration of prophylactic anti-D.

Therefore, the same minimum patient identification on antenatal samples and request forms is required as for pre-transfusion samples [BCSH-e, 1999] i.e.

- i] Surname/family name [correctly spelt]
- ii] First name[s] in full
- iii] Date of birth [not age or year of birth]
- iv] Unique identifier number e.g. Hospital number/ NHS number

The hospital and/or NHS numbers may not be readily available when antenatal samples are taken. In these circumstances, 'address' is a suitable alternative identifier if it is given on both the sample and the request form.

Recommendation 1:

Samples for antenatal screening are identified to the same standard as pre-transfusion samples (Good Practice Point [GPP])

Recommendation 2:

Samples should be dated, labelled and signed by the person taking them, in the presence of the patient who should be asked to confirm demographic details. Any labels pre-printed away from the phlebotomy procedure, e.g. Addressograph labels, should not be accepted on the specimen [BCSH-b, 2004, Level IV, Grade C].

It is essential that any previous administration of prophylactic anti-D in the current pregnancy, including date and dose, is recorded on the laboratory request form.

Clinical history, particularly of HDN and previous transfusions, is also essential information on the request form.

3. RECOMMENDATIONS FOR LABORATORY TESTING

3.1 All test procedures must be well established and validated in compliance with published guidelines [BCSH-f, 1995]. Ideally testing should be performed on automated equipment which ensures positive sample identification and with electronic transfer of results to the woman's computer record.

Recommendation 3:

ABO and D grouping must be performed in accordance with the guidelines for compatibility procedures in blood transfusion laboratories [BCSH-b,2004].(Level IV, Grade C)

3.2 D grouping

Monoclonal IgM anti-D grouping reagents which do not detect D^{VI} should be used and, unless samples are tested on secure automation, the reagents should be used in duplicate. Anti-CDE reagents should not be used for routine typing of pregnant women [BCSH-b, 2004].

The antiglobulin test should not be used to D type maternal samples. Women should not be classified as D positive on the basis of a weak positive result using a single anti-D reagent, or a pool of more than one reagent. If clear-cut positive results are not obtained, it is safer to classify the woman as D-negative until confirmation of D status is carried out by a red cell reference laboratory [BCSH-b, 2004].

Recommendation 4:

All pregnant women found to be D negative should be issued with blood group cards to inform them, and those responsible for their care, of the D negative status and the need for prophylactic anti-D (Level IV, Grade C).

3.3 Antibody screening.

Approximately 1% of pregnant women are found to have clinically significant red cell antibodies [Howard et al, 1998]. Of these, the commonest specificity is anti-D, although the universal introduction of RAADP is predicted to reduce this sensitisation rate [NICE, 2002]. However, with the introduction of RAADP there is a significant rise in positive antibody screening results, due to the detection of prophylactic anti-D [Dalton 2003; Parker 2003].

3.3.1 Screening methods.

The Indirect Antiglobulin Test [IAT] using reagent red cells suspended in LISS is the most suitable for detection of clinically significant red cell antibodies [BCSH-b, 2004]. Column agglutination methods, liquid-phase tube tests and solid-phase methods have been found to be suitable [Poole, 1996].

There is no additional value in using an enzyme technique in antibody screening [Clark et al, 1999].

Testing for high levels of immune anti-A or anti-B in pregnant women is not recommended as their presence neither predicts ABO HDN nor does it cause problems *in utero*. [Mollison et al a, 1997]

3.3.2 Reagent cells.

Cells used for antibody screening should comply with the recommendations of the guidelines for compatibility procedures in blood transfusion laboratories [BCSH-b, 2004].

The following antigens should be expressed on screening cells:

C,c,D,E,e,K,k,Fy^a, Fy^b, Jk^a, Jk^b,S, s, M, N, Le^a.

It is recommended that one of the screening cells should be R₁R₁ and another should be R₂R₂ and that the Fy^a, Fy^b, Jk^a, Jk^b, S and s antigens should be represented on reagent cells with homozygous expression. Screening cells must not be pooled.

It is not necessary to include screening cells which express low frequency antigens such as C^w, Kp^a or Lu^a [Garratty, 2003].

Recommendation 5:

The screening cells and methods used for red cell antibody screening should comply with the guidelines for compatibility procedures in blood transfusion laboratories; BCSH b 2004 (Level IV, Grade C)

4. ANTENATAL TESTING PROTOCOLS

See algorithm for samples and testing requirements.

4.1 Routine Testing

All pregnant women should have samples taken early in pregnancy, ideally at 10-16 weeks gestation, for ABO and D typing 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 5.3]

All pregnant women, whether D positive or D negative, should have a further blood sample taken at 28 weeks gestation for re-checking the ABO and D group and further screening for red cell allo-antibodies [National Collaborating Centre for Women's and Children's Health, 2003]. D positive women are just as likely as D negative women to form antibodies, other than anti-D, late in pregnancy [Thompson et al, 2003].

No further routine blood grouping or antibody screening is necessary after 28 weeks.

There is evidence that antibodies detected only in the third trimester do not cause HDN [Rothenberg et al, 1999; Heddle et al, 1993]. Further, and significantly, the introduction of RAADP has resulted in the detection of anti-D in samples taken after 28 weeks from D negative women [Dalton 2003; Parker 2003]. Since it is not possible to differentiate between prophylactic and immune anti-D there is the potential for confusion between the two [New et al, 2001].

Recommendation 6:

All pregnant women should be ABO and D typed and screened for the presence of red cell antibodies early in pregnancy and at 28 weeks gestation [National Collaborating Centre for Women's and Children's Health, 2003]. (Level III, Grade B)

Local policies must ensure that D-negative women eligible for RAADP have the third trimester antibody screening sample taken before the first RAADP injection is administered at 28 weeks. Samples taken after the injection could result in passive anti-D being detected which may be mistaken for immune anti-D [New et al, 2001].

4.2. Sensitising Episodes during pregnancy

This section is a synopsis of recommendations of the Guideline for administration of anti-D prophylaxis [BCSH-c, 2005].

See algorithm, Page x

In addition to RAADP, pregnant women who are D negative should be considered for anti-D prophylaxis for the following potentially sensitising episodes:

- Amniocentesis
- Cordocentesis
- Other in-utero therapeutic intervention/surgery (e.g. intrauterine transfusion, shunting)
- Ante partum haemorrhage (APH)
- Chorionic villus sampling
- Ectopic pregnancy
- External cephalic version
- Fall / Abdominal trauma
- Intrauterine death
- Miscarriage
- Termination of pregnancy

Before 12 weeks gestation, confirmed by scan, in uncomplicated miscarriage or mild and painless vaginal bleeding, anti-D should not be administered because the risk of fetomaternal haemorrhage [FMH] is minimal.

Between 12 and 20 weeks gestation, for any potential sensitising event outlined above, a sample should be tested to confirm that the woman is D negative and that she has not become sensitised to the D antigen. At least 250 iu anti-D immunoglobulin should be administered as soon as possible and certainly within 72 hours of the event. There is no need to assess the volume of FMH under 20 weeks gestation.

After 20 weeks gestation there is a requirement to assess the volume of FMH. If the acid elution technique is used and a FMH of more than 4mL is indicated, the test should be repeated using flow cytometry. At least 500 iu anti-D immunoglobulin should be administered intramuscularly as soon as possible, and certainly within 72 hours of the potentially sensitising event. If FMH of more than 4mL is confirmed by flow cytometry, more anti-D will be required [BCSH-c, 2005].

After the birth, a cord sample must be tested to obtain the ABO and D type of the baby. If this is not collected for any reason, a heel prick sample should be obtained as soon as possible.

Maternal samples for confirmatory ABO and D type and FMH testing should be collected after sufficient time has elapsed for any FMH to be dispersed in the maternal circulation. A period of 30-45 minutes is considered adequate. (Mollison *et al*, 1997)

Following birth of a D positive infant at least 500 iu anti-D must be administered to the woman if the FMH is 4 mL or less.

If the pregnancy is non-viable and no sample can be obtained from the baby, prophylactic anti-D must be administered to the woman.

4.3 Red cell antibodies detected in pregnancy:

When red cell antibodies are detected, further testing of maternal blood should be undertaken to determine the specificity, concentration, origin and level of antibody or antibodies, and the likelihood of HDN.

Anti-D, anti-c and anti-K are the antibodies most often implicated in causing haemolytic disease severe enough to warrant antenatal intervention.

4.3.1 Women with anti-D present

Distinguishing between prophylactic and immune anti-D

In addition to the administration of prophylactic anti-D following sensitising events the use of RAADP is increasing as the NICE recommendations [NICE, 2002] are being adopted. It is therefore inevitable that more antenatal samples containing low levels of anti-D will present the problem of determining whether the anti-D is prophylactic or immune.

Following administration of an intramuscular injection of anti-D immunoglobulin, serologically detectable levels of anti-D are quickly reached and peak blood levels are reached within three to seven days. The half-life of prophylactic anti-D immunoglobulin is approximately 3 weeks [Eklund *et al*, 1982]. Prophylactic anti-D can be detected by serological tests for several weeks: by IAT for up to 8 weeks or more and by other more sensitive techniques for up to 12 weeks and in exceptional cases for several months.

Immune anti-D becomes detectable approximately 4 weeks after exposure to D positive cells, and reaches a peak level after 6-8 weeks [Mollison *et al*, 1997].

Both prophylactic and immune anti-D are detectable by laboratory tests and cannot be distinguished. While prophylactic anti-D levels will fall with time, immune anti-D levels will usually remain stable or rise if there is re-stimulation of the antibody.

The level of anti-D in maternal samples post prophylaxis rarely exceeds 1 iu/mL unless a dose[s] of more than 1250 iu has been administered.

Procedure if prophylactic anti-D is suspected

- a. If there is a record of administration of anti-D within the past 8 weeks and the antibody reaction is weak, testing should be as for non-

sensitised women i.e. no antibody testing after 28 weeks and Rh prophylaxis should continue.

- b. If there is no record of anti-D administration or information regarding prophylaxis is not available the antibody should be monitored by both IAT and anti-D quantification as for immunised women i.e. at 4 weekly intervals to 28 weeks or at 2 weekly intervals if after 28 weeks. If the anti-D becomes undetectable by IAT and the quantified level is falling it is probably prophylactic. A rising or steady level indicates immune anti-D.

If there is significant doubt about the immune or passive nature of anti D, the sample should be referred for quantification.

In either case anti-D prophylaxis should continue unless it is established beyond doubt that the anti-D is immune.

The pregnant woman with anti-D should not be issued with an antibody card documenting the finding of anti-D until it is established that the anti-D is immune.

If the sample in which anti-D is detected is referred for routine antibody screening or is pre-transfusion, a panel of D negative cells, selected to provide all relevant red cell antigens, should be used to detect or exclude the presence of allo-antibodies of other specificities.

Recommendation 7:

Blood transfusion laboratories should keep a record of anti-D administration to provide a basis for distinguishing between immune and prophylactic anti-D (Level IV, Grade C).

5.3.2. Women with immune anti-D

Blood samples from women with immune anti-D should normally be tested at least monthly until 28 weeks gestation and every 2 weeks thereafter to monitor the level of anti-D and to identify any additional antibodies that may develop. The antibody level should be quantified, in iu/mL, using the national anti-D standard [National Institute for Biological Standards and Control (NIBSC),2003]. Each sample should be tested in parallel with the previous sample and the results compared to identify significant changes in antibody level.

Where the level is more than 1.0 iu/mL an increase of anti-D level of 50% or greater over the previous level indicates a significant increase, irrespective of the period of gestation.

Anti-D is the most frequent antibody responsible for serious HDN. The following levels of anti-D have been used to guide the management of pregnancies since the publication of the previous guideline [BCSH a, 1996; Nicolaides & Rodeck, 1992].

Anti-D	Less than 4 iu/mL	HDN unlikely
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Anti-D	4-15 iu/mL	Moderate risk of HDN
Anti-D	More than 15 iu/mL	High risk of hydrops fetalis

As a consequence of developments in the assessment of fetal anaemia and in the technique of IUT the significant anti-D level is that which triggers referral to a specialist feto-maternal unit. Non-invasive assessment can then be used to monitor fetal anaemia [Scheier et al, 2004]. A woman whose anti-D level is 4 iu/mL or greater and/or has a rising anti-D level and/or has a history of HDN affected offspring must be referred to such a unit. It should also be noted that HDN has been reported at levels less than 4 iu/mL [Bowell et al, 1982]. Once the referral to the feto-maternal unit has been made the value of subsequent samples for anti-D quantification is doubtful. A sample at 28 weeks should be tested for the presence of further red cell antibodies.

It is possible to determine the D type of the fetus from a maternal peripheral blood sample using polymerase chain reaction (PCR) – see section 7. [Daniels et al a, 2004]

4.3.3. Women with apparent anti-C + D, possible anti-G

A proportion of antibodies with apparent anti-C+D specificity but with disproportionately high anti-C titres may be demonstrated, by advanced serological techniques, to be anti-G. [Maley et al, 2001]. Since women with anti-G, without anti-D, should be eligible for RAADP and post-delivery anti-D immunoglobulin it is important that a reference centre should confirm examples of apparent anti-C+D specificity.

4.3.4. Women with immune anti-c present

Women with anti-c should be re-tested with the same frequency as women with anti-D, i.e. at least monthly to 28 weeks gestation and every 2 weeks thereafter. Samples from women with anti-c should be quantified with reference to the international anti-c standard [NIBSC 2003] with the previous sample tested in parallel, as for anti-D [above], and any additional antibodies should be identified.

Quantification of anti-c is useful in monitoring increases in the antibody concentration.

When account has been taken of previous history of HDN the following levels of anti-c are indicative of the need to refer to a specialist unit [Kozlowski et al, 1995].

Anti-c level:

Less than 7.5 iu/mL	Continue to monitor.
7.5 to 20 iu/mL	Risk of moderate HDN, refer to specialist unit.
More than 20 iu/mL	Risk of severe HDN Refer to specialist unit.

It is important to note that anti-c may cause delayed anaemia in the neonate.

4.3.5 Women with immune anti-K, or other Kell system antibodies.

HDN due to anti-K is characterised by low haemoglobin, but amniotic and/or cord bilirubin levels are not generally reported. The fetal anaemia associated

with anti-K may be due to the inhibition of K positive erythroid early progenitor cells [Vaughan et al, 1998] or to promotion of their immune destruction [Daniels et al b, 2003].

While it has been stated that the severity of HDN due to anti-K is not correlated with titre of the antibody reports of affected pregnancies are associated with antibodies with a titre of at least 32. [McKenna et al, 1999; Ahaded et al, 2000] However, samples from women with anti-K should be titrated by IAT when first identified in the pregnancy, as for any clinically significant antibody.

The majority of cases of anti-K in pregnant women are the consequence of previous K positive transfusions. The incidence of anti-K could be reduced by selecting K negative units for transfusion to females with potential for childbearing [Lee & de Silva, 2004]. Therefore selecting K-negative units for females under the age of 60 is considered good practice. However urgent transfusions should not be delayed if suitable K-negative units are not immediately available.

The transfusion history of women with anti-K should be established and a sample from the father of the fetus should be K typed. If the woman has not been transfused and the father is K positive, the patient should be referred to a specialist unit and titration of samples should be performed at monthly intervals to 28 weeks, and at fortnightly intervals thereafter. If the father is K negative and a confidential enquiry establishes paternity, no further samples are required until 28 weeks when the antibody should be titrated and further antibodies excluded.

The fetus can be K typed from an amniocentesis sample, but this sampling involves physical intervention with associated risks to the fetus and of stimulating the antibody level. See section 7, 'Fetal genotyping', for reference to the development of K genotyping from peripheral maternal blood samples.

Recommendation 8: *Cases of anti-D, anti-c and anti-K [unless the father is confirmed K negative] should be assessed at monthly intervals to 28 weeks gestation and at fortnightly intervals thereafter. Such cases must be referred to a specialist fetal medicine unit if the antibody reaches the critical level and/or the level is rising significantly. (Grade B)*

4.3.6. Women with other red cell antibodies

Only IgG antibodies are capable of entering the fetal circulation. Red cell antibodies with a significant IgG component are detectable by IAT. 'Cold reactive', IgM and low affinity antibodies to high frequency antigens [e.g. CR1 and CR4 related antibodies] have not been implicated in HDN.

In addition to anti-D, -c and -K, the following specificities are most commonly associated with HDN: anti-C [-Ce], -E [-cE], -Fy^a, and -Jk^a [Moise, 2000; Moran 2000; Goodrick et al, 1997]. However, many other specificities have been reported as the cause of HDN, and a summary, by blood group system, is given by Daniels et al [Daniels et al c, 2002]. In all these cases re-testing at 28 weeks generally provides sufficient information to determine management of

the pregnancy. A medical decision should be made regarding the more frequent testing of women with a previous history of children with HDN.

Where an antibody has been detected, testing of both booking and 28-week samples should include titration and testing by IAT against reagent cells heterozygous for the corresponding antigen. Careful attention to technique is necessary to minimise the variables in the method and titrating the national anti-D standard [NIBSC,2003] in parallel, as an internal control, is recommended [BCSH d, 1999]. Given the wide spread implementation of RAADP programmes prophylactic anti-D may be present in addition to alloantibodies and selecting D negative reagent cells for titration should be considered.

In general, a titre of 32 or greater is likely to cause HDN, although a clear-cut association between titre and HDN has not been established.

The presence of any further antibodies should be established and any clinically significant antibodies should be titrated as above.

Recommendation 9:

Clinically significant antibodies, other than anti-D, -c or -K, should be assessed, and other antibodies excluded, at 'first appointment' and at 28 weeks gestation. (level IIb Grade B)

Recommendation 10:

All women who have previously had an infant affected by HDN should be referred before 20 weeks to a specialist unit for advice and for assessment of fetal haemolysis, irrespective of antibody level. (Level IIa Grade B)

5. PATERNAL TESTING

Where a clinically significant antibody capable of causing HDN, particularly anti-D, anti-c or anti-K, is present in a maternal sample, determining the father's phenotype provides useful information to predict the likelihood of a fetus carrying the relevant red cell antigen. The complexities of paternal testing and the potential for misidentification of the father need to be acknowledged [National Collaborating Centre for Women's and Children's Health, 2003].

6. FETAL GENOTYPING

When a clinically significant antibody of high concentration is present, and/or the woman has a history of HDN and the father is heterozygous for the relevant antigen, it can be clinically relevant to determine the genotype of the fetus. Until recently fetal DNA for genotyping by PCR assay was obtained by amniocentesis or chorionic villus sampling. These invasive techniques carry a small risk of spontaneous miscarriage and may boost maternal antibody levels.

A technique is now available for the accurate determination of fetal D genotype from samples of maternal peripheral plasma.

The same testing service for fetal c and K type is under development and should be used as it becomes available.

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 woman's antenatal care of the likely significance of the antibody/ies, with respect to both the development of HDN and transfusion problems [National Collaborating Centre for Women's and Children's Health 2003]. Reports should also, where relevant, alert the clinician to the need to refer the woman to a specialist unit.

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

Recommendation 11:

Women with clinical significant red cell antibodies should be issued with a card giving details of the antibody. (GPP)

8. ACTION AT TIME OF BIRTH

8.1 D negative women with no immune anti-D

A maternal sample and a cord blood sample should be taken. The cord blood sample should be used to determine the infant's D group, thus identifying women who must receive post-delivery prophylactic anti-D immunoglobulin.

Since there is minimal evidence that D^{VI} on fetal red cells can cause maternal sensitisation, and since detecting D^{VI} on cord samples would require different reagents from those used on adult patients, with the potential for confusion and inappropriate testing of adult patients, testing cord samples for D^{VI} is not recommended, i.e. anti-D reagents for typing cord samples should not react with D^{VI}. Most examples of weak D antigen can be easily detected by selecting high affinity anti-D reagents. [BCSH b, 2004]

A test should be performed on the maternal blood sample to detect and estimate the volume of fetal cells present, so that additional anti-D immunoglobulin may be given if the feto-maternal haemorrhage [FMH] exceeds 4mL. Samples showing a FMH result of more than 4mL by acid elution technique, should be referred for a more accurate assessment of the volume of bleed by flow cytometry. The dose calculation for prophylactic anti-D is based on the volume of FMH. [BCSH c, 2006]

8.2. Direct Antiglobulin Test [DAT] on cord samples

8.2.1 Routine DAT on the cord samples of D positive infants born to D negative women

This is not recommended. It has been shown that following RAADP anti-D immunoglobulin can cross the placenta, enter the fetal circulation and bind to fetal D antigen sites. Consequently, up to 3-6% of D positive cord samples have been found to have a positive DAT [Dalton, 2003; Parker 2003] and this may result in unnecessary additional investigations being undertaken and in anxiety for the parents. There is evidence that prophylactic anti-D does not cause destruction of fetal/neonatal red cells [Maayan-Metzger et al, 2001].

8.2.2. DAT on infants of women who have IAT reactive red cell antibodies.

Whenever the maternal serum has been found to contain an immune, IAT reactive red cell antibody/ies a DAT should be done on the cord sample.

A positive DAT in itself is not diagnostic of HDN. However if it is positive, the infant's haemoglobin and bilirubin levels should be checked to diagnose/exclude HDN. Where the DAT is positive and the infant shows symptoms of HDN, a red cell eluate may be helpful to confirm the red cell antibody specificity. In cases of suspected HDN wherever possible the red cells from the cord should be tested for the corresponding antigen[s].

Infants who have been transfused *in utero* with units negative for the relevant antigen the DAT may be negative and the baby may type as antigen negative for several months after birth. [BCSH b, 2004]

Recommendation 12:

All infants born to women who have clinically significant antibodies should be closely observed for evidence of HDN. A DAT should be performed and if positive, haemoglobin and bilirubin levels should be measured. (Level IV, Grade C)

8.3. Pre and Post delivery testing of maternal samples

Routine antibody screening of immediate pre and/or post delivery samples is not required, as this information does not influence management of the pregnant woman or her infant. Blood grouping and antibody screening of maternal samples other than confirmatory D typing should be undertaken only if pre-transfusion compatibility tests are required.

9. FUTURE DEVELOPMENTS

The NICE guidance on the use of RAADP [NICE, 2002] endorses the importance of feasibility studies of mass testing for fetal blood group antenatally by analysis of circulating fetal DNA in maternal plasma. "If fetal Rh blood type could be determined before 28 weeks gestation, antenatal anti-D prophylaxis would be necessary only if the fetus was RhD positive, and knowledge of the father's blood group would no longer be required' It is anticipated that such large scale testing will become available in the near future [Daniels et al a, 2004].

10. AUDIT

Audits of 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 [Department of Health (DH) a 1997; DH b, 1998].

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None of the authors have declared a conflict of interest.

Task force membership at time of writing this guideline

F Boulton (Chair); D Stainsby (Secretary), H Cohen, M Rowley, B McClelland, H Qureshi, H Boralessa, K Wilson, C Elliot., A Blest (co-opted)

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Samples and Testing required in a viable pregnancy

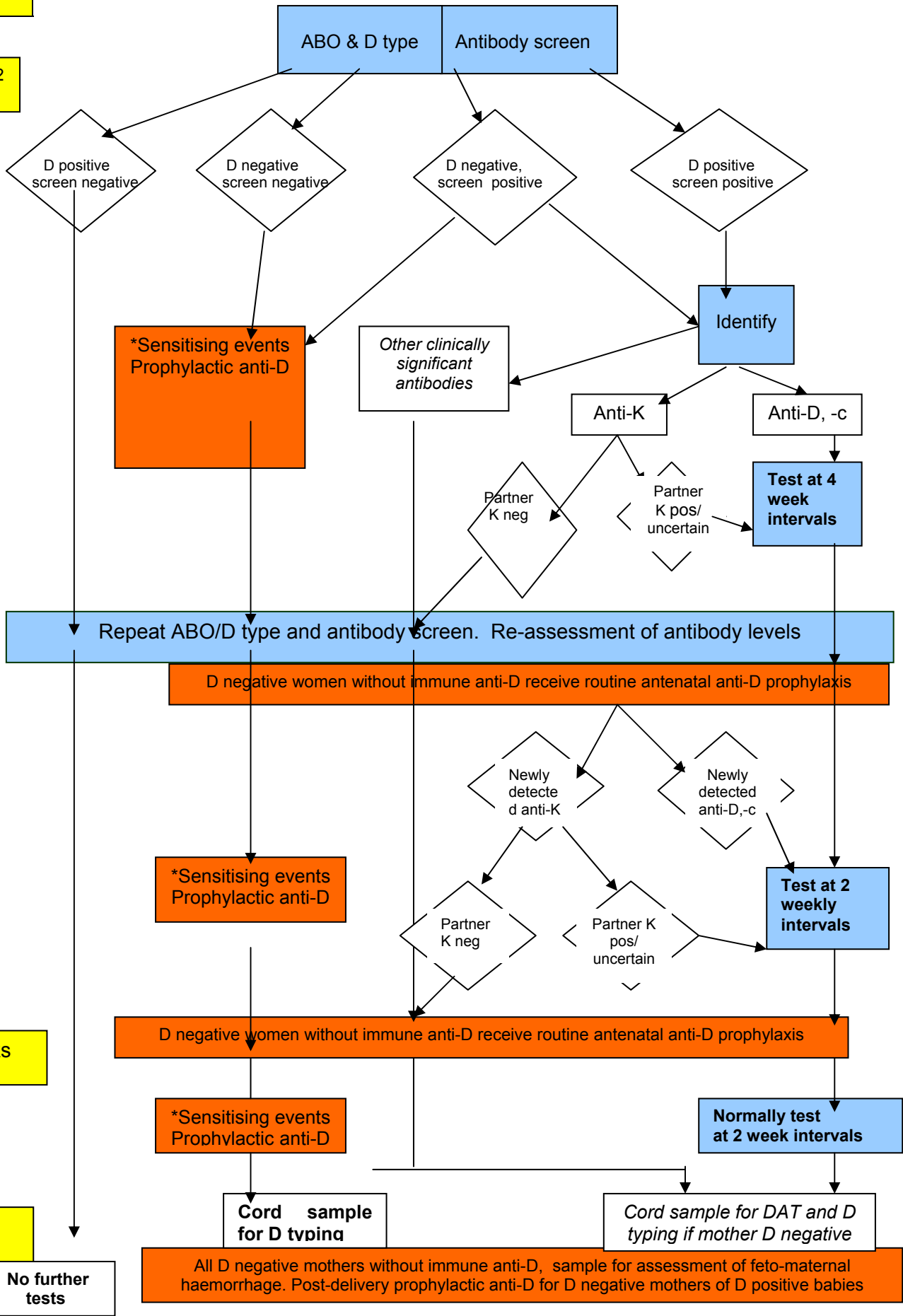
Gestation

Circa 12 weeks

28 weeks

34 weeks

Birth



***After 20 weeks, group, screen and Kleihauer test required**

Appendix 1

Level of evidence and grade of recommendations

Level Type of evidence

Ia

Evidence obtained from meta-analysis of randomised controlled trials.

Ib

Evidence obtained from at least one randomised controlled trial

IIa

Evidence obtained from at least one well-designed controlled study without randomisation

IIb

Evidence obtained from at least one other well-designed quasi-experimental study

III

Evidence obtained from well-designed non-experimental descriptive studies, such as comparative studies, correlation studies and case-control studies

IV

Evidence obtained from expert committee reports or opinions and/or clinical experience of respected authorities

Grade Recommendation (based on AHCPR)

A (evidence levels Ia, Ib)

Requires at least one randomised controlled trial as part of the body of the literature of overall good quality and consistency addressing the specific recommendation

B (evidence levels IIa, IIb, III)

Requires availability of well-conducted clinical studies but no randomised clinical trials on the topic of recommendation

C (evidence level IV)

Requires evidence from expert committee reports or opinions and/or clinical experience of respected authorities. Indicates absence of directly applicable studies of good quality