Guidelines for the use of prophylactic anti-D immunoglobulin

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Introduction

Prior to 1970 haemolytic disease of the newborn (HDN) due to anti-D was a significant cause of morbidity and mortality. By 1990, a reduction in mortality from 1.2 per 1000 births to 0.02 per 1000 births had been achieved in response to the introduction of immunoprophylaxis with anti-D immunoglobulin (Tovey, 1992). During that time the sensitisation rate dropped to about 1.2%. A further reduction to between 0.17 to 0.28% was achieved by introducing prophylaxis during the third trimester of pregnancy (Huchet *et al*, 1987; MacKenzie *et al*, 1999; Mayne *et al*, 1997; Tovey *et al*, 1983). These findings contributed to the National Institute for Clinical Excellence (NICE) recommendation that all D-negative pregnant women who do not have anti-D should be offered anti-D immunoglobulin routinely during the third trimester of pregnancy (NICE, 2002).

Objectives

The objective of this guideline is to provide healthcare professionals with practical guidance on the use of anti-D immunoglobulin as immunoprophylaxis to prevent sensitisation with anti-D, to update the previous recommendations of 1999 on the administration of anti-D (Lee *et al,* 1999) and to take into account the NICE publications and recommendations.

Methods

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 following key words were used: anti-D, pregnancy, antenatal, prophylaxis, rhesus, and RhD. 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 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).

Guideline update

This Guideline replaces the previous publication of Lee *et al,* 1999.

This guideline is divided into six sections: -

Section 1 deals with the issues relating to administration of anti-D
Section 2 deals with the prevention of anti-D formation in response to sensitising events.
Section 3 deals with routine prophylaxis.
Section 4 deals with pre-transfusion testing.
Section 5 deals with the prevention of anti-D formation in the event of recurrent uterine bleeding during pregnancy.
Section 6 deals with prophylaxis after the transfusion of D positive blood products.

SECTION 1: ADMINISTRATION OF ANTI-D IMMUNOGLOBULIN

1.1 Preparations available in the UK and route of administration of anti-D

Various anti-D preparations are available for use in the UK. Some are intended for intramuscular use only while others may be given by either intramuscular or intravenous route. Preparations intended for intramuscular use only must not be given intravenously.

The following preparations are available in the UK at the time of writing this guideline.

D-GAM ® (Bio Products Laboratory): available as 250, 500 and 2500 iu vials, for intramuscular use only.
Partobulin SDF ® (Baxter Bioscience): available as 1250 iu prefilled syringe, for intramuscular use only.
Rhophylac ® (ZLB Behring): available as 1500 iu prefilled syringe, for intramuscular or intravenous use.
WinRho SDF ® (Baxter Bioscience): available as 1500 i.u and 5000 iu vials, for intramuscular or intravenous use.

The dose of anti-D immunoglobulin recommended in this guideline is intended as a minimum dose for a specific clinical situation. The actual dose given may depend on the type of preparation used and limitations of its range of available doses. A dose of 500 iu, given intramuscularly is considered sufficient to treat a Feto Maternal Haemorrhage (FMH) of up to 4 mLs. Where it is necessary to give additional or larger dose of anti-D immunoglobulin, the dose calculation should be based on 125 i.u / mL if given by intramuscular route, and 67.5 i.u / mL if given by intravenous route. It is

acknowledged that manufacturers of some intravenous preparations recommend a different dose for a specific licensed indication. In these situations it is recommended that product literature is followed. When large or multiple doses are necessary, consideration should be given to limiting batch exposure.

The deltoid muscle is an appropriate and safe site for administration of the injection (Lee *et al,* 1999). Particular care must be taken if the gluteal region is used to ensure that the injection is given into muscle, as absorption may be delayed if the injection only reaches the subcutaneous tissues (Lee *et al,* 1999).

1.2 Documentation and audit trail of the issue and administration of anti-D immunoglobulin

The EU guide on good manufacturing practice recommends that records are kept to enable traceability of all blood products (including anti-D) from donors to recipients and vice versa (European Commission 2000).

There are distinct advantages in the hospital transfusion laboratory being involved in the issue and administration process, as the information will be stored automatically in the laboratory computer systems. However it is recognised that local arrangements may vary and other departments may already be responsible for the administration process. Whatever the local arrangements may be, it is essential that complete records of issue and administration are kept to allow traceability of anti-D to recipients.

1.3 Informed consent

Patient's informed consent should be obtained and recorded in the maternal and hospital case notes by the healthcare professional responsible for the administration of anti-D (RCOG, 2002).

Recommendation 1

Documentation accompanying the injection must include a report containing the following details: -

- ***Identity of the patient to include surname, forename, date of birth and a unique ID number with the date when the injection is to be given. (Level IIa, Grade B).***
- ***Identity and address of the GP surgery / antenatal clinic administering the injection. (Level IIa, Grade B).***

Details of the injection will include batch number and strength of dose and route of administration.

Recommendation 2

The details of the administration of anti-D must be recorded in the antenatal record. It is also important that these details are centrally recorded in the hospital blood bank computer so that this information is readily available should pre-transfusion testing be required.

SECTION 2: PREVENTION OF ANTIBODY FORMATION.

2.1 Potentially sensitising events for pregnant women who are D negative.

Pregnant women who are D negative must be considered for prophylactic anti-D for the following potentially sensitising events: -

- 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

2.2 Tests and treatment during pregnancy (Table I)

2.2.1 Before 12 weeks gestation, confirmed by scan, in uncomplicated miscarriage where uterus is not instrumented, or mild painless vaginal bleeding, prophylactic anti-D immunoglobulin is not necessary because the risk of feto-maternal haemorrhage (FMH) is negligible. However 250 iu prophylactic anti-D immunoglobulin should be given in cases of therapeutic termination of pregnancy, whether by surgical or medical methods, to confirmed D negative women who are not known to be already sensitised to D (RCOG, 2002).

2.2.2 Between 12 and 20 weeks gestation,

For any potentially sensitising event listed in 2.1., blood sample should be tested to ensure the woman is D negative and that she is not already sensitised with anti-D. Anti-D immunoglobulin, 250 iu, should be administered (RCOG, 2002).

2.2.3 After 20 weeks gestation

There is an additional requirement to assess the volume of FMH. If the Acid elution (Kleihauer) technique is used and a FMH of >4mL is indicated, the test should be repeated using flow cytometry. At least 500 iu anti-D should be administered intramuscularly (i.m) and additional anti-D given if the FMH is confirmed to be >4mL (RCOG, 2002).

It should be noted that acid elution technique may give a false positive result if a woman has high level of fetal haemoglobin (HbF). This issue can be resolved by using flow cytometry technique.

2.2.4 Following birth

A cord blood sample should be tested to obtain the ABO and D type of the baby. If this is not collected for any reason, a heel prick sample from the baby should be obtained as soon as possible (BCSH c, 2006).

Direct Antiglobulin Test (DAT) should not be performed on cord blood sample as a matter of routine since in a proportion of cases it may be positive because of antenatal prophylaxis with anti-D. However DAT should be performed if haemolytic disease of the newborn is suspected because of a low cord blood haemoglobin level &/or the presence of maternal red cell antibodies.

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, i.m. must be administered to the woman if the FMH is ≤ 4 mL. Additional dose of anti-D immunoglobulin is necessary for larger FMH and the dose to be administered by intramuscular route should be calculated as 125 i.u for each additional mL of FMH. In cases of very large FMH i.e., in excess of 80 mLs, intravenous anti-D should be considered.

Recommendation 3

If the pregnancy is non-viable and no sample can be obtained from the baby, prophylactic anti-D should be administered to the woman, if she is D-negative (level IV Grade C).

Recommendation 4

Following sensitising events anti-D should be injected as soon as possible and certainly within 72 hours of the event. However if this deadline cannot be met due to exceptional circumstances, some protection may be offered up to 10 days after the sensitising event (Lee et al, 1999; RCOG, 2002). (Level III Grade B).

2.2.5 Assessment of the volume of feto-maternal haemorrhage

This is required when a woman who is D negative experiences a potentially sensitising event after 20 weeks gestation and after the birth of a D positive baby. (RCOG, 2002). See Table 1.

Initial screening, using an EDTA sample, should be carried out by the acid elution technique (Kleihauer) or flow cytometry technique. If a FMH of a greater volume than that covered by the standard dose is indicated by acid elution, the volume of FMH must be confirmed by flow cytometry. This may involve referring the sample to a reference laboratory (BCSH a, 1999; Lloyd-Evans *et al* 1996).

Additional anti-D may be required. A 500 iu, i.m. dose is sufficient for 4mL of fetal cells. The additional dose of anti-D should be calculated on the basis of an extra 125 iu for each mL of fetal cells present.

A follow-up maternal sample 72 hours after the intramuscular administration of anti-D (48 hours if anti-D is given intravenously) should be tested to assess the removal of fetal cells following a feto-maternal haemorrhage of >4mL. More anti-D may be necessary if fetal cells remain (BCSH a, 1999).

A clinical decision may need to be made in determining the dose and frequency of more injections dependant upon the volume of residual fetal cells detected 72 hours after the original injection (48 hours if anti-D was administered intravenously).

Recommendation 5

It is essential to assess the volume of FMH to calculate the appropriate anti-D dosage for administration. (level IIb Grade B).

SECTION 3: MANAGEMENT OF A ROUTINE PROPHYLAXIS SCHEME.

3.1 Administration of routine antenatal anti-D prophylaxis (RAADP)

This section takes account of the publication of the NICE guidance which recommends that RAADP is offered to all D negative non-sensitised pregnant women at 28 and 34 weeks gestation at routine antenatal visits (NICE, 2002). A dose of at least 500 iu, i.m. is recommended on each occasion.

A single dose of 1500 iu anti-D, given i.m. at 28 weeks, may be an effective alternative RAADP regimen that potentially offers cost and logistic benefits. However more evidence is required to establish its comparative efficacy.

Use of routine *antenatal* anti-D prophylaxis should not be affected by previous anti-D prophylaxis for a sensitising event early in the same pregnancy. Likewise *postpartum* anti-D prophylaxis should not be affected by previous routine antenatal anti-D prophylaxis or by antenatal anti-D given for a sensitising event.

3.2 Management of RAADP scheme

Information regarding the administration of RAADP must reach the transfusion laboratory promptly in order that information is available should a pregnant woman require pre-transfusion testing. (Good Practice Point [GPP]). This is essential because it is not possible to differentiate between administered prophylactic anti-D and immune anti-D in laboratory tests.

The following recommendations for practice have emerged following the publication of the NICE guidance (Chilcott *et al*, 2003: NICE, 2002).

- Identification of women eligible for RAADP involves training and regular retraining of personnel responsible. This training must be carried out to ensure that all eligible women are correctly identified and their informed consent obtained. (Grade A).
- Information leaflets should be made available to pregnant women to help with the informed consent process (RCOG, 2002) (Grade C).
- Written requests for the injections, with suitable identification of the recipient, should be forwarded in a timely manner to the unit responsible for issuing the injection. (GPP).

Recommendation 6

The RAADP scheme should be regarded as supplementary to any anti-D administered for sensitising episodes listed in section 2.1 (RCOG, 2002). (Level IIb Grade B).

3.3 Antenatal screening sample

Recommendation 7

It is important that the 28-week antibody screening sample is taken prior to the first routine prophylactic injection being given. This forms the second screen required in pregnancy under the BCSH Guidelines for Blood Grouping and Red Cell Antibody Testing during pregnancy. (BCSH c, 2006) (Level III, Grade B)

SECTION 4: PRETRANSFUSION ANTIBODY SCREENING

4.1 Pretransfusion testing post anti-D injection

Antibody screens on maternal samples may detect circulating passive anti-D following injections.

Recommendation 8

The specificity of anti-D detected post-injection should be confirmed using a panel of D negative reagent cells. This test should also be used to establish the presence or absence of any other clinically significant antibodies, especially in transfused patients (BCSH c, 2006) (Grade B).

4.2 Anti-D present in pre-transfusion samples

The anti-D present may be passive or immune. Passive anti-D rarely exceeds 1iu /mL unless a larger than standard dose has been given, and the level falls with time.

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.

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

Recommendation 9.

If there is a record of anti-D injection within the past eight weeks and the level is below 1 iu/mL a further sample should be tested at 28 weeks and prophylaxis should continue. If there is no record of anti-D injection the antibody should be monitored as for immune anti-D i.e. at four weekly intervals to 28 weeks and at fortnightly intervals thereafter. If the anti-D level is falling, it is probably passive whereas if it is steady or rising it is probably immune. Prophylactic anti-D should continue in either case unless it is established that the anti-D is immune (BCSH c, 2006) (Level II b, Grade B).

SECTION 5: PREVENTION OF ANTI-D FORMATION IN THE EVENT OF RECURRENT UTERINE BLEEDING IN D- NEGATIVE WOMEN DURING PREGNANCY

5.1 Recurrent uterine bleeding before 12 weeks gestation

Evidence that women are sensitised after uterine bleeding in the first 12 weeks of pregnancy where the fetus is viable and the pregnancy continues is scant (Ghosh et al, 1994).Therefore anti-D immunoglobulin is not necessary in women with threatened miscarriage with a viable fetus where bleeding completely stops before 12 weeks gestation. However it may be prudent to administer 250 iu anti-D Immunoglobulin where bleeding is heavy or repeated or where there is associated abdominal pain particularly if these events occur as gestation approaches 12 weeks (Level IV, *Grade C recommendation*). The period of gestation should be confirmed by ultrasound.

5.2 Recurrent uterine bleeding between 12 and 20 weeks gestation

D-negative women with recurrent PV bleeding between 12 and 20 weeks gestation should be given 250 iu anti-D immunoglobulin at a minimum of 6 weekly intervals (Level IV, Grade C).

5.3 Recurrent uterine bleeding after 20 weeks gestation

Anti-D immunoglobulin 500 iu should be given at a minimum of 6 weekly intervals. Estimation of FMH by acid elution technique should be carried out at 2 weekly intervals. If the 2 weekly FMH is positive, additional dose of anti-D immunoglobulin (500 iu minimum, more if FMH exceeds 4mLs) should be offered regardless of the presence or absence of passive anti-D in maternal plasma, and FMH should be retested after 72 hours (Level IV, Grade C).

SECTION 6: MANAGEMENT OF TRANSFUSION OF D-POSITIVE BLOOD COMPONENTS.

6.1 D positive platelet transfusions

Whenever possible, D negative platelets should be transfused to D negative pre-menopausal women who need a platelet transfusion. Occasionally, if the appropriate product is not available or would cause unacceptable delay, it may be necessary to transfuse D positive platelets. In these circumstances, prophylaxis against possible Rh alloimmunisation by red cells contaminating the platelet product should be given (Menitove, 2002).

A dose of 250 iu anti-D immunoglobulin should be sufficient to cover up to five adult therapeutic doses of D positive platelets given within a 6 week period (BCSH b, 2003) (Grade B). In severely thrombocytopenic patients with platelet counts of less than 30 x 10^9/L, anti-D should be given subcutaneously to avoid the risk of haematoma following i.m. injection.

It is not necessary to administer anti-D immunoglobulin to D-negative females without childbearing potential, or males who receive D positive platelets (BCSH b, 2003: Menitove, 2002).

6.2 Inadvertent transfusion of D positive blood to D negative pre-menopausal females

When less than 15 mL have been transfused, the appropriate dose of anti-D immunoglobulin should be given. When more than 15mL have been transfused, it is preferable to use the larger anti-D immunoglobulin i.m. preparation (2500 iu). The dose should be calculated on the basis that 500 iu of anti-D will suppress sensitisation by 4 mL of D positive red cells (RCOG, 2002).

When two units or more of D-positive blood have been transfused, a red cell exchange transfusion should be considered to reduce the load of D positive red cells in circulation and the dose of anti-D immunoglobulin required to suppress immunisation. In this situation, the patient should be counselled regarding the implications of both non-intervention (for future pregnancies) and of treatment, including any hazards from receiving donated blood, the exchange procedure itself and of larger doses of anti-D including intravenous anti-D (RCOG, 2002).

A single blood-volume red cell exchange transfusion will achieve a 65-70% reduction in D-positive red cells; a double volume exchange will achieve an 85-90% reduction. Shortly after the exchange transfusion, the residual volume of D-positive red cells should be estimated using flow cytometry. Intravenous anti-D Immunoglobulin is the preparation of choice, achieving adequate plasma levels immediately and being more effective microgram for microgram at clearing red cells. The dose to be administered should assume that 600 iu of anti-D i.v. will suppress immunisation by 10mL fetal red cells. Intramuscular preparations of anti-D immunoglobulin must not be given intravenously. An appropriate combined dose of i.v. and i.m. anti-D should be determined in discussion with a specialist in Transfusion Medicine. Follow-up tests for D positive red cells should be undertaken every 48 hours and further anti-D given until there are no detectable D positive red cells in circulation.

Free anti-D in mother's serum does not necessarily reflect adequate prophylaxis and anti-D immunoglobulin treatment should be continued until D positive red cells are no longer detectable (RCOG, 2002).

Passive anti-D given in large doses may be detectable for up to 6 months or longer, and tests for immune anti-D may not be conclusive for several months.

Future developments

The ability to obtain D type from fetal genetic material in a maternal peripheral blood sample during the first trimester of pregnancy has been reported recently (Daniels *et al*, 2004). The introduction of

this test as routine would mean that the current practice of administering prophylactic anti-D to women carrying D negative babies would become unnecessary.

Audit

Audits of practice should to 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 [DH a, 1997: DH b, 1998].

Declarations of interest

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)

Table 1

Recommendations for antenatal and postnatal tests and the prevention of sensitisation.

Gestation	Summary of tests and treatment
< 12 weeks	No action for uncomplicated miscarriage or painless vaginal bleeding. In all other cases check ABO and D type to confirm D negativity. Confirm absence of anti-D. Issue and administer 250 iu anti-D, i.m
12 weeks | 20 weeks	For all potentially sensitising episodes ABO and D type to confirm D negativity. Confirm absence of immune anti-D. Issue and administer 250 iu anti-D, i.m.
20 weeks |	For all potentially sensitising episodes ABO and D type to confirm D negativity. Confirm absence of immune anti-D. Assess FMH. Issue and administer at least 500 iu anti-D, i.m., depending on the size of FMH.
28 weeks	**First RAADP.** Issue and administer at least 500 iu prophylactic anti-D. The routine sample for blood group and antibody screen as required by BCSH Guidelines (BCSH c, 2006) **must** be taken prior to this injection.
34 weeks	**Second RAADP.** Issue and administer at least 500 iu anti-D.
| BIRTH	TESTS ON BABY – Establish ABO and D type. MATERNAL TESTS – Check ABO and D type. Assess FMH if baby is D positive. Issue and administer at least 500 iu anti-D to the mother if baby is D positive. More anti-D may be required depending upon the size of any FMH.

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

REFERENCES

British Committee for Standards in Haematology (BCSH – a) Guidelines for the estimation of feto-maternal haemorrhage. Transfusion Medicine 1999 **9** 87-92.

British Committee for Standards in Haematology (BCSH - b) Guidelines for the use of Platelet transfusions. BJH 2003; **122**.10-23.

British Committee for Standards in Haematology (BCSH - c). Guidelines for blood grouping and red cell antibody testing in pregnancy 2006 (in preparation)

Chilcott, J., Lloyd Jones, M., Wight, J., Wray, J., Beverley, C., Tappenden, P. (2003) A review of the clinical effectiveness and cost-effectiveness of routine anti-D prophylaxis for pregnant women who are rhesus-negative. *Health Technology Assessment*, vol. **7**, No.4.

Critical Appraisal Skills Program (CASP, 2004), Milton Keynes Primary Care NHS Trust; document assessable from http://www.phru.nhs.uk/casp/reviews.htm (accessed on 22/09/2005)

Daniels, G., Finning, K., Martin, P., Soothill, P. (2004) Fetal blood grouping genotyping from DNA from maternal plasma: an important advance in the management and prevention of haemolytic disease of the fetus and newborn. *Vox Sanguinis*, ***87***, 225-232.

Department of Health 1997 (DH) The new NHS, Modern Dependable. White Paper, London.

Department of Health (DH) 1998 A first class service – quality in the new NHS. London.

European Commission (2000), Manufacture of medicinal products derived from human blood or plasma –Revision of Annexe 14 to the EU Guide to Good Manufacturing Practice.

Ghosh, Murphy WG. Implementation of the rhesus prevention programme: a prospective study. Scott Med J 1994; **39**:147-9.

Huchet, J., Dallemagne, S., Huchet, C., Brossard, Y., Larsen, M., Parnet-Mathieu., F. (1987). [Ante-partum administration of preventive treatment of Rh-D immunisation in rhesus-negative women. Parallel evaluation of transplacental passage of fetal blood cells. Results of a multicentre study carried out in the Paris region]. J Gynecol Obstet Biol Reprod 1987; **16**: 101-111

Lee, D., Contreras, M., Robson, S.C., Rodeck, C.H., Whittle, M.J. (1999) Recommendations for the use of anti-D immunoglobulin for Rh prophylaxis. *Transfusion Medicine*, **9**, 93-97.

Lloyd-Evans, P., Kumpel, B.M., Bromelow, I., Austin, E., Taylor, E. (1996) Use of directly conjugated monoclonal anti-D (BRAD-3) for quantification of fetomaternal haemorrhage by flow cytometry. *Transfusion*, **36**, 432-437.

MacKenzie, I.Z., Bowell, P., Gregory, H., Pratt, G., Guest, C., Entwistle, C.C. (1999)

Routine antenatal rhesus D immunoglobulin prophylaxis: the results of a prospective 10 year study. *British Journal of Obstetrics and Gynaecology*, **106**, 492-497.

Mayne, S., Parker, J.H., Harden, T.A., Dodds, S.D., Beale, J.A. (1997) Rate of RhD sensitisation before and after implementation of a community based antenatal prophylaxis programme. *British Medical Journal*, **315**: 1588.

Menitove, J.E. (2002) Immunoprophylaxis for D negative patients receiving platelet transfusion from D negative donors Transfusion **42**:136-138.

Mollison, PL., Engelfriet, CP., Contreras, M. (1997) Blood Transfusion in Clinical Medicine 10th edition p318.

National Institute for Clinical Excellence (NICE). (2002) Full guidance on the use of routine antenatal anti-D prophylaxis for RhD-negative women. *Technology Appraisal Guidance* No.41.

Royal College of Obstetricians and Gynaecologists. (RCOG revised May 2002). Clinical green top guidelines: - Use of anti-D immunoglobulin for Rh prophylaxis (22).

Tovey, L.A.D., Townley, A., Stevenson, B.J., Taverner, J. (1983) The Yorkshire antenatal anti-D trial in primigravidae. *Lancet*, **2**, 244-246.

Tovey, L.A.D. (1992) Towards the conquest of Rh haemolytic disease: Britain's contribution and the role of serendipity. *Transfusion Medicine*, **2**, 99-109.