SUMMER 2003
ISSUE 13

Blood Matters

Quarterly information for hospitals served by the National Blood Service

NATIONAL BLOOD SERVICE

NHS

Editorial

From the inception of blood transfusion therapy, there has been great concern about the serious consequences of incompatible transfusions. As a result, for many decades most research and development was confined to red cell immunohaematology (RCI). The National Blood Service (NBS) RCI Reference Laboratories have always provided a high quality service but the NBS restructuring in 1999-2000 has enabled changes to be made to standardise and remove any variability in the handling and reporting of hospital referrals. The recent changes in both laboratory testing and clinical advice have enabled more consistent investigation and reporting irrespective of the centre, the region and the requestor. All clinically significant RCI reports are now authorised by an NBS consultant. Ten RCI Clinical Policies have been established which cover a variety of topics and in the near future those considered useful for hospitals will be circulated to all Trusts.

Work undertaken in a laboratory bench-marking exercise spawned a harmonisation project within RCI. There were 17 specific initiatives within this project, including the harmonisation of computer profiles, reports, stationery and laboratory methods.

Providing results in an electronic format has proved to be a challenge. Establishing a mechanism for direct transfer to hospital computer systems has been hampered by the fact that international EDI (Electronic Data Information) standards have no data definitions for blood transfusion and the NBS SACIT (Standing Advisory Committee for Information Technology) standards cover only donors and donation data and do not as yet cover reference laboratory test results.

An alternative approach to EDI has therefore been developed, using web browser technology in co-operation with the NHS Information Authority (NHSIA). RCI reports are entered into the browser each day and in order to gain access to RCI reports, all that a requestor needs is a PC with Internet access, and authorisation by the hospital Caldicott guardian. This facility has been successfully piloted by a number of hospitals and is now available to all hospitals served by the NBS.

In making these changes, RCI has made every effort to comply with BCSH and other UK Guidelines and by eliminating variability in the service moves towards best practice to comply with the principles of clinical governance.

This edition of Blood Matters is dedicated to RCI issues. It aims to provide the reader with an update on topical issues and ends with a CPD Questionnaire to act as an 'aide-memoire' or provide an opportunity for reflective learning.

The development of the International Blood Group Reference Laboratory (IBGRL) is described. They provide a service for NBS and overseas laboratories to solve difficult serological problems. A useful table of what blood to select for patients with common and rare red cell antibodies is included, and the UK NEQAS article eloquently expresses its value as a tool for learning and improving practice. Then follows a group of articles on haemolytic disease of the newborn, from the NICE recommendations to the audit of antenatal transfusion practice. These provide many helpful hints on how best to manage some of the everyday laboratory problems and how to obtain help and advice when needed. Finally, there is a section on Frequently Asked Questions, but if there are still questions you would like answers to, please do not hesitate to contact either the authors directly or the editors of this issue.

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The International Blood Group Reference Laboratory (IBGRL)

Red Cell Reference Services

The Blood Group Reference Laboratory was established by the Ministry of Health in 1946 and had two main functions:

- to provide a centralised service for the production of blood grouping reagents
- to provide a red cell reference service to the then newly formed National Blood Transfusion Service.

The research was mainly in the discovery of new blood groups, their population distribution and the development of new test methods for blood typing. It was also a centre for the training of transfusion specialists from the UK and abroad in blood grouping methods. The first director, Dr Arthur Mourant, collaborated with Drs Coombs and Race in the development of the antiglobulin test, which still remains the most important test in the serology laboratory, although it now appears in many different guises!

In 1953 the BGRL gained World Health Organisation (WHO) recognition for its red cell reference work and became the IBGRL. In the mid 1960s the National and International Panels of Rare Blood Donors were established in collaboration with the International Society of Blood Transfusion.

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Many changes have taken place over the years including cessation of reagent production and expansion of reference and R&D activities. The laboratory, originally based in London and then Oxford, is currently located at the NBS Bristol Centre.

Today, the IBGRL is a unit of the Bristol Institute of Transfusion Sciences (BITS) within the NBS, under the Diagnostics Development and Research (DDR) directorate. Red cell reference services are provided to the NBS, Welsh, Northern Ireland and Scottish Blood Services and, under the auspices of WHO, to blood transfusion laboratories worldwide. IBGRL is a WHO collaborating centre in immunohaematology and one of its primary roles is to provide specialist diagnostic services where quality and cost-effectiveness are maximised by provision from a single specialist centre. The number of samples referred in 2002 was 899, a record high and an approximately 10% increase over the previous two years. The percentage of UK to overseas samples remains constant at approximately 65%.

The red cell reference department undertakes antibody and antigen investigations of a non-routine and complex nature when the referring laboratory has been unable to elucidate the problem.

Antibody investigations are usually from a patient whose serum reacts with all red cells and for whom compatible donors are difficult or impossible to find. The incompatibility may be due to a complex mixture of antibodies or an antibody to a high frequency antigen produced by a patient with a rare red cell phenotype. Sometimes both occur in the same patient. Antibody investigations are occasionally from a patient whose serum contains an antibody to a single example of donor cells or baby/father cells in case of maternal antibody. These are antibodies to low incidence antigens and do not cause crossmatch problems but may rarely cause HDN.

Antibody confirmation is undertaken when the referring laboratory needs verification of a suspected antibody specificity and rare cells are needed to exclude the presence of additional underlying antibodies to 'common' blood groups, i.e. D, Fy^a, K, Jk^b etc.

Red cell antigen investigations are carried out when a phenotype is difficult to determine or an uncommon polymorphism is suspected. All blood group systems are included here and cells can be from patient or donor. The Rh system forms the largest single group, notably D antigen investigations.

Clinical significance of an antibody can often be deduced from the specificity or mode of reactivity or both. Functional assays such as the chemiluminescence test (CLT) is sometimes helpful to predict the clinical significance of an antibody.

Compilation and maintenance of the Rare Donor Panels continues to be undertaken at IBGRL. We can provide the location of donors with a rare type and contact details of the local Blood Centre. These details are available on the Internet to authorised laboratories.

Training in serological techniques and approach to complex investigations is given to transfusion specialists from UK and overseas.

Research is undertaken as an extension to complex case studies. The unique resources of IBGRL are used to advance knowledge in human blood groups and collaborate with academic groups worldwide.

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Guidance For The Selection Of Blood For Patients With Common And Rare Red Cell Antibodies

(Table from NBS RCI Clinical Policy 2003)

Antigen-negative red cells
Anti-A, -B, -A,B
Anti-M (active at 37°C), -S, -s, -U
All Rh antibodies (except anti-C^w)
Anti-Lu^b, -Lu3
Kell antibodies (including anti-K, -k, -Kp^b, -Js^a, -Js^b, -Ku) but not anti-Kp^a , -Ul^a and -K17)
All Duffy antibodies (anti-Fy^a, -Fy^b, -Fy3, -Fy5)
All Kidd antibodies (anti-Jk^a, -Jk^b, -Jk3)
Anti-Wr^b
Anti-Sc1
Anti-Co^a
Anti-H (in O_h individuals)
Anti-Kx
Alloanti-I (active at 37°C)
Anti-P, -PP1P^k
Anti-Vel, -AnWj

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Red cells compatible by IAT at 37°C
Anti-A_1
Anti-N (active at 37°C), -En^a, antibodies to low frequency MNS antigens (anti-'Mi^a')
Anti-P1 (active at 37°C)
Anti-Lu^a
Anti-C^w
Anti-Le^a, -Le^b, -Le^{a+b}
Anti-Kp^a, Ul^a, -K17
Anti-Wr^a
Anti-Yt^b
Anti-Xg^a
Anti-Do^a, -Do^b
Anti-Di^a
Anti-Co^b
Anti-H/HI in para-Bombay, use ABO identical
Anti-HI (in patients with common ABO phenotypes)
Anti-In^a
Autoanti-I
Serologically least incompatible red cells, but antigen-negative red cells for strong examples of the antibody
Antibodies to other (not anti-Lu^b or -Lu3) high frequency Lutheran antigens
Anti-Yt^a
Anti-Gy^a, -Hy, -Jo^a
Cromer antibodies
Anti-In^b
Anti-Lan, -At^a, -Jr^a
Anti-Di^b
ABO/D compatible, least incompatible red cells
Anti-LW^a, -LW^{ab} (use D–)
Chido/Rodgers antibodies
Gerbich antibodies
Knops antibodies
Anti-JMH
Anti-Er^a
Anti-LKE
Anti-Emm, -PEL, -ABTI
Anti-Sd^a (avoid Sd(a^{+++}) donors)
Anti-Sc3
Anti-Co3
Anti-Ok^a
Anti-MAM

When a phenotype that is normally not available in routine stock is required, the RCI laboratory must be consulted. In selected cases (clinical urgency or non availability of suitable units) the RCI consultant will advise on the best alternative.

Learning From UK NEQAS (BTLP)

What goes through your head when the NEQAS package drops on your desk? Something like 'Oh no, not NEQAS again already, I'll never find time to do it', or 'We mustn't make another mistake or the consultant will be down on us like a ton of bricks' or 'We must make sure at all costs that we don't get penalty points'. Perhaps some of you think 'Here's an opportunity to give confidence to one of the more junior members of staff', or 'Here's a chance to learn about weaknesses in our system'. In reality you probably don't specifically say any of these things but your attitude towards NEQAS is important if you as an individual and the UK as a whole is to benefit from the results.

The aims of all UK NEQAS exercises are primarily educational and if undertaken in the right spirit, satisfactory performance should boost confidence within the laboratory. Less than satisfactory performance should provoke a review of processes and procedures to try and establish what went wrong and what corrective action is required to prevent a similar occurrence with a clinical sample.

EQA is an important part of the laboratory quality system, but does not replace internal quality monitoring of reagents, equipment or staff. The samples can never truly mirror real patient samples and sometimes mistakes are made that do not reflect clinical practice. However, NEQAS does provide powerful data based on large numbers of UK laboratories by way of questionnaires as well as exercise material.

There has been a huge improvement in proficiency in the UK in the last two decades, particularly with respect to antibody screening, antibody identification and crossmatching. This is likely to be largely due to a move towards more robust and standardised testing systems, to an improvement in the quality of reagents, and to a reduction in the use of insensitive procedures and techniques, e.g. pooled screening cells and NISS IAT [1].

The new IAT technologies, particularly when automated, should give rise to more standardised results than those obtained by tube tests, with their disparate use of consumables and reagents, and subjective reading. The more important question now is why did 2% of laboratories using a particular technology miss an antibody in the crossmatch, whilst the other 98% detected it? Some answers

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can be found by direct communication with the laboratories in question and some from the data obtained from questionnaires, resulting in learning points for all participants.

The 1996 BCSH guidelines[2] state that D typing should be performed in duplicate. Many use cards/cassettes manufactured by DiaMed and Ortho that fulfil the guidelines by providing one anti-D and one anti-CDE reagent. Two NEQAS exercises have highlighted interpretation errors using this reagent: an r'r cell was reported as D positive or D variant (defined as partial or weak D on the NEQAS result form) in exercises 01R2 and 02R2 by three and four participants respectively. There is no advantage in using anti-CDE for routine patient typing and the revised guidelines (2003)[3], recommend that it is not used routinely.

Some of the errors seen in NEQAS relating to new technology may be explained by lack of compliance with manufacturers' instructions and lack of quality assurance measures. Results of a questionnaire distributed to all users of column agglutination techniques in 1999 (when only 13% were using automation) were cause for concern. 19% of DiaMed and 1% of BioVue users incubate for less time than is recommended. To make matters worse 14% use reactants straight from the fridge but do not increase incubation times to compensate for the time taken to reach 37°C. Approximately 7% do not use the recommended diluent for antibody screening, whilst up to 7% use non-volumetric pipettes for dispensing serum and red cells. Less than half include a positive control with each batch of screens, one third do not monitor staff proficiency and only 60% perform any checks on the temperature of the incubator.

Even automation has brought new problems: There have been several examples of automated systems missing a strong antibody in the antibody screen (00E4, 01E6, 01E8, 01E10, 03E3). None of these failures have been reproducible and although the exact cause is unknown, it has been speculated that bubbles in the samples have caused air to be aspirated instead of serum. This has lead to the recommendation in the revised guidelines[3] that all patient samples are centrifuged prior to automated testing. There have also been a few instances of the automation being inappropriately overridden: in 03E3, anti-D+Jk^b was missed in the antibody screen (anti-Jk^b titre 16) because the operator had ignored a warning given by the AutoVue that the final liquid level was too low, presumably due to a partial aspiration of the serum.

There have been some significant differences in sensitivity between the users of different IAT technologies, however, with the exception of NISS IAT, these have nearly all related to crossmatching using heterozygous cells[1]. The main recommendation is therefore to use screening cells that bear homozygous expression of those antigens that express dosage. Of much more concern than small differences in sensitivity are the ABO and D typing errors, the more serious antibody identification errors and the failure to detect strong antibodies in the screen, particularly where electronic issue is used.

NEQAS results provide only a snapshot of a laboratory's performance at any given time and the true level of proficiency in the UK may be masked by circumstances. This is particularly true with antibody identification of mixtures, where the ease of identification is dependent on the panel(s) of cells in use at the time; overall error rates for the period April 2000 to March 2002 were 2.5% with a range of 0-6.1%. However, in a recent exercise (00E3), 14% participants did not record the presence of the anti-S or anti-Fy^a in sample 3; the majority of the errors were in mistaking the reaction pattern for anti-M, which it happened to fit perfectly with one panel in common use.

By linking results with techniques and procedures, UK NEQAS can identify specific strengths and weaknesses, driving change. National guidelines are reinforced and the need for new guidelines identified.

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NICE Recommendations for Routine Antenatal Anti-D Prophylaxis and Impact on Transfusion Laboratories

Anti-D immunoglobulin (anti-D Ig) was first used for Rh prophylaxis in the UK in 1969. Since then deaths due to anti-D haemolytic disease of the newborn (HDN) have fallen from 46 per 100,000 births in 1969 to less than 2 per 100,000 births in 1990. However, in the UK, nearly 1000 women

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continue to become sensitised each year either as a result of anti-D Ig not being given following the delivery of a D positive infant, after other sensitising events during pregnancy, or due to sensitisation from silent bleeds that occur during pregnancy. Studies have shown that the sensitisation rate during a normal pregnancy can be reduced 5-fold from 1% to less than 0.2% if anti-D Ig is given at 28 weeks and 34 weeks gestation[1,2] and in North America routine antenatal anti-D prophylaxis (RAADP) has been standard practice for over 15 years.

In May 2002, the National Institute for Clinical Excellence[3] (NICE) recommended that RAADP should be offered to all D negative pregnant women in the UK who do not have pre-existing anti-D and that a dose of at least 500iu anti-D should be given at 28 weeks gestation followed by a second dose at 34 weeks.

Following the NICE recommendation, several queries have been raised about the impact of RAADP on serological testing during pregnancy. These problems mostly revolve around the fact that when the anti-D level is low, serological tests cannot differentiate between immune anti-D and circulating anti-D immunoglobulin (passive anti-D). Clearly, it is dangerous to misinterpret one as the other. Misinterpreting passive anti-D as immune may label a woman as being sensitised and deprive her of essential prophylaxis and if immune anti-D is mistaken for passive anti-D, appropriate follow-up may not be undertaken putting the fetus or infant at risk of HDN.

How to resolve the dilemma whether the anti-D is immune or passive

AVOID THE PROBLEM![4]

- Undertake the second antenatal screening test at 28 weeks gestation.
- Consider D negative women who do not have anti-D in their booking sample to be eligible for RAADP.
- Take the 28 week sample **before** giving the first dose of RAADP (passive anti-D can be detected by IAT within minutes of the injection being given).
- Eliminate screening tests at 34-36 weeks gestation.

  Paragraph 3.2 of the BCSH Guidelines for Blood Grouping and Red Cell Antibody Testing during Pregnancy states, "where no red cell antibodies are detected at booking, all pregnant women should be retested once during 28-36 weeks gestation. Some workers believe that RhD negative women should have at least 2 tests performed during this period, one of which should be at 34-36 weeks. In view of the lack of scientific evidence, this recommendation is optional, but immunisation during late pregnancy is unlikely to result in an antibody that will reach a level sufficiently high to cause HDN requiring treatment".

MAINTAIN AN AUDIT TRAIL OF ANTI-D Ig

This is a mandatory DOH requirement for all blood products and applies to anti-D Ig too. Where the pharmacy issues anti-D Ig, it is important for the laboratory to be given this information so that the BMS knows which woman has received prophylaxis, the date of administration and dose given. GPs, midwives and staff in antenatal clinics should be informed to ensure that these details are included on lab request forms.

If passive anti-D is suspected:

- When information is available that prophylaxis has been given in the previous 8 weeks: routine testing should be as for non-sensitised women. No antibody testing is required after 28 weeks and Rh prophylaxis must continue.
- When information regarding prophylaxis is *not* available, there is no option but to monitor the antibody by both IAT and anti-D quantitation. After a dose of 1250iu anti-D or less, the anti-D is <1iu/ml and passive anti-D is generally not detectable by IAT after 8 weeks. A rising or steady level indicates immune anti-D while a falling level signifies passive anti-D.

Pretransfusion testing:

- When anti-D is detected in pre-transfusion, whether it be prophylactic or immune anti-D, it is necessary to test for the presence or absence of other alloantibodies. 2 or 3 suitable rr cells may be selected from an antibody identification panel or an NBS "rr screening – cell" set used to exclude clinically significant antibodies, except anti-D. In any case, most laboratories will already have these procedures in place to handle samples from women who have been given anti-D Ig for a sensitising event before 28 weeks and need transfusion subsequently.

Cord sample testing following RAADP

- Anti-D immunoglobulin, being IgG, can pass through the placenta and enter the fetal circulation and may coat D positive fetal red cells and give a positive DAT (7-8% Whittington Hospital, London and 3% in Derby).[5] However, these DAT positive red cells survive normally and there has been no report of fetal or neonatal anaemia or HDN.

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- Difficulty with D typing of DAT positive samples may occur due to false positive reactions. The use of a high affinity monoclonal anti-D reagent that is not potentiated will overcome this problem. Unpotentiated reagents may give discrepant results but only if the DAT is strongly positive due to anti-D binding to all or most of the D sites. This will not occur following RAADP as only small amounts of anti-D immunoglobulin will enter the fetal circulation.

Everything needs to be done to avoid misinterpreting passive anti-D for immune, and immune anti-D for passive. It is hoped that the above will help laboratories overcome most of the serological problems that may be encountered following the implementation of RAADP and ensure that both mother and baby are protected.

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Prenatal Testing Of Fetal RhD Blood Group Using Maternal Blood

The Molecular Diagnostics Service at the International Blood Group Reference Laboratory (IBGRL), of the National Blood Service, undertakes blood group genotyping using molecular genetic methods. These tests are performed to determine the blood group of the fetus when the mother has a high level of antibody known to be capable of causing severe haemolytic disease of the fetus (HDFN).

Maternal anti-D is the most common cause of HDFN, followed by anti-K (K1) and anti-c. Samples from pregnant women with high levels of these antibodies and whose partners are heterozygous for the offending antigen, are referred by Fetal Medicine Units to IBGRL for fetal blood group genotyping.

Until recently, fetal DNA has been obtained by amniocentesis or chorionic villus sampling (CVS). These invasive procedures impart a small risk of spontaneous miscarriage and can boost the maternal antibody level. Over the last few years, IBGRL has developed a technique by which the fetal RhD blood group can be accurately determined from the cell-free fetal DNA found in maternal plasma.[1] Maternal blood is centrifuged to completely remove all cellular material and DNA is extracted from just under 1ml of plasma. A highly sensitive fluorescence-based real-time polymerase chain reaction (PCR) assay is then used to detect fetal *RHD* gene sequences. As with the conventional PCR-based techniques to determine fetal RhD status from amniocytes/CVS, the real-time PCR assay is designed to prevent false positive results due to the presence of the *RHD* pseudogene common in black D negative individuals.[2] The fetal RhD blood group has now been predicted from maternal plasma in 200 cases with 100% accuracy and over half of all referrals to IBGRL for fetal RhD typing are for the non-invasive test using maternal blood.

Fetal DNA can be detected in maternal plasma from the first trimester, but because the amount of fetal DNA increases throughout pregnancy, fetal RhD typing is usually performed after 16 weeks. The origin of the fetal DNA in maternal plasma appears to be the placenta and not the destruction of fetal blood cells *in vivo*. Unlike DNA from amniocytes or CVS, the DNA obtained from maternal plasma is a mixture of both maternal and fetal DNA. This means that only fetal DNA sequences that are different to that of the mother can be distinguished, i.e. paternally inherited genes or polymorphisms. Therefore, when the fetus is predicted to be RhD negative, there is a theoretical possibility of a false negative result due to the lack of fetal DNA in the sample. Assays to detect common insertion/deletion polymorphisms in the human genome are therefore currently being validated to provide a suitable control to confirm the presence of fetal DNA when no *RHD* sequences can be detected.[3]

At present testing for D using maternal blood is only available to Fetal Medicine Units. The ability to determine fetal D type from maternal

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blood gives an advantage to obstetricians managing pregnancies at risk from HDFN. If the fetus is D positive, then invasive procedures that could increase maternal anti-D levels may be delayed and careful non-invasive monitoring for signs of fetal anaemia can be performed. If the fetus is D-negative the woman requires less intensive monitoring without invasive interventions.

In May 2002, the National Institute for Clinical Excellence (NICE) recommended that all non-sensitised D-negative pregnant women should be given Routine Antenatal Anti-D Prophylaxis (RAADP) at 28 and 34 weeks of pregnancy.[4] Without a screening test, up to 40% D negative women carrying a D negative fetus are given RAADP without any benefit. NICE have endorsed research to develop a test to determine fetal D type before 28 weeks and researchers are exploring ways to scale up maternal plasma testing. In the future, it may be possible to screen all D negative pregnant women (approximately 105,000 in England and Wales) to identify those carrying a D positive fetus.

Other antibodies, particularly anti-K and anti-c also cause HDFN and IBGRL have designed tests to establish the fetal K and c status using maternal plasma. Validation of these tests will be undertaken in the near future in collaboration with obstetric, midwifery, hospital blood transfusion laboratory and NBS red cell immunohaematology teams in more than one centre before they are offered as part of the routine service.

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Management of Large (D+ve) Feto-maternal Haemorrhages in D Negative Women

The feto-maternal haemorrhage (FMH) at delivery is <4ml in 99.6% women. In the UK, the Kleihauer test is used to assess the volume of the FMH in D negative women who deliver a D positive baby. The Kleihauer test is reliable for measuring FMHs that are <4ml and BCSH Guidelines recommend that when the FMH is >4ml an additional check is undertaken using a different technology. The second technology used most commonly in the UK is Flowcytometry (FC). The great advantage of FC is that it measures the volume of D positive cells (rather than fetal cells) and is also very reliable and accurate when the FMH is >4ml.

Because more than 99% women have a FMH <4ml, most hospitals test the cord D type shortly after delivery, administer the standard dose of anti-D immunoglobulin to mothers of D positive infants and mother and baby are discharged. The Kleihauer test is usually undertaken as a batch during the next 24-48 hours to identify the small number of women who need additional prophylaxis. To prevent D sensitisation, prophylaxis must be given within 72 hours of delivery and the NBS provides a 24 hour FC service when an urgent result is required. We recommend that FC is undertaken in all cases where the Kleihauer shows a FMH >4ml. Hospitals should contact their local RCI laboratory as soon as a >4ml FMH is identified and forward the post-delivery maternal sample for FC. When FC results are available the local RCI consultant will advise if additional anti-D is required and when a repeat sample should be taken to establish clearance of all D positive red cells. In general, the dose of anti-D Ig will be selected to minimise the number of intramuscular injections required. Where several intramuscular injections are required the injections should be given at different sites to prevent local reactions. If the FMH is over 100ml red cells, intravenous anti-D will be provided. The total dose of intravenous anti-D Ig may be infused as a single dose over a period of 3-5 minutes.

Follow-up tests

The purpose of follow-up testing is to ensure that the correct dose has been given. When FC has been undertaken to check the Kleihauer result, the volume of the FMH is likely to be measured more accurately and it is very likely that the correct dose was given. Nevertheless it is important to undertake follow-up tests to demonstrate clearance of D positive red cells.

When IM anti-D Ig is given, it takes 48-72 hours for a maximum blood level to be reached and sampling after 72 hours to show clearance is optimal. When IV anti-D Ig has been given, clearance of D positive

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red cells begins immediately and a sample taken 48 hours later to show clearance, is optimal.

Where follow-up testing is required, we recommend that Kleihauer testing is undertaken by the hospital and samples are only referred to the local RCI laboratory if any fetal red cells are seen. Please note that the above advice differs from the BCSH guidelines[1] which were published in 1999 when a 24 hour FC service was not available widely for the estimation of D positive red cells.

When follow-up testing shows residual D positive red cells, this could mean either that the dose of anti-D Ig was inadequate, that anti-D Ig was not administered, or that clearance of coated D positive red cells is slow due to RE blockade. Fortunately, these instances are rare and further anti-D Ig should be given, as it has been shown that prophylaxis given up to 9 days later can prevent sensitisation.

Where the FMH is >4ml, please contact your local RCI consultant for advice. Good communications are essential to ensure that women receive the best protection.

When D positive red cells are still detectable in the circulation, the presence of free passive anti-D must not be taken as proof that the dose of anti-D has been adequate. It has been shown that in spite of free anti-D being present, circulating D positive red cells can result in sensitisation.[2]

Monitoring The Newborn Infant

The blood volume of the newborn is approx 80ml/kg (red cell volume 60ml/kg). When the FMH is large, the infant's Hb should be checked. Where there has been a chronic feto-maternal haemorrhage over a long period, the infant's Hb may be disproportionately high compared to the volume of the FMH. In these cases, the woman should be counselled that prophylaxis may not be effective.

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Audit on Antenatal Transfusion Practice

Blood grouping and antibody screening are routinely carried out on pregnant women to identify D negative women who require anti-D prophylaxis and pregnancies with a risk of fetal and neonatal haemolytic disease. In 1996 the BCSH issued guidelines for blood grouping and red cell antibody testing during pregnancy[1] and in 1999 the BBTS and the Royal College of Obstetricians made recommendations for the use of anti-D immunoglobulin for Rh prophylaxis.[2]

We decided to audit practice of NHS hospitals against national guidelines and where there was non-compliance to identify areas in the guidelines that require further clarification. A postal questionnaire was sent through the National Blood Service (NBS) Clinical Audit and Effectiveness Steering Group to Consultant Haematologists with responsibility for blood transfusion in 282 hospitals which provided antenatal services. Completed questionnaires were received from 147 (52%) hospitals.

The questionnaire contained 16 questions related to frequency of testing, confirmation of antibodies at the time of titration/quantitation, testing partner's samples, timing of pre-transfusion samples, advice to obstetricians, postnatal anti-D prophylaxis and Kleihauer testing. We describe some of the interesting findings.

The audit demonstrated wide variation in practice and compliance with Guidelines. There was 97% adherence to some recommendations, whilst the compliance in other areas was as low as 43%.

- Follow-up testing of RhD positive women without red cell antibodies:

  The BCSH Guidelines recommend testing D positive women at booking and once at 28-36 weeks gestation.

  20% of hospitals did not test D positive women after the booking visit. This could result in failure to identify clinically significant antibodies (especially anti-c/-K), which are capable of causing serious haemolytic disease. On the other hand, 13% of hospitals tested women more than once in the third trimester. These tests are unnecessary and result in increased workload, inconvenience for the mother and a waste of NHS resources.

- Rh D negative women with no red cell antibodies detected at booking:

  BCSH guidelines recommend retesting once during 28-36 weeks but gives the option of doing two tests during the third trimester, one of which should be at 34-36 weeks.

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There was 97% compliance with 47% of hospitals taking the former and 50% taking the latter option. However, when no antibodies are detected at 28 weeks, there is little data to support the added value of testing at 34-36 weeks and when routine antenatal prophylaxis (RAADP) is implemented, passive anti-D is likely to be detected after 28 weeks and would result in difficulties in differentiating immune from passive anti-D. This would lead to unnecessary additional hospital visits, laboratory tests and anxiety for patients.

Two questions were designed to find out how many hospitals took into consideration a history of anti-D prophylaxis, anti-D level and the duration of the presence of anti-D, to differentiate immune from passive anti-D. The survey showed that almost all respondents appreciated the value of the history of anti-D prophylaxis, while there was no consensus on the level of anti-D or the duration of the anti-D in the plasma in differentiating immune from passive anti-D.

- Kell antibodies:

  BCSH Guidelines, whilst recommending that women with Kell antibodies should be retested monthly up to 28 weeks and fortnightly thereafter, also state that 'Kell related antibodies may affect the fetus regardless of titre'. Even though these two recommendations were not consistent with each other, 86% of the respondents indicated that they would carry out follow-up testing according to the recommended protocol.

- Referral to a Fetal Medicine Unit:

  Inconsistencies were observed in the way obstetricians were advised about referring a woman with a high risk of haemolytic disease of the fetus to a fetal medicine unit. 78% of haematologists relied on the clinical advice from the NBS to advise the obstetrician, whilst the others left it to the obstetricians to make their own decision. All RCI reports contain clinical advice regarding the management of pregnancies with atypical red cell antibodies.

This audit has identified areas of non-compliance and unnecessary laboratory testing and also highlighted areas that require clarification when BCSH Guidelines are reviewed. A full report of the audit has been sent to all respondents.

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References

1. BCSH Blood Transfusion Task Force (1996). Guidelines for blood grouping and red cell antibody testing during pregnancy. *Transfusion Medicine*, 6, 71-74.
2. BBTS and RCOG Guidelines (1999). Recommendations for the use of anti-D immunoglobulin for Rh prophylaxis. *Transfusion Medicine*, 9, 93-97.

Frequently Asked Questions

Q. Why perform a cord DAT?

A. (a) To identify infants at risk of HDN.
A positive DAT is not diagnostic of HDN but a positive DAT is a good screening test to identify infants who should have their haemoglobin and bilirubin checked in order to diagnose or exclude HDN. Except in ABO HDN, a negative DAT signifies that there is no haemolytic disease. (In ABO HDN the DAT may be negative.)

(b) An essential test in the investigation of neonatal jaundice and anaemia due to any cause, e.g. ?AIHA, ?HDN due to ABO incompatibility or other antibody which has not been detected because the corresponding antigen was absent on antibody screening cells (e.g. anti-C^w, -Wr^a).

Q. When is *routine* cord DAT testing required?

A. When any clinically significant [IAT reactive] red cell antibody is present in maternal blood.

The BCSH antenatal guidelines recommend performing a cord DAT if the baby of a D negative woman is D positive so that HDN due to late-developing anti-D can be diagnosed. Routine Antenatal Anti-D Prophylaxis [RAADP] will minimise the risk of HDN greatly and removes the need for DAT testing. If DAT testing is continued after RAADP is implemented, up to 8% of healthy babies will have a positive DAT due to passive anti-D, which could result in further unnecessary investigations.

Q. Is there a danger in using 2 screening cells neither of which expresses C^w and Kp^a in routine antenatal and pretransfusion testing?

A. No.

Using 3 antibody screening cells is no more beneficial for patients and much more expensive for laboratories. The main argument for using 3 cells is to ensure expression of C^w and Kp^a. Although anti-C^w and –Kp^a can cause haemolysis, HDN requiring treatment is very rare . Other antigens, such as A and B groups

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CPD Questionnaire

Q1 **Routine antenatal anti-D prophylaxis (RAADP), if given at 28 weeks and 34 weeks gestation during a normal pregnancy, has been shown to reduce sensitisation rate:-**

a) from 2% to less than 0.4%

b) from 1% to less than 0.2%

c) from 0.5% to less than 0.02%

Q2 **RAADP:-**

a) has never been used in the UK

b) is a novel clinical intervention

c) has been a standard practice for over 15 year in some countries

Q3 **Passive anti-D:-**

a) can easily be differentiated from immune anti-D

b) can be detected by IAT within minutes of injection

c) does not cross the placenta

Q4 **Fetal DNA in maternal plasma:-**

a) cannot be detected in the first Trimester

b) the quantity increases throughout pregnancy

c) has an origin from destruction of fetal blood cells in vivo

Q5 **Fetal DNA in maternal plasma:-**

a) can easily distinguish maternally inherited genes

b) distinguishes only paternally inherited genes

c) is a test available to all obstetricians

Q6 **Fetal Maternal Haemorrhage is less than 4 ml in:-**

a) 89.6% of deliveries

b) 98.6% of deliveries

c) 99.6% of deliveries

Q7 **After IM anti-D Ig is given, optimal sampling to demonstrate clearance of fetal cells is:-**

a) 24 hours

b) 48 hours

c) 72 hours

Q8 **Prophylaxis up to:-**

a) 5 days can prevent sensitisation

b) 9 days can prevent sensitisation

c) 13 days can prevent sensitisation

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and anti-Wr^a are capable of causing severe HDN but are not expressed on screening cells! As with HDN due to ABO incompatibility, the trigger for investigation should be jaundice or anaemia of the newborn.

Neither anti-Kp^a or C^w have been reported to cause significant haemolytic transfusion reactions.

Q. Should an anti-D which detects D^{VI} be used for typing cord samples?

A. RCI does not recommend this.
D^{VI} fetal cells have never been reported to have stimulated anti-D in a D negative mother. The same anti-D reagents used for pretransfusion tests are recommended for use for typing cord samples. This is much safer too, as it will avoid a BMS using the D^{VI} reagent (or kit) for patient typing by mistake.

Q. Why does the NBS now advise that the second antenatal sample after booking should be taken at 28 weeks rather than at any time between 28-34 weeks?

A. To rationalise testing protocols. More Trusts are implementing RAADP and D negative women will need to attend a clinic at 28 weeks for the first RAADP injection. It is important that the third trimester sample is taken BEFORE the anti-D is injected. This small change will minimise the number of clinic visits for D negative women and also streamline antenatal protocols if both D positive and D negative women are tested at 28 weeks gestation.

Q. The NBS has recently introduced a two part 'tear-off' report format for users of the routine antenatal screening programme. Is it necessary to always use the tear-off request form?

A. No, you may use a regular request form but the tear-off form will save time and effort both for you and for the NBS! The tear-off request form must not be used for 'booking' samples, because microbiology tests cannot be requested on this form.

Blood Matters Questionnaire

We would like to thank everyone who completed a Blood Matters questionnaire which was enclosed in the last edition of Blood Matters. We are currently analysing the results of the feedback, and will use this to further improve future editions of Blood Matters.

NEWS & SNIPPETS

JPAC Website:

http://www.transfusionguidelines.org.uk/

This site presents the guidelines for the Blood Transfusion Services in the United Kingdom, covering the whole transfusion chain from donor selection to clinical use of blood components and donor selection, testing and processing of tissues.

Diary Dates

- ISBT VII Regional European Congress, 5-9 July, Istanbul, Turkey. *www.isbt2003.org*
- 14th Congress European Society of Haemapheresis & Haematherapy, 10-13 September, Prague, Czech Republic. *www.congress.cls.cz/esfh2003*
- BBTS Annual Scientific Meeting, 2-5 October, Manchester. *www.bbts.org.uk*
- NBS Clinical Audit Conference, 'Using clinical audit to demonstrate personal clinical effectiveness', Wednesday 17 September 2003, Stonebridge Manor, Coventry. Further details available from John Grant-Casey on *john.grantcasey@nbs.nhs.uk* or 0772027 5388
- 6th SHOT Symposium 26th September
  The 6th SHOT report will be launched on 17th July and copies and summaries will be distributed to all hospitals. An electronic copy of the report will also be available on the new SHOT website, *www.shotuk.org*

  A joint symposium in conjunction with the CMO's National Blood Transfusion Committee is to be held on 26th September at the Royal College of Physicians. The programme and registration details will be circulated shortly, and may also be found on the website.

Handy Hints

Have you any 'Handy Hints' to add to a Better Blood Transfusion Tool Kit? We are looking for examples that have made a real difference to blood usage and better blood transfusion that we can share across the whole blood transfusion community.
An example of such a handy hint (taken from article in a previous edition) could be:

Investigation of every event where blood has been wasted

- All staff involved interviewed
- Clinical incident report completed
- Line Manager / Clinical Director informed
- Incident discussed at the HTC
- Procedures and protocols revised, if necessary

Please send your example to the Editor for possible inclusion in the next edition of Blood Matters.

Feedback

We are always interested in your comments and feedback on Blood Matters. We are constantly striving to improve Blood Matters and your suggestions help us in this task. If you have any comments, please contact Chris Hartley on *chris.hartley@nbs.nhs.uk*, or phone 01923 486837 or fax 01923 486801.

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