GUIDELINES
FOR
BLOOD GROUPING & ANTIBODY SCREENING
IN THE
ANTENATAL & PERINATAL SETTING
FOREWORD

The introduction of antenatal RhD-Ig immunoprophylaxis in Australia has raised a number of significant issues, some related to the timing of RhD-Ig injections but most significantly in laboratory testing and reporting. The requirement for antenatal prophylaxis continues to be reviewed in New Zealand.

Current guidelines, in both countries, recommend that all RhD negative females should have red cell antibody screening performed at their initial antenatal visit and at least once between 28 and 36 weeks gestation.

Some recent Australian cases of significant adverse fetal outcomes have highlighted the importance of clinicians ensuring the correct timing of the RhD-Ig injection and providing information of such administration on the laboratory request form, and interpretation by the laboratory of red cell antibody screening results in the context of the RhD-Ig antenatal prophylaxis program.

These cases have reportedly been attributed to a lack of clarity as to whether the presence of RhD antibodies was due to active alloimmunisation or prophylaxis. Evidence suggests that in these cases, the RhD-Ig was given prior to blood sample collection thus further complicating laboratory findings and reporting.

In light of these reported cases, ANZSBT in conjunction with the RhD-Ig Joint Consultative Committee (JCC) comprising representatives from all key stakeholders, including,

<table>
<thead>
<tr>
<th>RANZCOG</th>
<th>ARCBS</th>
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<tr>
<td>RCPA</td>
<td>ACMI</td>
<td>CSL Limited</td>
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</table>

met to review the current issues these cases have highlighted and to review and suggest recommended changes to the previous guidelines, chiefly on testing and reporting.

Again these revised guidelines lay out a consensus approach to cover this area of practice however they may not cover all aspects and individual laboratories may have validated alternative protocols in place.

However AZNSBT again considers the principles of these guidelines can be useful throughout Australasia to the benefit of patients, requesting medical officers and laboratory staff.

These guidelines are the considered opinion of the Council of the Australian and New Zealand Society of Blood Transfusion. They are not intended as prescriptive statements but best practice guides.

All correspondence should be directed through the Secretariat of the Society.

Ken Davis
President ANZSBT
March 2007
INTRODUCTION

The objectives of immunohaematology testing in the antenatal and perinatal setting are essentially to minimise the incidence and severity of Haemolytic Disease of the Newborn (HDN) by:

- identifying RhD negative females
- identifying females with clinically significant alloantibodies to red cell antigens
- assisting in the diagnosis and management of HDN both during pregnancy and following delivery

With the introduction of antenatal RhD-Ig immunoprophylaxis, RhD-Ig is usually administered during pregnancy to prevent alloimmunisation to RhD, and again at delivery. Tests can be performed to determine the dose required.

In females who are alloimmunised, the role of the testing laboratory is to determine antibody specificity and, when potentially clinically significant antibodies are present, to monitor antibody levels either by titration or quantitation [quantitation is currently limited to measurement of antibodies to the RhD and c antigens].

Where HDN is present, it is the role of the laboratory to provide the appropriate blood components for transfusion to the affected fetus or newborn infant. In cases where HDN is suspected but the maternal serum appears to lack antibodies of aetiological significance, or is unavailable, laboratories are required to exclude or identify an immunological basis for the infant’s clinical condition. This entails the testing for ABO incompatibility between mother and child, although on occasions, the maternal serum may be found to contain an antibody to a low frequency antigen, paternally derived.

Laboratory methodology has also changed with an increasing use of column technology. The sensitivity of this newer technology requires laboratories to reassess what constitutes a significant reaction score as compared to the previously used tube technology.

These revised guidelines are designed to assist laboratories undertaking antenatal and perinatal testing as well as providing recommendations on transfusion support for fetuses affected by haemolytic disease of the newborn (HDN).

Close communication between laboratories and clinicians will facilitate appropriate diagnosis and management.

All laboratories need to be mindful of the possible medico-legal implications of the report they generate and the information it provides. It is recommended that laboratories choose a more minimal report approach where no or minimal information is provided regarding RhD-Ig administration.
1 ROUTINE TESTING

1.1 First antenatal visit
All females should be typed for ABO and RhD as early as possible during each pregnancy, preferably at their first trimester visit.

ABO typing is done primarily to aid in patient identification and a record of the maternal ABO type can be useful should the newborn infant develop clinical signs and symptoms consistent with ABO HDN. The results should not conflict with historical records and any discrepancy must be fully investigated and resolved.

Controls shall be used to prevent false typing of RhD negative females as RhD positive. Testing for a weak expression of RhD is not required, however, if performed should be done according to the test manufacturer’s instructions. If a decision is made to include a test for weak D and the test is clearly positive the woman should be regarded as RhD positive and treated as such. When such testing is undertaken care must be taken to ensure that clinically significant D variants are not typed as RhD positive.

Antibody screening must be undertaken and in the event that the antibody screen is positive then further testing must be performed to identify the presence of any clinically significant red cell antibodies.

Antiglobulin testing should be done to detect those antibodies with the potential to cross the placenta and cause HDN.

Methods such as LiSS, PEG, column agglutination, and solid phase adherence used to detect unexpected antibodies during pretransfusion testing may also be used for antenatal antibody screening. The use of enzyme treated red cells or polyspecific anti-human globulin is not recommended as both methods may promote unwanted positive reactions, although they may be useful in special situations to assist antibody identification.

Detection of any antibody at the first antenatal visit is abnormal and should trigger further clinical assessment [this should include previous transfusion history and recent administration of RhD-Ig]. The clinical significance of the antibody should be assessed by reference to section 4.1.

1.2 Testing at 28 weeks gestation
All RhD negative females should have an antibody screen at 28 weeks. For RhD positive females the decision to repeat the antibody screen at 28 weeks is dictated by individual circumstances and the judgement of the clinician / obstetrician

For RhD negative females receiving RhD-Ig, the blood sample must be collected prior to the injection being given. If RhD-Ig has already been given for an earlier sensitising event, antibody screening must still be performed and the date of administration of RhD-Ig must be clearly stated on the request form to assist with interpretation of the result.

It is acceptable for Rh-Dlg to be given immediately after the blood sample has been taken, before results are available. This is because the vast majority of RhD negative females will not be sensitised and this is the most practical approach for optimising patient care.

Summary - routine antenatal testing is designed to:
• determine the blood group - in particular to identify RhD negative females who may require the administration of prophylactic RhD-Ig both during pregnancy and after delivery
• detect the presence of red cell antibodies in particular those that have potential for causing HDN
• monitor the level [by titration or quantitation] of clinically significant antibodies or to detect any further antibodies that may form during pregnancy
• provide compatible blood for intrauterine or intrapartum transfusion when necessary
## ROUTINE TESTING TIMETABLE

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Recommended prenatal testing</th>
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<tr>
<td><strong>Testing and condition</strong></td>
<td><strong>Timing</strong></td>
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<td>Blood Group [ABO &amp; RhD]</td>
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<td>Initial visit</td>
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<tr>
<td>Other</td>
<td>For pretransfusion testing</td>
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<tr>
<td><strong>Antibody screening</strong></td>
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<tr>
<td>All pregnancies</td>
<td>Initial visit</td>
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<td>RhD negative females</td>
<td>For those RhD negative females who will receive RhD-Ig [at 28 weeks or at the time of any sensitising event], the blood sample must be collected prior to its administration</td>
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<tr>
<td>Other</td>
<td>For pretransfusion testing</td>
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<tr>
<td><strong>Antibody identification</strong></td>
<td>Upon initial detection</td>
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<tr>
<td><strong>Antibody titration/quantitation</strong></td>
<td>Rh antibodies and other potentially significant antibodies capable of causing HDN</td>
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<tr>
<td></td>
<td>In the event that clinically significant antibodies are detected, further testing is indicated at intervals no greater than 4 weeks. Seek specialist advice.</td>
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</table>

Adapted from Judd, Transfusion, 2001 [with permission from AABB]

### 2 SPECIFIC CONSIDERATIONS WHEN ANTI-D DETECTED

The introduction of antenatal RhD-Ig immunoprophylaxis in Australia has raised a number of significant issues; some related to the timing of RhD-Ig injections and more significantly in laboratory testing and reporting following suspected or confirmed RhD-Ig administration.

Some recent Australian cases of significant adverse fetal outcomes have highlighted the importance of clinicians ensuring the correct timing of administration and impact on subsequent interpretation of red cell antibody screening in the context of the RhD-Ig antenatal prophylaxis program.

Consequently the following recommended testing and reporting protocols should be followed where ‘anti-D’ is detected:

1. Perform antibody screening using a current standard approved two or three cell screening set by standard laboratory protocol.
2. If this screen is negative no further testing is required.
3. If antibody screen is positive and there is no evidence of, or prophylaxis cannot be confirmed, a full antibody investigation is required.
4. If the screen is positive following confirmed prophylaxis, set up an “RhD-negative screening set”. [these cells (r’, r”, r) other than presence of RhD must comply with the current ANZSBT Guidelines for Pretransfusion Laboratory Practice].
5. If the ‘RhD-negative screening set’ is negative, anti-D has been identified
6. If the ‘RhD-negative screen’ set is positive, proceed as normal with an extended panel investigation and report findings as per normal laboratory practice.

**Suggested criteria for using the ‘RhD-negative set’:**
- the patient is RhD antigen negative
- the laboratory is aware of the administration of RhD-Ig prophylaxis
- result of current standard antibody screen is positive and typical of anti-D
- there is no record/history (other than anti-D) of an unexpected antibody at initial testing
3. INTERPRETATION OF PRESENCE OF ANTI-D

3.1 For LABORATORY:
(1) Any 'anti-D' with score \( \geq 2 \) (0-4 grading scale) or \( \geq 8 \) (0-12 grading scale) requires:
- antibody titration
- referral for quantitation if appropriate and available
- follow up antibody testing at 4 weeks, or earlier, if clinically indicated

(2) For an 'anti-D' with score \( <2 \) (0-4 grading scale) or \( <8 \) (0-12 grading scale):
- where there is a confirmed history of RhD-Ig administration within the previous 6 weeks, report as ‘most likely to be due to recent RhD-Ig administration’
- where there is no confirmed history of RhD-Ig administration, or RhD-Ig given > 6 weeks ago: treat as immune and investigate as in (1) above

3.2 For CLINICIANS to interpret results:
The laboratory results should not be reviewed in isolation but should take into account the clinical history including the presence or absence of potentially sensitising events and recent administration of RhD-Ig.

FLOW CHARTS

a] Confirmed RhD-Ig administration

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Confirmed history of RhD-Ig administration

Within the previous 6 weeks
  Score \( \geq 2 \) (0-4 grading scale) or \( \geq 8 \) (0-12 grading scale)
    Titre and repeat in 4 weeks (or earlier if clinically appropriate)
    Example Report Y
  Score \( <2 \) (0-4 grading scale) or \( <8 \) (0-12 grading scale)
    Assume RhD-Ig prophylaxis
    Example Report X

More than 6 weeks ago
  Regardless of score, titre and repeat
  Example Report W
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b] No evidence of RhD-Ig administration

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No evidence of RhD-Ig administration
  Titre and repeat in 4 weeks (or earlier if clinically appropriate)
  Example Report Z
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EXAMPLE REPORTS

Report W:
Results: “Anti-D detected. Titre XX. Quantitation XX”.

Interpretation
“These laboratory results should not be reviewed in isolation but in association with the clinical history including the presence or absence of potentially sensitising events and recent administration of RhD-Ig.

Clinical information available indicates administration of RhD-Ig greater than 6 weeks ago. These results are suggestive of an immune response and should be considered clinically significant. Further samples should be obtained for repeat testing and refer to a specialist obstetrician to guide further clinical management.”

Report X:
Results: “Anti-D detected [weak reaction]”

Interpretation
“These laboratory results should not be reviewed in isolation but in association with the clinical history including the presence or absence of potentially sensitising events and recent administration of RhD-Ig.

Clinical information available indicates the recent administration of RhD-Ig [within the past six weeks]. These results suggest that the anti-D detected may be passive in nature. However the possibility of an early immune response cannot be excluded by serology alone.”

Report Y:
Results: "Anti-D detected. Titre XX. Quantitation XX".

Interpretation
“These laboratory results should not be reviewed in isolation but in association with the clinical history including the presence or absence of potentially sensitising events and recent administration of RhD-Ig.

Clinical information available indicates the recent administration of RhD-Ig [within the past six weeks] however the reactions are stronger than expected. These results may be suggestive of an immune response and should be considered clinically significant. Further samples should be obtained for repeat testing and refer to a specialist obstetrician to guide further clinical management.”

Report Z:
Results: “Anti-D detected. Titre XX. Quantitation XX”.

Interpretation
“These laboratory results should not be reviewed in isolation but in association with the clinical history including the presence or absence of potentially sensitising events and recent administration of RhD-Ig.

Clinical information available indicates no history of administration of RhD-Ig. These results are suggestive of an immune response and should be considered clinically significant. Further samples should be obtained for repeat testing and refer to a specialist obstetrician to guide further clinical management.”

Some Notes:
1. any injection of RhD-Ig must be given just after a blood sample is collected where antibody screening is to be requested
2. once antenatal RhD-Ig has been given, the passive anti-D may be detectable for >6 weeks
3. following any potentially sensitising event antenatally, the appropriate dose of RhD-Ig should be given [irrespective of prior administration of prophylactic RhD-Ig]. Tests to estimate the extent of any FMH, after the first trimester, should be performed to assess the need for additional RhD-Ig
4. post-delivery, RhD-Ig should still be given to any eligible woman [RhD negative woman with an RhD positive baby] even if anti-D antibody is detected at delivery unless it has been clearly documented that she is already alloimmunised
4 ALLOIMMUNISATION AND PREGNANCY

4.1 When clinically significant antibodies are detected during pregnancy

These antibodies [see glossary] should be identified and assessed for the potential to cause HDN. Antibodies that cause HDN are reactive by the indirect antiglobulin test and are IgG. Antibodies can be grouped according to their likelihood of causing HDN, as follows:

Group 1  
Anti - D, - c, - E, - e, - C, - K, - k, - Fy\textsuperscript{a}  
These antibodies are commonly associated with clinical HDN. Those most often associated with moderate to severe HDN are anti-D, anti-c and anti-K. Other less frequently encountered antibodies, may also cause clinical HDN.

Once the antibody has been assessed as having the potential to cause clinical HDN, the titre/quantitation of antibody should be determined by a standardised technique (eg. the titration method in Appendix 1). Antibody investigation and titre/quantitation should be repeated every 4 weeks until 32 weeks gestation, then every 2 weeks until delivery. When a clinically significant rise in titre/quantitation occurs, the results of antibody monitoring aid the clinician in determining when to initiate fetal monitoring such as ultrasound, amniocentesis or cordocentesis.

Group 2  
Anti, - C\textsuperscript{w}, - Fy\textsuperscript{b}, - Jk\textsuperscript{a}, - Jk\textsuperscript{b}, Jk\textsuperscript{3}, - S, - s, -M, Ge\textsuperscript{a}  
These antibodies may cause a positive DAT but therapy, if necessary, is likely to be limited to phototherapy.

**Titration of Non-Rh Antibodies.** These titrations should be undertaken only after discussion with the obstetrician as to the significance of the results and how the data obtained will affect patient management. There is little data available concerning critical titres for non-Rh antibodies encountered in pregnancy.

Group 3  
Anti-P1, - N, - H, - Le\textsuperscript{a}, - Le\textsuperscript{b}, - Le\textsuperscript{a+b}, - Lu\textsuperscript{a}, - Lu\textsuperscript{b}, - Sd\textsuperscript{a}, - HLA  
These antibodies are not documented to cause clinical HDN.

4.2 When clinically significant antibodies are detected at first antenatal presentation

All women who are immunised to group 1 or group 2 antibodies require further investigation and management, including paternal phenotype.

All females who have previously had an infant affected by HDN, other than that related to ABO, should be referred to a specialist centre as soon as possible and preferably before 20 weeks gestation irrespective of antibody level.

4.3 Females with anti-D or anti-c

It is imperative that all relevant information is available to assist in the management of an Rh alloimmunised patient. Information required includes:

- previous history, eg transfusion, pregnancies, anti-D prophylaxis
- previously affected pregnancies, eg IUT, neonatal exchange transfusion, jaundice
- paternal blood group and phenotype

[If RhD immunoglobulin is administered during pregnancy it is currently impossible to distinguish between the passive immunity secondary to the administration of prophylactic anti-D from low-level anti-D resultant from alloimmunisation, by serological testing].

Antibody level should be measured at the time the antibody is first detected during pregnancy and every 4 weeks thereafter preferably by quantitation. Each sample should be tested in parallel with the previous sample and the results compared. If there is a significant rise in titre (at least 2 dilutions) or quantitation, follow up testing should be performed.

Although it is documented that anti-D/anti-c titrations/quantitations do not always correlate well with the severity of HDN, it is still the only method available for many laboratories.

If performed by the titration method, (see appendix 1) a titre of 32 or higher indicates the need for clinical assessment by an obstetrician experienced in the management of pregnancies complicated by HDN.
Anti-D/anti-c quantitation (IU/mL) using a standard anti-D/anti-c reference serum is more reproducible and correlates better with the severity of HDN. Laboratories performing anti-D/anti-c quantitation should provide guidelines as to the significance of results. Some laboratories may provide further assessment using bioassays.

Following any intrauterine transfusion, the maternal sample should be screened/panelled prior to the next transfusion to determine whether additional antibodies have been formed, particularly if complete phenotype compatible blood has not been used (see 4.8).

Once intrauterine transfusion has been commenced, the further measurement of titre/quantitation is of little diagnostic value.

4.4 Females with Red Cell Antibodies Other Than Anti-D or anti-c
Only IgG antibodies can cross the placenta and cause HDN. Antibodies can be grouped according to their likelihood of causing HDN[refer 4.1]. Those antibodies not implicated in HDN need not be monitored.

Antibodies which have a significant IgG component, detectable by indirect antiglobulin methods, should be titrated every 4 weeks, throughout pregnancy, by the method detailed in appendix 1 using, where possible, a pool of red cells with homozygous expression of the relevant antigen.

The antibody other than RhD & Rhc that is most likely to cause HDN is anti-K.

4.5 Females with anti-K antibodies
Several studies have now shown that fetal and neonatal disease related to maternal anti-K and anti-D differs in that:

- in contrast to anti-D, previous obstetric history is not predictive of disease severity related to anti-K antibodies
- there is poor correlation between antibody titre and outcome
- amniotic fluid spectrophotometric estimation (OD 450 nm) of bilirubin concentration is of limited value since haemolysis is not a dominant feature. **MCA Doppler is now seen as the standard of care to measure fetal anaemia**
- hyperbilirubinaemia is not a feature of the disease in affected neonates

Erythroid suppression rather than haemolysis is the predominant mechanism in producing fetal anaemia related to maternal anti-K.

The following are suggested recommendations for monitoring females with anti-K antibodies:

- check the paternal K antigen status
- if paternal phenotype is K-positive or unknown, **amniocentesis is the preferred approach for fetal genotyping**. If the fetus is K negative treat the patient as for an unaffected pregnancy
- if the fetus is K positive and fetal anaemia is present an intrauterine transfusion protocol should be implemented

4.6 Titration/Quantitation
The purpose of titrating potentially significant antibodies is not to predict the severity of HDN. This is done to determine when to monitor for HDN by non-serological means such as MCA Doppler measurements for fetal anaemia. Titration studies should be performed as suggested in Appendix 1. The use of enzyme-treated red cells, LISS or other enhancement means for titration purposes is contra-indicated.

4.7 Blood Group Status of the Fetus
It is worthwhile phenotyping red cells from the putative father whenever the potential for HDN exists. On the basis of the probable genotypes that may be deduced, it is possible to predict the likelihood that the fetus carries the antigen which corresponds to the maternal antibody specificity. (See Judd 2001).

4.8 Intrauterine transfusion
Blood selected for intrauterine transfusion should be less than 7 days old. Frozen washed cells may also be used.
Blood selected should also be:
- ABO and Rh compatible with both the mother and the fetus. If the group of the fetus is unknown then group O RhD negative low haemolysin or washed cells are preferable
- antigen negative for the relevant maternal antibody
- preferably matched to the maternal phenotype such that the mother is not exposed to any of the major blood group antigens - Fy, Jk, K and S - which are not present on her red cells
- leucodepleted
- whenever possible CMV antibody negative
- irradiated and used within 24 hours
- red cells for intrauterine transfusion should have a minimum haematocrit of 70%

5 TESTING / MANAGEMENT AT DELIVERY

5.1 Mother/Infant
Table 2 highlights the tests necessary in the management of mother and infant at delivery.

ABO and RhD typing and a direct antiglobulin test on the infant’s blood are recommended if the mother was not tested for ABO and RhD and unexpected antibodies during pregnancy.

In the absence of maternal alloimmunisation during pregnancy, no testing of cord blood samples is required unless it assists in diagnosis, neonatal care or determining candidacy for RhD immunoglobulin.

In the absence of fetomaternal ABO incompatibility but with clinical evidence of HDN (i.e. a positive DAT), an antibody in the maternal serum to a low incidence antigen should be considered.

5.2 Protocols
Maternal sample
If a group and antibody screen has not been previously performed or if blood transfusion or RhD immunoglobulin is required, a pre or post delivery sample should be tested.

Cord sample
A cord sample should be taken from the babies of RhD negative females, females with known antibodies or in cases where there is insufficient documentation of maternal blood group or antibody status. The cord sample should be tested for blood group and direct antiglobulin test [#]. Elution studies may be useful. Haemoglobin and bilirubin estimation should also be performed if DAT is positive.

When the cord blood sample of the baby of an RhD negative woman is RhD positive, RhD immunoglobulin administration is indicated. When the cord blood is RhD negative, it is recommended that testing for the presence of the weak RhD antigen by the indirect antiglobulin test be performed. If positive, RhD immunoglobulin is indicated.

[#] The direct antiglobulin test is indicated when there are clinical signs of jaundice or anaemia in the infant and where the mother is known to have a clinically significant antibody. An elution should be performed to confirm the identity of the antibody coating the cord red cells.

NOTE:
- RhD-Ig, being IgG, can cross the placenta and enter the fetal circulation and may coat RhD positive fetal cells and give a positive DAT. However, these DAT positive red cells survive normally and there has been no report of fetal or neonatal anaemia or HDN
- Difficulty with RhD 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 along with appropriate controls may overcome this problem
5.3 Fetomaternal Haemorrhage (FMH)
As soon as practical and preferably within 72 hours after delivery, a maternal sample should be taken from all RhD negative females who have delivered an RhD positive baby and who have not preformed anti-D, to determine the extent of the fetomaternal haemorrhage. If an FMH greater than that covered by the standard dose of RhD immunoglobulin has occurred, further RhD immunoglobulin should be given.

RhD Immunoglobulin-VF 625IU, ARCBS/CSL Bioplasma’s RhD immunoglobulin should afford protection against a FMH of 6ml (12mL whole blood) of RhD positive red cells. For haemorrhages greater than 6mL, the recommended dose is 100 IU per mL RhD positive red blood cells.

Where large volumes of RhD immunoglobulin need to be administered or the patient has a specific contra indication to intramuscular injections, an intravenous [IV] RhD-Ig preparation should be considered. Currently WinRho 600IU, Cangene/Baxter’s immunoglobulin is available from the ARCBS for the IV route of administration.

TESTING AT DELIVERY

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<thead>
<tr>
<th>TABLE 2</th>
<th>Recommended testing at delivery</th>
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<td>Indication</td>
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<td>Blood Group [ABO/RhD]</td>
<td>To obtain concordant results of tests on two samples, or if pretransfusion tests requested</td>
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<tr>
<td>Antibody detection</td>
<td>When pretransfusion tests requested</td>
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<tr>
<td>Antibody identification</td>
<td>First detection of alloantibody, RhD negative panel should be used if RhD immunoglobulin given during pregnancy</td>
</tr>
<tr>
<td>Titration studies</td>
<td>Not indicated</td>
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<tr>
<td>FMH testing</td>
<td>All RhD Neg females who deliver an RhD Pos infant</td>
</tr>
<tr>
<td>Testing to diagnose HDN</td>
<td>ABO/RhD and tests for unexpected antibodies if not done during the admission for delivery</td>
</tr>
<tr>
<td>Test maternal plasma against paternal RBC’s if there are no unexpected antibodies found by routine reagent screening/panel cells</td>
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Cord/infant blood

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Infants born to RhD Neg females</td>
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<tr>
<td>Infants born to females with potentially significant antibodies</td>
</tr>
<tr>
<td>No maternal alloimmunisation but infant with clinical signs and symptoms of HDN</td>
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Adapted from Judd, Transfusion, 2001 [with permission from AABB]

Appendix 1 - Titration Method

1. Prepare master dilutions of the serum/plasma using a minimum volume of 250µL and a diluent of 5% protein in Buffered Saline (pH 7.0 - 7.2).
2. Prepare a 3% washed cell suspension in Buffered Saline (pH 7.0 - 7.2). These cells should be a pool of equal volumes of cells homozygous for the antigen being tested.
3. Transfer 200µL of serum/plasma or serum/plasma dilution into a tube. Add 50uL of the cell suspension.
4. Mix and incubate at 37°C for 30 minutes.
5. Wash 3 times in PBS and add AHG, mix, spin and read.
6. The end point is read as the last tube showing a score 5 (1+) reaction.

Developed by a NICE (National Immunohaematology Continuing Education) consensus forum.
## Blood Group Systems and Related Antibodies

<table>
<thead>
<tr>
<th>SYSTEM</th>
<th>ANTIBODY</th>
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<tr>
<td>Rh</td>
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