

Guidelines for Blood Grouping & Antibody Screening in the Antenatal & Perinatal Setting

2nd Ed. 2004

Prepared by

**Scientific Subcommittee of the
Australian & New Zealand Society of Blood Transfusion Inc**



Australian & New Zealand
Society of Blood Transfusion Inc

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Foreword

Several changes have occurred since the last edition in 1999.

Routine antenatal RhD immunoprophylaxis has been recommended in Australia since 2002; there have been changes in reagents, in test methods, and advancement in knowledge about the molecular basis and structure of blood group antigens, especially those of the Rh blood group system.

The introduction of antenatal RhD immunoprophylaxis has stimulated numerous questions on the approach to, and the frequency of, immunohaematology testing of mother and neonate.

In response to requests from members, and other health care professionals, for educational material and recommendations for antenatal and perinatal testing, a working party of the ANZSBT Scientific Subcommittee was tasked with re-evaluating past practice in this area with a view to considering what changes, if any, might be considered current best practice.

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

Whilst antenatal RhD immunoprophylaxis has been introduced in Australia [from November 2002] it has not, at the time of printing of these guidelines, been formally recommended in New Zealand.

However the SSC 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 Scientific Subcommittee and 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 to the Chairman of the Scientific Subcommittee through the Secretariat of the Society.

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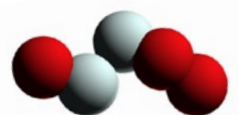
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Introduction

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:

1. Identifying RhD negative women,
2. Identifying women with clinically significant alloantibodies to red cell antigens
3. Assisting in the diagnosis and management of haemolytic disease of the newborn [HDN] both during pregnancy and following delivery.

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

In women 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.

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.

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 HDN.

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

1. Recommended Testing Procedures During Pregnancy

1.1 ABO and RhD Testing

All women should be tested 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 women as RhD positive.

Testing for a weak expression of RhD is optional, 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.

1.2 Antibody Screening

Initial testing

All women, regardless of RhD type, should be tested during each pregnancy for clinically significant unexpected antibodies at their first visit and any alloantibodies detected should be fully identified. Antiglobulin testing should be done with anti-IgG 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 pre transfusion testing may also be used for antenatal antibody screening. The use of enzyme treated red cells or polyspecific anti-human globulin is not advocated as both promote unwanted positive reactions, although they may be useful in special situations to assist antibody identification.

Testing at 28 weeks gestation

The value of performing an antibody screen at 28 weeks has been questioned by Judd [2001]. This is particularly relevant in the context of an active antenatal prophylaxis program. The risk of occurrence of RhD alloimmunisation between the first trimester visit and 28 weeks is quite low and there is a paucity of cases in which anti-D not detected at the first visit has developed during pregnancy and caused significant HDN requiring medical intervention. However the cost effectiveness of repeat testing has not been studied.

Recent guidelines from the American College of Obstetricians and Gynaecologists state that the decision to repeat the antibody screen at 28 weeks is dictated by individual circumstances and the judgement of the obstetrician.

Third Trimester Testing of RhD positive women

In most cases RhD positive women need to be screened for antibodies only once during pregnancy, i.e. at their initial visit. Data cited by Judd [2001], in two large studies, found a very low incidence of potentially significant antibodies detected for the first time at delivery. In both studies no significant neonatal morbidity resulted.

Additional testing is appropriate, however, when there is a history of significant antibodies, blood transfusions, or traumatic deliveries.

Testing After RhD Ig Administration

Requests to perform titration studies to differentiate between passive immunity to RhD (secondary to administration of RhD immunoglobulin) from alloimmunisation (after a presumed or known sensitising event) are not recommended. Detection of anti-D found for the first time following a sensitising event, or at delivery following administration of RhD immunoglobulin, should be assumed to be due to the administration of passive anti-RhD until proven otherwise.

The mother should be assessed for a FMH and remains a candidate for post-partum RhD immunoglobulin administration.

1.3 Summary

Routine antenatal testing is designed to:

1. Determine the blood group – in particular to identify RhD negative women who may require the administration of prophylactic RhD immunoglobulin both during pregnancy and after delivery.
2. Detect the presence of red cell antibodies in particular those that have potential for causing HDN.
3. Monitor the level of clinically significant antibodies or to detect any further antibodies that may form during pregnancy.
4. Provide compatible blood for intrauterine or intrapartum transfusion when necessary.

1.4 Protocols

All pregnant women, both RhD Positive and RhD Negative, should be tested in the first trimester for blood group and clinically significant red cell antibodies. Testing for the presence of the weak RhD phenotype by the indirect antiglobulin test is optional [see 1.1].

Antenatal screening for high titre ABO antibodies is not recommended as it has little or no predictive value in ABO HDN.

When no clinically significant antibodies are detected at the first trimester the value of repeat testing in all pregnancies at 28 weeks or later has been questioned [Judd 2001].

However repeat testing of RhD negative women at 28 weeks prior to administering RhD immunoprophylaxis is becoming the accepted protocol in most Australian centres, as is the elimination of the antibody screen at 34–36 weeks.

In New Zealand, in the absence of routine antenatal prophylaxis, normal practice is to test RhD negative women at 28 and 36 weeks gestation.

Testing Timetable

Table 1. Recommended Prenatal Testing

Testing and condition	Timing
ABO & Rh (test for weak D optional)	
All pregnancies	Initial visit
Other	For pretransfusion testing
Antibody screening	
All pregnancies	Initial visit
RhD Neg pregnancies	Additionally at least once between 28 and 36 weeks. In the context of routine antenatal prophylaxis this should be undertaken prior to administration of the first dose of RhD immunoglobulin at 28 weeks
Other	For pretransfusion testing
Antibody identification	
Unexpected antibodies present	Upon initial detection
Confirmatory testing	At time of titration
Antibody titration/quantitation	
Rh antibodies	Upon initial detection Repeat at 18–20 weeks gestation. Further testing will be influenced by titre/quantitation and specificity of antibody
Other potentially significant antibodies	As above, with discussion with obstetrician

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

2. Suggested Protocol For Antibody Screening In RhD Negative Women Following Antenatal RhD Immunoprophylaxis.

Antenatal prophylaxis with RhD Immunoglobulin in Obstetrics have been approved by the Australian Department of Health and Ageing (DHA) [2002].

This is a three stage implementation program which will eventually result in universal antenatal prophylaxis with RhD Immunoglobulin [ADGG] at 28 and 34 weeks to RhD negative women.

If the previous “ASBT Guidelines for Blood Grouping and Antibody Screening During Pregnancy [June 1999]” are followed, antenatal prophylaxis will result in an increased number of positive antibody screens. This in turn will increase the amount of follow up testing required from laboratories.

However, if these new guidelines are followed, this expected increase may not eventuate.

To assist laboratories the following protocol is suggested.

2.1 Protocol for antibody screening in RhD negative women [including those women receiving ADGG prophylaxis].

1. Perform antibody screening using a current standard approved two or three cell screening sets 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 prophylaxis, a full antibody investigation is required.
4. If the screen is positive following prophylaxis, set up an “RhD–negative screening set”. [these cells (r', r''), r) need to comply with current ANZSBT Pretransfusion Guidelines]. If in any doubt, a full cell panel should be performed.
5. If the ‘RhD–negative screening set’ is negative, anti–D has been identified. If ADGG prophylaxis cannot be confirmed then treat as a normal positive antibody screen, ie. titre/quantitation should be performed.
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 ADGG 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

2.2 Reporting an antibody screen that indicates ‘anti-D detected’ in this situation.

Antenatal

If clinical notes or laboratory records clearly state “Anti-D prophylaxis given at/on.....”, then report as follows:

“Anti-D detected, likely to be consistent with administration of passive [prophylactic] anti-D given at 28 or 34 weeks”.

If no [or insufficient] clinical notes provided report as follows:

“Anti-D antibody alone is detected in this antenatal sample from this RhD negative woman. This may be indicative of either passive immunity secondary to the administration of prophylactic anti-D or alloimmunisation. If this antibody is not the result of prophylaxis, then further antenatal serology should be performed at four weekly intervals through to 36 weeks gestation and thereafter at two weekly intervals until term. If the antibody titre is more than or equal to 16, referral to a maternal-fetal medicine specialist is suggested.”

Postnatal

If no [or insufficient] clinical notes provided, report as follows:

“Anti-D antibody alone is detected in the serum sample from this RhD negative woman post delivery. There is no way to differentiate between passive immunity secondary to the administration of prophylactic anti-D or alloimmunisation. If the baby is RhD positive, postnatal anti-D prophylaxis should be administered [even if antenatal prophylaxis was given] along with assessment of fetomaternal haemorrhage.”

[These suggested report comments are provided as examples. Each laboratory should formulate their own reports under the guidance of the officer in charge.]

2.3 'RhD-negative screening set'

This set of cells shall express all the significant antigens, other than RhD, so as to comply with ANZSBT Pretransfusion Guidelines 4th Ed. [2002], clause A 1.2.2 and A 1.2.3.

A proposed configuration could be (r', r'', r).

Some Notes:-

1. The DHA has recommended antenatal prophylactic administration of ADGG (625IU) be given to any non-sensitised RhD negative mother. ADGG should be administered at 28th and 34th week gestation.
2. The 1st injection of ADGG should be given just after a blood sample is taken for the routine 28th week antibody screen.
3. Once antenatal ADGG has been given, the passive anti-D may be detectable for >6 weeks.
4. Following any potentially sensitising event antenatally, the appropriate dose of ADGG should be given [irrespective of prior administration of prophylactic ADGG]. Tests to estimate the extent of a any FMH, after the first trimester, should be performed to assess the need for additional ADGG.
5. Post-delivery, ADGG 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.

3. Alloimmunisation and Pregnancy

3.1 When clinically significant antibodies are detected during the first trimester

These antibodies 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

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.

Group 2 Anti, - C^w, - Fy^a, - Fy^b, - Jk^a, - Jk^b, Jk3, - S, - s, - M, Ge^a

These antibodies may cause a positive DAT but therapy, if necessary, is likely to be limited to phototherapy.

Group 3 Anti-P1, - N, - H, - Le^a, - Le^b, - Le^{a+b}, -Lu^a, - Lu^b, - Sd^a, - HLA

These antibodies are not documented to 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 36 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.

3.2 When clinically significant antibodies are detected at first antenatal presentation

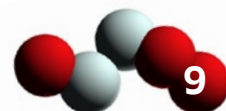
All women who have previously had an infant affected by HDN, other than that related to ABO, should be referred to a specialist centre as soon as possible and preferably before 20 weeks gestation irrespective of antibody level. Fetal haemolysis may need to be assessed by amniocentesis or fetal blood sampling and the need for intrauterine transfusion (IUT) determined.



PREV



NEXT



3.3 Women 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 tested at the first visit 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 16 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/paneled prior to the next transfusion to determine whether additional antibodies have been formed, particularly if complete phenotype compatible blood has not been used (see 3.9).

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

3.4 Women 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. Those antibodies not implicated in HDN need not be monitored.

Antibodies which have a significant IgG component detectable by indirect antiglobulin methods should be titred every 4 weeks by the titration method using, where possible, red cells of the same phenotype throughout pregnancy.

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

3.5 Women with anti-K antibodies

Several studies have now shown that fetal and neonatal disease related to maternal anti-K and anti-D differ 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 underestimates the severity of the disease.
- 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 women with anti-K antibodies:

- check the paternal K antigen status.
- if paternal phenotype is K-positive or unknown refer for chorionic villus sampling (CVS) at 10 - 12 weeks or cordocentesis at 18 weeks gestation. 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.

3.6 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 spectrophotometric analysis of amniotic fluid. 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.

3.7 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.

3.8 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).

3.9 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 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 24hours.
- red cells for intrauterine transfusion should have a minimum haematocrit of 70%.

4 Management At Delivery

4.1 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 (ie. a positive DAT), an antibody in the maternal serum to a low incidence antigen should be considered.

4.2 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 women, women 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.

If the direct antiglobulin test is positive, it may indicate fetomaternal incompatibility [NB: see note below]. Antibody elution from the neonatal red cells can be performed to confirm the identity of the antibody coating the cord red blood cells.

Note:

RhD immunoglobulin, 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 may overcome this problem.

4.3 Fetomaternal Haemorrhage (FMH)

As soon as practical and preferably within 72 hours after delivery, a maternal sample should be taken from all RhD negative women 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.

CSL RhD immunoglobulin 625IU /WinRho 600IU should afford protection against a FMH of 6ml (12mls whole blood) of RhD positive red cells.

Testing At Delivery

Table 2 Recommended testing at delivery

	Indication
Maternal Blood	
ABO/RhD	To obtain concordant results of tests on two samples, or if pretransfusion tests requested
Antibody detection	When pretransfusion tests requested
Antibody identification	First detection of alloantibody, RhD negative panel should be used if RhD immunoglobulin given during pregnancy
Titration studies	Not indicated
FMH testing	All RhD Neg women who deliver an RhD Pos infant
Testing to diagnose HDN	ABO/RhD and tests for unexpected antibodies if not done during the admission for delivery Test maternal plasma against paternal RBCs if there are no unexpected antibodies found by routine reagent screening/panel cells
Cord/Infant Blood	
Infants born to RhD Neg women	RhD status, including test for weak D
Infants born to women with potentially significant antibodies	ABO/RhD, and DAT
No maternal alloimmunisation but infant with clinical signs and symptoms of HDN	If fetomaternal ABO incompatibility exists an infant eluate should be performed. If no fetomaternal ABO incompatibility exists, maternal plasma or infant eluate should be tested against paternal RBCs

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).
3. These cells should be a pool of equal volumes cells homozygous for the antigen being tested.
4. Transfer 200µl of serum/plasma or serum/plasma dilution into a tube. Add 50ul of the cell suspension.
5. Mix and incubate at 37 degrees C for 30 minutes.
6. Wash 3 times in PBS and add AHG, mix, spin and read.
7. 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.

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