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GUIDELINES FOR LABORATORY ESTIMATION OF FETOMATERNAL HAEMORRHAGE

Australian & New Zealand
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Guidelines for the Laboratory Estimation of Fetomaternal Haemorrhage

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Prepared by the:

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Foreword

The ANZSBT Council is delighted to publish this second edition of the *Guidelines for the laboratory estimation of fetomaternal haemorrhage* which like the previous 2002 edition were prepared by the ANZSBT Transfusion Science Standing Committee (TSSC).

The revision was prompted by Australia's National Blood Authority (NBA) release of the *Prophylactic use of RhD Immunoglobulin in Pregnancy Care* guidelines which replace the NBA *Guidelines on the Prophylactic Use of RhD Immunoglobulin (Anti-D) in Obstetrics*. [1, 2]

This new edition is now consistent with the format of other ANZSBT guidelines and focuses on the laboratory testing and reporting of results.

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1. National Blood Authority. (2021). *Prophylactic use of RhD immunoglobulin in pregnancy care*. (https://www.blood.gov.au/anti-d-0)
2. National Blood Authority. (2003). *Guidelines on the Prophylactic Use of Rh D Immunoglobulin (Anti-D) in Obstetrics*. (https://www.blood.gov.au/system/files/documents/glines-anti-d.pdf)

Contents

Introduction

These guidelines are intended to provide guidance for pathology service providers performing fetomaternal haemorrhage (FMH) testing.

Detecting and quantifying the volume of a fetal haemorrhage is important in determining the appropriate dose of RhD immunoglobulin (RhD Ig) for RhD negative women following a sensitising event. FMH testing may also be used when assessing fetal welfare.

The laboratory should be aware of the current requirements in complementary standards, or guidelines; for example, those published by the National Pathology Accreditation Advisory Council (NPAAC), the Australian and New Zealand Society of Blood Transfusion (ANZSBT), The Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG), Australia's National Blood Authority (NBA), the Australian Commission on Safety and Quality in Health Care (ACSQHC), the National Association of Testing Authorities (NATA) or International Accreditation New Zealand (IANZ).

Terminology

These guidelines are primarily informative and reflect what the TSSC believes is the minimum acceptable level of practice. Guidance is provided in the form of recommendations, the strength of which is indicated by the following (modal) terms:

Must Indicates a strongly recommended practice where compliance would be expected.

Should Indicates a recommended practice where compliance would be expected but alternative practices may be acceptable.

May Indicates a practice that is permitted within the context of the guidelines.

Section 1
Indications for testing

1.1 General principles

1.1.1 Indications for FMH testing and subsequent recommendations for dosing of RhD Ig (see section 5) are based on the NBA *Guidelines for the prophylactic use of RhD immunoglobulin in pregnancy care.*[3]

1.1.2 A test to detect and quantify fetomaternal haemorrhage (FMH) should be performed on all RhD negative women, who deliver an RhD positive baby or following a sensitising event beyond 20 weeks of pregnancy, to determine the appropriate dose of RhD Ig.

- ⓘ Prior to 20 weeks gestation the recommended doses of RhD Ig are sufficient to cover the maximum FMH of a 20-week gestation singleton fetus.[4]
- ⓘ FMH assessment may be requested at clinical discretion between 13 and 20 weeks.

1.1.3 The first dose of the RhD Ig should be given without waiting for the result of FMH testing.

1.1.4 Other indications for FMH testing include investigation of fetal welfare:

- unexplained fetal hydrops or severe fetal anaemia;
- reduced fetal movements with a normal ultrasound;
- near-term fetal death; and
- unexplained stillbirth.

1.1.5 **Urgent** FMH testing might be indicated for investigating fetal welfare or in major trauma **and** when test results will change the patient's clinical management. In this instance, results may be required outside of routine working hours.

1.1.6 FMH testing is NOT indicated in the following settings:

- antepartum haemorrhage in RhD positive women;
- diagnosis of placental abruption;
- sensitising events up to and including 20 weeks gestation;
- women with preformed anti-D alloantibody;
- RhD negative mother with RhD negative baby (unless for investigation of fetal welfare);
- minor trauma in pregnancy irrespective of RhD type; and
- evaluation of unexplained abdominal pain in late pregnancy.

3. National Blood Authority. (2021). *Guidelines for the prophylactic use of RhD immunoglobulin in pregnancy care*. (https://www.blood.gov.au/anti-d-0)

4. Working Party of the British Committee for Standards in Haematology, Transfusion Taskforce. (2009). *Guidelines for the estimation of fetomaternal haemorrhage*. (https://b-s-h.org.uk/guidelines/)

2

Section 2
Specimens

2.1 General principles

2.1.1 Patient and specimen identification, labelling, transport and collection procedures must comply with applicable standards and/or guidelines e.g. in Australia NPAAC *Requirements for Medical Pathology Services*.[5]

2.1.2 The patient identifiers recorded on the request and specimen label must agree.

2.1.3 The request should include relevant clinical details and date and time of the infant's delivery, or the gestational age (in weeks and days) in the case of an antenatal sensitising event.

2.1.4 The laboratory should have a policy for managing out of hours urgent requests and for managing specimens accompanying patients transferred from other hospitals, facilities or external locations outside of their jurisdiction.

2.2 Specimen collection

2.2.1 A maternal EDTA specimen should be collected *and* tested within 72 hours of the infant's delivery or other sensitising event. The use of clotted specimens is not recommended.

ⓘ Care should be exercised at delivery to ensure the maternal and cord specimens are correctly identified and labelled.

2.2.2 Before collecting the maternal specimen for FMH testing following delivery or other sensitising event it is advisable to allow time for the fetal red cells to become distributed through the maternal circulation. A period of 30 to 45 minutes should be sufficient.[6]

2.2.3 The specimen should be collected prior to administration of RhD Ig.

2.2.4 The indication for testing should be documented on the request form.

2.3 Transport and storage

2.3.1 If there is a delay between collecting the specimen and delivering it to the laboratory, transport and storage conditions must ensure the specimen's viability is maintained. Specimens should be kept between 2°C and 8°C during transit.

2.4 Specimen integrity

2.4.1 Clotted, leaking or grossly haemolysed specimens should not be used for FMH testing as they may cause a false negative result or underestimate the size of the FMH.

2.5 Specimen processing

2.5.1 Specimens should be processed, and results reported within 72 hours of delivery or other sensitising event to ensure timely administration of a supplementary dose of RhD Ig for fetal bleeds greater than that covered by the standard 625 IU dose of RhD Ig.

5. National Pathology Accreditation Advisory Council. (2018). *Requirements for Medical Pathology Services*. 3rd edition. (https://www1.health.gov.au/internet/main/publishing.nsf/Content/health-npaac-publication.htm).

6. Working Party of the British Committee for Standards in Haematology, Transfusion Taskforce. (2009). *Guidelines for the Estimation of Fetomaternal Haemorrhage*. (https://b-s-h.org.uk/guidelines/)

Section 3
Test methods

3.1 General principles

3.1.1 In the Kleihauer-Betke test fetal red cells are differentiated from adult red cells by the relative resistance of fetal haemoglobin (HbF) to acid elution compared with adult haemoglobin (HbA).[7] In a counterstained blood film fetal red cells are stained bright pink against a background of "ghost" adult (maternal) red cells.

3.1.2 Flow cytometry can also be used for detecting fetal red cells in maternal blood.[8, 9] In this method fluorescent-labelled anti-HbF or anti-D antibodies are used to detect and enumerate fetal red cells.

3.1.3 Commercially manufactured FMH test kits and reagents are available for both Kleihauer-Betke and flow cytometry testing. When using commercial testing kits the manufacturer's instructions should be followed.

ⓘ Any changes to the manufacturer's instructions may result in the test being considered an in-house in vitro diagnostic medical device (IVD) which would require additional validation.[10]

3.2 Factors influencing interpretation and reporting of FMH tests

3.2.1 A variety of technical and patient factors may affect the accuracy and reproducibility of FMH tests resulting in the under- or overestimation of FMH, and therefore an inappropriate dose of RhD Ig being administered.

3.2.2 The formulae used to calculate FMH volume typically assume a 70 kg woman has a red cell volume of 1800 mL. The actual maternal red cell volume will depend upon factors such as body mass index (BMI), haematocrit and gestational age. A significant increase in BMI or maternal red cell volume may result in an underestimate of the FMH.

3.2.3 Increased levels of HbF in maternal red cells during pregnancy may lead to overestimation of FMH. In up to 36% of pregnant women, the level of maternal HbF rises above the upper limit of the reference interval at about 8 weeks gestation and may persist for the duration of the pregnancy and early post-partum period.[11]

3.2.4 Increased levels of HbF are also seen in women with haemoglobinopathies and hereditary persistence of fetal haemoglobin (HPFH).[12]

3.2.5 Limitations of the **Kleihauer-Betke** test include: variations in technique by individual operators and between different operators, thickness of the blood film, sensitivity of haemoglobin elution to pH, time and temperature and subjectivity in the examining the stained blood film.

3.2.6 Although **flow cytometry** offers improved accuracy:

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7. Kleihauer E, Braun H, Betke K. (1957) Demonstration von fetalem Hämoglobin in den Erythrocyten eines Blutausstrichs. *Klin Wochenschr 35*: 637-38. (https://doi.org/10.1007/BF01481043).
8. Nelson M, Popp H, Horky K, Forsyth C, Gibson J. (1994). Development of a flow cytometric test for the detection of D positive fetal cells after fetomaternal hemorrhage and a survey of the prevalence in D negative women. *Immunohematology, 10*:55-59. (https://www.exeley.com/immunohematology/doi/10.21307/immunohematology-2019-819).
9. Nelson M, Zarkos K, Popp H, Gibson J. (1998). A flow-cytometric equivalent of the Kleihauer test. *Vox Sang 1998; 75*: 234– 41. (https://doi.org/10.1046/j.1423-0410.1998.7530234.x).
10. Therapeutic Goods Administration Australia. *Medical Devices & IVDs*. (https://www.tga.gov.au/medical-devices-ivds)
11. Corcoran D, Murphy D, Donnelly JC, Ainle FN. (2014). The prevalence of maternal F cells in a pregnant population and potential overestimation of foeto-maternal haemorrhage as a consequence. *Blood Transfusion, 12*(4):570–574. (http://www.bloodtransfusion.it/articolo.aspx?idart=002784&idriv=97)
12. Thein SL, Menzel S. (2009). Discovering the genetics underlying foetal haemoglobin production in adults. *British Journal of Haematology, 145*(4). (https://onlinelibrary.wiley.com/doi/10.1111/j.1365-2141.2009.07650.x)

- Tests using anti-HbF do not distinguish between HbF of fetal or maternal origin particularly when the mother's red cells have HbS or hereditary persistence of fetal haemoglobin (HPFH).
- In tests using anti-D, RhD Ig administered prior to collecting the maternal specimen may bind to and block antibody binding sites on the fetal red cells preventing detection thereby underestimating the volume of FMH.
- The comparative sensitivities of tests using anti-D or anti-HbF may differ.

3.2.7 Calculations assume fetal red cells are 22% larger than adult red cells and the fetal haematocrit is 0.51.

3.3 Kleihauer-Betke test (acid elution method)

3.3.1 Blood films

3.3.1.1 Thin blood films should be prepared in accordance with the laboratory's standard method.

ⓘ To assist in achieving thin films it is recommended that an aliquot of the maternal specimen is diluted 1:2 or 1:3 with phosphate buffered saline before preparing the film.[13]

3.3.2 Staining

3.3.2.1 Effective staining is dependent on a number of factors including:

- temperature of the reagents;
- age, quality and pH of the stain;
- thorough washing of the slides;
- ensuring the back of each slide is wiped after staining to remove any excess stain deposit; and
- avoiding lengthy delays during the staining process to avoid fixation of haemoglobin.

3.3.3 Controls

3.3.3.1 Positive and negative control slides must be tested in parallel with the patient slides to ensure the staining process is adequate and clearly differentiates between adult and fetal red cells, as well as standardising the counting of fetal red cells. The following controls are recommended:

- **Positive**: 0.25% fetal red cells (in adult male blood of the same blood group).
  The positive control is set to detect the equivalent of a 6 mL bleed of fetal red cells in the maternal circulation. This is the maximum volume of FMH covered by the standard 625 IU dose of RhD Ig.
- **Negative**: 100% adult male blood.

3.3.3.2 An 0.25% concentration of fetal red cells is equivalent to a 1:400 ratio of fetal to adult red cells (for example, approximately 5 µL of cord blood in 2 mL of adult blood).

3.3.3.3 To prepare a more accurate mixture, the required volumes of adult blood or cord blood can be calculated by utilising the red cell count (RCC) of the original cord and adult blood specimens:

$$\text{i.e. } 0.25\% = 0.0025 = \frac{\text{Volume cord blood (mL) x cord blood RCC}}{\text{Volume adult blood (mL) x adult specimen RCC}}$$

For example, to calculate the required volume of adult blood if using 5 µL (0.005 mL) of cord blood:

$$\text{Volume of adult blood (mL)} = \frac{0.005 \text{ x cord blood RCC}}{0.0025 \text{ x adult speciment RCC}}$$

3.3.3.4 Add the calculated volume of cord blood to the chosen volume of adult blood, mix well and make thin blood films as per standard method.

13. Working Party of the British Committee for Standards in Haematology, Transfusion Taskforce. (2009). *Guidelines for the Estimation of Fetomaternal Haemorrhage*. (https://b-s-h.org.uk/guidelines/)

3.3.3.5 It is recommended that the control slides are examined first to ensure the staining and preparation process are satisfactory. If the controls are not acceptable then new control and patient slides should be prepared.

3.3.4 Examination of the blood film

3.3.4.1 Use of an eye piece graticule is recommended to aid with counting.

3.3.4.2 It is recommended that the examination process is separated into **screening** (see 3.3.5) and **quantification** (see 3.3.6).

3.3.4.3 Examination of the film and counting of fetal and adult red cells requires sufficient cells to be counted to ensure statistical validity at the low end of desired sensitivity.

3.3.4.4 In the area of the film to be screened count the number of adult cells in one high power field (10x eyepiece and 40x objective). There must be at least 100 adult cells present for screening to proceed.

3.3.4.5 If the cell count is acceptable then proceed to screening (see 3.3.5) but if not, new slides should be made.

3.3.5 Screening

3.3.5.1 The initial screen should examine 25 low power fields (using a 10x eyepiece and 10x objective) or 50 low power fields (using a 10x eyepiece and 20x objective).

3.3.5.2 The positive and negative controls should be checked to verify suitability of staining on low power. The patient film should be thoroughly scanned on low power for the presence of any fetal cells (stained bright pink).

3.3.5.3 If a total of less than 10 fetal cells are seen across the screened fields, then it can be assumed and therefore reported, that the FMH is <2 mL (with a note indicating no further testing is required). An FMH <2 mL will be covered by the standard dose of RhD Ig (625 IU).

3.3.5.4 If 10 or more fetal cells are seen then a quantification must be performed by viewing the film at a higher power (see 3.3.6).

3.3.6 Quantification

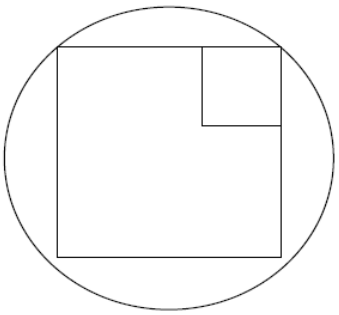
3.3.6.1 Quantification should be performed when initial screening (see 3.3.5) estimates the FMH to be ≥2 mL. For accuracy a minimum of 10,000 maternal red cell ghosts should be screened by high power (using a 10x eyepiece and 40x objective).

3.3.6.2 Quantification may be performed using either a **Miller Square** (see 3.3.6.3) or an **Indexed Square** (see 3.3.6.4)

3.3.6.3 Using a Miller square

3.3.6.3.1 Examine the film using high power and a Miller square (see figure 1).

Figure 1: Miller square



3.3.6.3.2 Select an area of the film where cells are touching but not overlapping.

3.3.6.3.3 Count the number of adult cells (a) present in the small square, including all cells which overlap the left and upper edges but not those overlapping the right and lower edges.

3.3.6.3.4 Count the number of bright pink fetal cells (F) in the large square, treating cells which overlap the edges as per above. Include those within the small square.

3.3.6.3.5 Move across the slide so that the next area falling within the counting grid is continuous with the preceding one and repeat the procedure. Ensure enough squares are screened so that a minimum of 10,000 cells are counted.

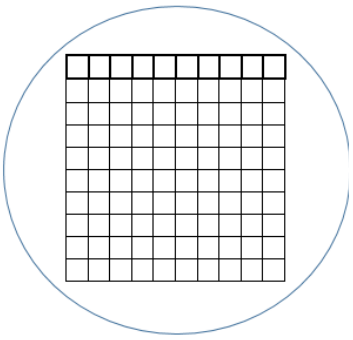
3.3.6.3.6 Assume the total adult cells counted A = (a x 9) and the total fetal cells counted = F.

3.3.6.4 Using an Indexed square

3.3.6.4.1 Examine the film using high power and an Indexed square (see figure 2).

3.3.6.4.2 Use the same process as 3.3.6.3 above, except adult cells (a) are counted in 10 squares of a 10 x 10 grid. The total number of adult cells (A) = (a x 10).

Figure 2: Indexed square



3.3.7 Calculation of FMH volume using Kleihauer-Betke test

3.3.7.1 Calculate the volume of FHM using Mollison's formula (shown below) which makes the following assumptions:[14]

- Fetal red cells are approximately 22% larger than maternal red cells.
- Only 92% of fetal red cells stain brightly.
- Average maternal red cell volume is 1800 mL.

The FMH is calculated as follows:

$$\text{Volume of FMH (mL)} = \frac{F}{A} \times 1800 \times \frac{122}{100} \times \frac{100}{92}$$

OR simplified to:

$$\text{Volume of FMH (mL)} = \frac{F}{A} \times 2400$$

Where: F = Number fetal red cells and A = Number of adult red cells

3.4 Flow cytometry

3.4.1 Selection of antibodies

3.4.1.1 The decision to test using anti-HbF or anti-D will be influenced by laboratory and patient factors:

- **Anti-HbF** may be used in antenatal or post-partum patients or where the fetal/neonate RhD group is either unknown or weak RhD. It may also be used for RhD positive patients.
- **Anti-D** may be used for patients who are RhD negative, post-partum where the neonate is known to be RhD positive. Anti-D may also be used for confirming FMH in patients with increased HbF.

14. Mollison PL. (1972) Quantitation of transplacental haemorrhage. *British Medical Journal, 3*: 31-34. (https://www.bmj.com/content/3/5817/31)

3.4.1.2 If fluorochrome anti-D is used and the maternal specimen is found to be RhD positive, the flow cytometer must be thoroughly flushed, and any subsequent specimens must be rerun.

3.4.2 Controls

3.4.2.1 Commercial controls are available.

3.4.2.2 Positive and negative control specimens should be run in parallel with each assay. The following controls are recommended:

- **Positive**: 0.25% fetal red cells (in adult male blood of the same blood group).
  If using fluorochrome anti-D the fetal red cells should be RhD positive. The positive control is set to detect the equivalent of a 6 mL bleed of fetal red cells in the maternal circulation. This is the maximum volume of FMH covered by the standard 625 IU dose of RhD Ig,

ⓘ Depending on the local method or testing kit used, the laboratory may choose to run different positive controls. For example, either a single dilution (such as 0.5% fetal red cells) or a combination of low (0.2%) and high (1.0%) controls.

- **Negative**: 100% male adult red cells (which should be RhD negative if using fluorochrome anti-D).

3.4.3 Calculation of FMH volume using flow cytometry

3.4.3.1 Calculate the volume of FHM using Mollison's formula (shown below) which makes the following assumptions:

- Fetal red cells are approximately 22% larger than maternal red cells.
- Average maternal red cell volume is 1800 mL.

The FMH is calculated as follows:

$$\text{Volume of FMH (mL)} = \frac{\text{Percentage of fetal red cells}}{100} \times 1800 \times \frac{122}{100}$$

OR simplified to:

Volume of FMH (mL) = Percentage of fetal red cells x 18 x 1.22

Section 4
Confirmatory and follow-up testing

4.1 Confirmation of Kleihauer-Betke test by flow cytometry

4.1.1 If the fetus is RhD positive or unknown RhD type and the Kleihauer-Betke test indicates an FMH of ≥2 mL, the laboratory may consider performing a confirmatory estimation of the FMH volume by flow cytometry. When 10 or fewer fetal cells are seen in Kleihauer-Betke test the result is reported as an FMH <2 mL and no further testing is necessary (see 3.3.5.3).

ⓘ The laboratory may choose to set a higher (than 2 mL) FMH threshold for flow cytometry referral.

4.2 Monitoring clearance of fetal red cells

4.2.1 Follow up testing is recommended to monitor for adequate clearance of fetal red cells for FMH ≥6mL and provide guidance for administration of additional RhD Ig. Collect and test a repeat specimen within 72 hours of intramuscular (IM) or 48 hours of intravenous (IV) administration of RhD Ig.

4.2.2 If fetal cells are detected in the follow-up specimen, additional RhD Ig will be required. Perform a repeat FMH test on a specimen collected within 72 hours of administering the additional RhD Ig. This cycle should be repeated until fetal red cells are no longer detected.

4.3 Ongoing or recurrent uterine bleeding

4.3.1 For recurrent or ongoing uterine bleeding in an RhD negative woman beyond 20 weeks gestation which is judged clinically to represent the same sensitising event, an FMH estimation should be performed every 6 weeks.

4.3.2 Further testing may be indicated where ongoing bleeding is suggestive of a new presentation or there is a significant change in the pattern or severity of bleeding.

4.3.3 If fetal red cells are detected, RhD Ig should be administered and a repeat FMH estimation performed.

ⓘ Since RhD Ig only clears RhD positive fetal red cells, the ongoing presence of fetal red cells despite RhD Ig administration may be due to the fetus being RhD negative.

4.3.4 A flow cytometry test using anti-D will determine whether the ongoing fetal cells are RhD positive. Alternatively, if available, non-invasive prenatal testing (NIPT) testing may be performed to determine the RhD status of the fetus.

Section 5
RhD immunoglobulin dosing

5.1 Standard dosing

5.1.1 For more information on dosing refer to the NBA's *Prophylactic use of RhD immunoglobulin in pregnancy care* and in particular *Appendix C Dosing of Rh D immunoglobulin following fetomaternal haemorrhage quantitation* and consider seeking transfusion medicine specialist advice.

5.1.2 Dosing is based on CSL Behring Rh(D) Immunoglobulin-VF (available in 250 IU and 625 IU vial sizes).

ⓘ Other products may be available from time to time, and these might have different formulations and/or potency. Always consult the manufacturer's product information to check the appropriate RhD Ig dosing.

5.1.3 One 625 IU dose of RhD Ig administered by IM injection will cover an FMH of up to 6mL of fetal RhD positive red cells.

ⓘ In the first 12 weeks of pregnancy a dose of 250 IU is indicated. Between 13 and 20 weeks of pregnancy a single standard 625 IU dose of RhD Ig is sufficient to cover the maximum expected FMH.

5.1.4 The initial dose of the RhD Ig should be given without waiting for the result of FMH testing.

5.1.5 An increase in the maternal BMI may result in an underestimate of the true FMH because calculation of FMH volume assumes a 70kg woman with a red cell mass of 1800mL. However, there is no evidence to support alternative dosing requirements for women with a high BMI (>30).

5.2 Dosing for FMH >6 mL

5.2.1 An additional dose of RhD Ig is required for FMH >6 mL. The recommended dose is 100 IU RhD Ig per mL of fetal red cells in excess of the 6 mL covered by the initial 625 IU dose, rounded up to the nearest whole vial or vials.

5.2.2 Refer to the Rh D Immunoglobulin dosing recommendations for FMH >6 mL.[15] When multiple vials of intramuscular Rh D Ig are required, consider alternate dosing with an intravenous preparation. Specialist advice is recommended for any large FMH.

5.2.3 Repeating FMH testing is recommended 72 hours post IM administration (or 48 hours post IV administration) of RhD Ig, to check for clearance of fetal red cells and to determine if further dose/s are required (Refer to Section 4.2).

15. National Blood Authority. (2021). *Prophylactic use of RhD immunoglobulin in pregnancy care.* 2021. (https://www.blood.gov.au/anti-d-0)

Section 6
Reporting of results

6.1 Reporting

6.1.1 Reports should include: the test method and the volume of FMH (in mL), and may include the recommended dose of RhD Ig.

ⓘ Reporting the number or % fetal red cells detected is not recommended, as these are a possible source of confusion when interpreting results. The % fetal red cells may however be recorded in the laboratory information system (LIS).

6.1.2 The report should include a statement to guide dosing, for example:

"*If the patient is RhD negative, a single 625 IU dose of RhD immunoglobulin, administered by intramuscular (IM) injection, will protect against an FMH of up to 6 mL of RhD positive fetal red cells.*"

If the mother is RhD positive or the infant is known to be RhD negative, this statement can be omitted.

6.1.3 For FMH >6 mL the report should also include guidance on providing an additional dose of RhD Ig, for example:

"*Follow national guidelines or specialist advice regarding additional doses of IM or IV RhD Ig, and follow-up testing.*

For FMH>6 mL an additional dose of RhD immunoglobulin is required. The recommended dose is 100 IU per mL of fetal red cells in excess of the 6 mL covered by the standard initial 625 IU dose of RhD immunoglobulin. The additional dose should be rounded up to the nearest full vial or vials.

Repeating FMH testing is recommended 72 hours post IM administration (or 48 hours post IV administration) of RhD Ig, to check for clearance of fetal red cells and to determine if further dose/s are required.

A specimen has been referred for flow cytometry [if applicable]."

6.1.4 The laboratory may consider provision of additional information on their reports e.g.

- Advice regarding use of IM versus IV RhD Ig when multiple injections of IM RhD Ig are indicated.
- Indicating that guidance should be sought for larger bleeds and in cases where the test assumptions may not hold true. For example, from guidelines, product information, Lifeblood/New Zealand Blood Service and/or transfusion medicine specialists as appropriate.

6.1.5 If the maternal HbF is known to be elevated, and the laboratory uses the Kleihauer-Betke method or flow cytometry with anti-HbF, then additional comments may be included expressing caution with interpretation of the results.

6.1.6 If an anti-D flow cytometry method is used, laboratories may wish to include a comment that this method is not suitable for testing RhD positive mothers.

6.1.7 Each laboratory should have a policy for notification of clinically significant results.

6.2 Example Reports

6.2.1 No fetal red cells or < 2 mL fetal red cells detected

Fetomaternal Haemorrhage (FMH) Assessment

Method:
Kleihauer-Betke

Estimated FMH Volume:
<2 mL

Comment:
If the patient is RhD negative administer a standard dose of RhD immunoglobulin. A single 625 IU dose of RhD immunoglobulin administered by intramuscular (IM) injection, will protect against an FMH of up to 6 mL of RhD positive fetal red cells.

No further testing required.

6.2.2 Fetomaternal haemorrhage ≤ 6 mL

Fetomaternal Haemorrhage (FMH) Assessment

Method:
[i.e.] Kleihauer-Betke [or] Flow cytometry

Estimated FMH Volume:
[e.g.] 3.7 mL

Comment:
If the patient is RhD negative, a single 625 IU dose of RhD immunoglobulin administered by intramuscular (IM) injection, will protect against an FMH of up to 6 mL of RhD positive fetal red cells.

[If applicable] A specimen has been referred for confirmation by flow cytometry.

6.2.3 Fetomaternal haemorrhage > 6 mL

Fetomaternal Haemorrhage (FMH) Assessment

Method:
[i.e.] Kleihauer-Betke [or] Flow cytometry

Estimated FMH Volume:
[e.g.] 10.9 mL

Comment:

FMH >6 ML FETAL RED CELLS

If the patient is RhD negative, a single 625 IU dose of RhD immunoglobulin administered by intramuscular (IM) injection, will protect against an FMH of up to 6 mL of RhD positive fetal red cells.

Follow national guidelines or specialist advice regarding additional doses of IM or IV RhD Ig, and follow-up testing.

For FMH > 6 mL an additional dose of RhD immunoglobulin is required. The recommended dose is 100 IU per mL of fetal red cells in excess of the 6 mL covered by the standard initial 625 IU dose of RhD immunoglobulin. The additional dose should be rounded up to the nearest full vial or vials.

Repeating FMH testing is recommended 72 hours post IM administration (or 48 hours post IV administration) of RhD immunoglobulin, to check for clearance of fetal red cells and to determine if further dose/s are required.

[If applicable] A specimen has been referred for confirmation by flow cytometry.

Section 7
Quality assurance

7.1 Laboratory accreditation

7.1.1 FMH testing must be performed in an accredited medical testing laboratory. The agencies responsible for accreditation in Australia and New Zealand are:

- **Australia**
  National Association of Testing Authorities (NATA)/Royal College of Pathologists of Australasia (RCPA) Laboratory Accreditation Program; accrediting against NPAAC standards and *AS ISO 15189 Medical Laboratories - Requirements for quality and competence*. [16, 17, 18]
- **New Zealand**
  International Accreditation New Zealand (IANZ); accrediting against *ISO 15189 Medical Laboratories – Requirements for quality and competence*. [19, 20]

7.2 Commercial test kits

7.2.1 Commercially manufactured test kits and reagents are available for both Kleihauer-Betke and flow cytometry FMH testing.

7.2.2 Changes to the manufacturer's instructions may result in the test being considered an in-house in vitro diagnostic device (IVD) which would require additional validation.

- In **Australia** modifications to a commercial test kit or reagent must comply with the requirements of the Therapeutic Goods Administration (TGA) as they apply to the creation of in-house in vitro diagnostic medical devices (IVD) and must include the development of a standardised method for testing.[21]
- In **New Zealand** commercial testing kits should be CE marked with an initial verification of method performed, following the manufacturer's instructions.

7.2.3 Laboratories must have a documented procedure for acceptance testing of test kits or reagents to verify performance before introduction into routine use.

7.3 Quality assurance programme

7.3.1 Laboratories must participate in an accredited external quality assurance programme. Laboratories should choose a programme accredited to *ISO/IEC 17043 Conformity assessment – General requirements for proficiency testing*.[22]

16. National Association of Testing Laboratories (NATA) (https://www.nata.com.au/)
17. National Pathology Accreditation Advisory Council (NPAAC) (https://www1.health.gov.au/internet/main/publishing.nsf/Content/health-npaac-publication.htm)
18. Standards Australia. (2013). *AS ISO 15189-2013 Medical laboratories – Requirements for quality and competence*. (https://infostore.saiglobal.com/en-au.)
19. International Accreditation New Zealand (IANZ) (https://www.ianz.govt.nz/);
20. International Organization for Standardization. (2012). *ISO 15189: 2012 Medical Laboratories - Requirements for quality and competence*. (https://www.iso.org/standards.html).
21. Therapeutic Goods Administration. *Medical Devices & IVDs*. (https://www.tga.gov.au/medical-devices-ivds)
22. International Organization for Standardization. (2010). *ISO/IEC 17043 Conformity assessment – General requirements for proficiency testing*. (https://www.iso.org/standards.html).

7.3.2 All staff must participate in at least two exercises per year, which can include a laboratory replicated testing programme to supplement the external quality assurance programme, if required due to large staffing numbers.

7.3.3 Mechanisms must be in place to ensure staff are trained and have ongoing competency assessment relevant to the organisations standard operating procedures.

7.4 Quality control

7.4.1 The laboratory must have procedures for monitoring the validity of FMH testing performance.

7.4.2 Any equipment used as part of the testing process must be maintained and calibrated.

Abbreviations

ACSQHC	Australian Commission on Safety and Quality in Health Care
ANZSBT	Australian & New Zealand Society of Blood Transfusion
AS	Australian Standard
BCSH	British Committee for Standards in Haematology
BSH	British Society for Haematology
BMI	Body mass index
CE	CE mark
EDTA	Ethylenediaminetetraacetic acid
FMH	Fetomaternal haemorrhage
HbA	Haemoglobin A
HbF	Haemoglobin F
HPFH	Hereditary persistence of fetal haemoglobin
IANZ	International Accreditation New Zealand
ISO	International Organisation for Standardisation
IM	Intramuscular
IU	International units
IV	Intravenous
IVD	In vitro diagnostic device
IVIg	Intravenous immunoglobulin
LIS	Laboratory Information System
NATA	National Association of Testing Authorities
NEQAS	National External Quality Assurance Scheme
NBA	National Blood Authority
NIPT	Non-invasive prenatal testing
NPAAC	National Pathology Accreditation Advisory Council
NZS	New Zealand standard
RANZCOG	Royal Australian and New Zealand College of Obstetricians and Gynaecologists
RCC	Red cell count
RCPA	Royal College of Pathologists of Australasia
RhD Ig	RhD immunoglobulin
TSSC	Transfusion Science Standing Committee

Glossary

Australian Standard (AS)	Precedes document number of standards issued by Standards Australia
British Society for Haematology (BSH)	British Society for Haematology
CE mark	Administrative marking that indicates conformity with health, safety, and environmental protection standards for products sold within the European Union (EU) and European Economic Area (EEA)
International Accreditation New Zealand (IANZ)	New Zealand national accreditation body
International Organization for Standardisation (ISO)	International standard-setting body composed of representatives from national standards bodies
In vitro diagnostic device (IVD)	A medical device is an in vitro diagnostic medical device (IVD) if it is a reagent, calibrator, control material, kit, specimen receptacle, software, instrument, apparatus, equipment or system, whether used alone or in combination with other diagnostic goods for in vitro use
Kleihauer-Betke test	Test used to detect and quantify fetomaternal haemorrhage
Laboratory	The blood bank or pathology laboratory responsible for performing FMH testing
National Association of Testing Authorities (NATA)	Australia's national accreditation body
National Pathology Accreditation Advisory Council (NPAAC)	Australian body that advises the Commonwealth, state and territory health ministers on matters relating to the accreditation of pathology laboratories. NPAAC has a key role in ensuring the quality of Australian pathology services and responsible for the development and maintenance of standards and guidelines for pathology practices
New Zealand standard (NZS)	Precedes document number of standards issued by Standards New Zealand
UK NEQAS	Provider of external quality assurance programmes used to monitor the quality of laboratory testing and reporting

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