Guideline for the prophylactic use of Rh D immunoglobulin in pregnancy care

2024

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Sections

1. Guideline Amendments

When changes are made to this guideline a new version is created.

Changes that are made that do not impact clinical guidance, are recorded as a minor version eg v1.1, v1.2, and changes that are made to clinical guidance, are recorded as a major version eg v2.0, v3.0.

Details of major guideline versions are captured in the Info box below. Should you wish to see publication dates of both major and minor versions, click the (!) button above and select 'Version history and subscription'.

Info Box

Major Version No.	Release Date	Section Updated	Details	Last date of evidence search
v1.0	6 March 2024	Guideline Release	Minor content updates to 2021 version published on the NBA website 28 May 2021	28 September 2021

2. Summary of clinical guidance

Summary of recommendations and expert opinion points

The Expert Reference Group (ERG) developed:

- recommendations (Rs) based on a systematic review, graded as either strong or weak and for or against an intervention
- expert opinion points (EOPs) for guidance that was outside the scope of the systematic review, and for guidance that was amended or carried over from the *Guidelines for the use of Rh (D) immunoglobulin (anti-D) in obstetrics in Australia* (2003) *[1]*. EOPs are based on consensus among the members of the ERG.

A more detailed description is provided in Methodology

Printable Guideline summary for health professionals

Summary of recommendations and expert opinion points		Section
Blood group and antibody screening in all pregnant women		
EOP1	All women should have an ABO / Rh D type and antibody screen performed no later than 10 weeks gestation *[2]*. Rh D positive pregnant women do not require Rh D immunoglobulin.* * If the mother has a weak or variant Rh D type, consult a haematopathologist in regard to interpretation of results and management.	6.1
EOP2	If antibody screening identifies anti-D in an Rh D negative pregnant woman, consideration of clinical history and laboratory findings is required to determine whether the anti-D is likely to be preformed (due to sensitisation) or passive (due to administration of Rh D immunoglobulin in the past 12 weeks).* In cases of likely preformed anti-D antibodies, seek specialist obstetric advice, manage as Rh D sensitised and consider NIPT for fetal Rh D status. * See EOP3	6.1
EOP3	Rh D immunoglobulin should not be given to Rh D negative pregnant women with preformed anti-D antibodies. However, if it is unclear whether the anti-D detected in the mother's blood is preformed (due to sensitisation) or passive (due to administration of Rh D immunoglobulin in the past 12 weeks), the treating clinician should be consulted. If there is continuing doubt, Rh D immunoglobulin should be administered.	6.1
Non-invasive prenatal testing for fetal *RHD* in all Rh D negative pregnant women		
R9	The ERG **recommends** the testing of maternal blood to determine fetal *RHD* genotype in all Rh D negative pregnant women to enable targeted antenatal Rh D immunoprophylaxis.* (*Strong recommendation, high certainty of evidence about the accuracy of the test*) * The ERG's recommendation on the use of NIPT for fetal *RHD* is not a policy statement on funding and supply arrangements for the national provisions of NIPT for blood group genotyping to determine the Rh D status of the fetus. As of February 2024, NIPT for fetal Rh D status is not widely available in Australia. Universal Rh D immunoprophylaxis should be maintained until NIPT is widely accessible. Further details are provided on the NBA website.	6.3
R10	The ERG **recommends** that test sensitivity be at least 99% in order to minimise the number of Rh D positive fetuses being missed by the test. (*Strong recommendation, high certainty of evidence about the accuracy of the test*)	6.3

	Summary of recommendations and expert opinion points	Section
R11	The ERG **recommends** NIPT for fetal *RHD* from 11^{+0} weeks of pregnancy because of higher test accuracy than at earlier weeks. (*Strong recommendation, high certainty of evidence about the accuracy of the test*)	6.3
Targeted immunoprophylaxis in Rh D negative pregnant women		
R6	The ERG **recommends** that antenatal Rh D immunoprophylaxis in Rh D negative pregnant women with no preformed anti-D antibodies be targeted to those predicted to be carrying an Rh D positive fetus, based on NIPT for fetal *RHD*. This applies to both routine and sensitising event immunoprophylaxis, if the result of fetal *RHD* genotyping is available.* (*Strong recommendation, low certainty of evidence about the size of effect*) * See EOP3 and EOP8	6.3
R7	If fetal Rh D status is not available or is uncertain, the ERG **recommends** that antenatal Rh D immunoprophylaxis be offered to Rh D negative pregnant women with no preformed anti-D antibodies. (*Strong recommendation, low certainty of evidence about the size of effect*)	6.3
Routine antenatal immunoprophylaxis in Rh D negative pregnant women		
R1	The ERG **recommends** access to antenatal Rh D immunoglobulin for the prevention of Rh D alloimmunisation in Rh D negative pregnant women with no preformed anti-D antibodies.* (*Strong recommendation, low to very low certainty of evidence about the size of effect*) * See R6	6.1
Routine dosage regimens in Rh D negative pregnant women		
R2	The ERG **recommends** that administration of Rh D immunoglobulin 625 IU at 28 and 34 weeks of pregnancy* continue in Rh D negative pregnant women with no preformed anti-D antibodies unless NIPT for fetal *RHD*^ has predicted that they are not carrying an Rh D positive fetus. The ERG does not currently suggest changing to a single dose of Rh D immunoglobulin 1500 IU. (*Weak recommendation, low to very low certainty of evidence about the size of effect*) * A woman's pregnancy care schedule and clinical discretion may warrant the administration of Rh D immunoglobulin within 2 weeks before or after the recommended 28 and 34 weeks of pregnancy. However, if the second dose of Rh D immunoglobulin is given before 34 weeks and the pregnancy goes beyond the due date, the risk of inadequate anti-D coverage at birth increases. ^ All women should have an ABO / Rh D type and antibody screen performed no later than 10 weeks gestation. Women who are Rh D negative should be retested at 28 weeks unless NIPT for fetal *RHD* has predicted that they are not carrying an Rh D positive fetus. The specimen should be collected before giving prophylactic Rh D immunoglobulin; however, the immunoglobulin can be given before the results are available *[2]*.	6.1
Sensitising event immunoprophylaxis in the first 12 weeks of pregnancy in Rh D negative women		
R3	After the following sensitising events in the first 12 weeks of singleton or multiple pregnancy: miscarriage, termination of pregnancy (after 10 weeks gestation), ectopic	6.2.2

	Summary of recommendations and expert opinion points	Section
	pregnancy, molar pregnancy and chorionic villus sampling, the ERG **recommends** that a dose of Rh D immunoglobulin 250 IU be given to all Rh D negative women with no preformed anti-D antibodies to prevent Rh D alloimmunisation. (*Strong recommendation, very low certainty of evidence about the size of effect*)	
R4	In the setting of termination of pregnancy before 10 weeks of gestation there is **insufficient evidence to suggest** the routine use of Rh D immunoglobulin *[3][4][147].* *(Discretionary [weak] recommendation, expert consensus)*	6.2.2
EOP4	For sensitising events in the first 12 weeks of pregnancy where there is any uncertainty with gestational age, consider Rh D immunoglobulin. Consider ultrasound to confirm gestational age.	6.2.2
R5	In Rh D negative women with an ongoing pregnancy who have uterine bleeding in the first 12 weeks of pregnancy there is **insufficient evidence to support** the routine use of Rh D immunoglobulin. However, where the bleeding is repeated, heavy or associated with abdominal pain or significant pelvic trauma, immunoprophylaxis may be considered in women with no preformed anti-D antibodies. (*Qualified [weak] recommendation, expert consensus*)	6.2.2
EOP5	At all times when Rh D immunoglobulin is being administered for a sensitising event, it should be given as soon as practical within 72 hours. If delayed beyond 72 hours, the dose should be given up to 10 days from the sensitising event, but may have lower efficacy.	6.2.2
EOP6	For repeated sensitising events in the first 12 weeks of pregnancy, there is no evidence to guide practice. Specialist obstetric consultation is advised regarding further administration of Rh D immunoprophylaxis. For new sensitising events a repeated dose of Rh D immunoglobulin may be indicated. For ongoing uterine bleeding alone, a repeat dose of Rh D immunoglobulin (250 IU if during the first 12 weeks and 625 IU if after) may be appropriate after an interval of 6 weeks *[5][6]*.	6.2.2
Sensitising event immunoprophylaxis beyond the first 12 weeks of pregnancy in Rh D negative women		
EOP8	A dose of Rh D immunoglobulin 625 IU should be offered to every Rh D negative woman with no preformed anti-D antibodies, unless NIPT for fetal *RHD* has predicted the fetus to be Rh D negative, to ensure adequate protection against alloimmunisation for the following indications after 12^{+6} weeks of pregnancy: • genetic studies (chorionic villus sampling, amniocentesis and cordocentesis) • abdominal trauma considered sufficient to cause FMH, even if FMH testing is negative • each occasion of revealed or concealed antepartum haemorrhage. Where the woman suffers unexplained uterine pain the possibility of concealed antepartum haemorrhage (and the need for immunoprophylaxis) should be considered • external cephalic version (successful or attempted) • miscarriage or termination of pregnancy.	6.2.3
EOP9	For sensitising events after 20 weeks of pregnancy, the magnitude of FMH should be assessed, and further doses of Rh D immunoglobulin administered if required.* * The first dose of the Rh D immunoglobulin should be given without waiting for the result of the test for FMH. See Point 4.3 of the British Committee for Standards in Haematology *Guidelines for the estimation of fetomaternal haemorrhage [7].* See Dosing of Rh D immunoglobulin following fetomaternal haemorrhage quantitation	6.2.3

	Summary of recommendations and expert opinion points	Section
EOP10	For ongoing uterine bleeding alone beyond 12 weeks gestation a further dose of Rh D immunoglobulin (625 IU) may be appropriate at 6 weekly intervals *[8]*. New sensitising events should be managed with a further dose of Rh D immunoglobulin (625 IU) and assessment of FMH (after 20 weeks or where otherwise indicated) with additional dosing to cover large volume FMH if required (100 IU for each mL of fetal red cells beyond 6 mL).* * See Dosing of Rh D immunoglobulin following fetomaternal haemorrhage quantitation	6.2.3
EOP11	In reference to antenatal sensitising events after 20 weeks of pregnancy and after giving birth, a maternal sample to assess the volume of FMH should be taken before administration of Rh D immunoglobulin. At no time should Rh D immunoglobulin be delayed based on, or pending, the results of testing to quantitate FMH. Between 13 and 20 weeks of pregnancy, the magnitude of FMH may be assessed at clinical discretion.	6.2.3
EOP12	The magnitude of the FMH should be assessed by a method capable of quantifying a haemorrhage of ≥ 6 mL of fetal red cells (equivalent to 12 mL of whole blood). Flow cytometry is accepted as the most accurate quantitative test for FMH and is the method of choice for quantitation if readily available. Where FMH quantitation shows that FMH greater than that covered by the dose already administered has occurred, an additional dose or doses of Rh D immunoglobulin sufficient to provide immunoprophylaxis must be administered as soon as practical within 72 hours.* If delayed beyond 72 hours, the dose should be given up to 10 days from the sensitising event, but may have lower efficacy. * See Dosing of Rh D immunoglobulin following fetomaternal haemorrhage quantitation	6.2.3
EOP13	For large bleeds ≥ 6 mL of fetal red cells (equivalent to 12 mL of whole blood), follow-up testing should be performed on a sample collected 48 hours post intravenous Rh D immunoglobulin administration or 72 hours post intramuscular Rh D immunoglobulin administration, to determine whether further dosing is required. Supplemental Rh D immunoglobulin should be administered if the test for FMH is still positive.* If testing for fetal cells is negative on a follow-up sample, no further testing is required. * See Dosing of Rh D immunoglobulin following fetomaternal haemorrhage quantitation	6.2.3
Targeted immunoprophylaxis in postnatal Rh D negative women		
R8	The ERG currently **recommends** that postnatal Rh D immunoprophylaxis (Rh D immunoglobulin 625 IU) continue to be administered to all Rh D negative women with no preformed anti-D antibodies who have a baby who is predicted to be Rh D positive based on NIPT for fetal *RHD*, or cord blood or neonatal Rh D typing.* The cord blood or neonatal testing should be performed regardless of the results of NIPT for fetal *RHD*, but need not delay administration of Rh D immunoprophylaxis when the fetus has been shown to be *RHD* positive by NIPT testing.	6.3

	Summary of recommendations and expert opinion points	Section
	If the baby is Rh D positive, administer Rh D immunoglobulin even if the NIPT predicted an Rh D negative baby. *(Strong recommendation, high certainty of evidence)* * If the newborn has a weak or variant Rh D type, consult a haematopathologist in regard to interpretation of results and management.	
High body mass index (BMI)		
R12	The ERG **does not currently support** an increased dose of Rh D immunoglobulin or changes in laboratory testing on the basis of high BMI in Rh D negative pregnant women. *(Weak recommendation, very low certainty of evidence about the size of effect)*	6.4
EOP7	Rh D immunoglobulin must be given by deep intramuscular injection. For women with a BMI of more than 30, particular consideration should be given to factors that may affect the adequacy of the injection (e.g. the site of administration and the length of the needle used).	6.4

ABO: ABO blood group system; BMI: body mass index; EOP: expert opinion point; ERG: Expert Reference Group; FMH: fetomaternal haemorrhage; IU: international units; NIPT: non-invasive prenatal testing; R: recommendation.

anti-D - refers to circulating antibodies; *RHD* - refers to genotype; Rh D immunoglobulin - refers to the product; Rh D positive/negative - refers to blood type.

Summary of guidance on the use and timing of pathology testing

Test	Timing	Target group	Section
ABO/Rh D type and antibody screen	First visit (no later than 10 weeks)	All pregnant women	6.1
NIPT for fetal *RHD*	From 11^{+0} weeks of pregnancy	All Rh D negative pregnant women	6.3
Magnitude of FMH*	After 20 weeks of pregnancy At delivery	Rh D negative women following birth or a sensitising event during pregnancy (after 20 weeks)	6.2.3
Rh D type and antibody screen (Retest)	28 weeks (prior to administration of Rh D immunoglobulin)	Rh D negative pregnant women (unless NIPT for fetal *RHD* has predicted that they are not carrying an Rh D positive fetus)	6.1
Cord blood or neonatal testing for Rh D type and direct antiglobulin test	At delivery	All babies of Rh D negative women	6.2.3
Follow up testing for large FMH*	48 hours post IV Rh D immunoglobulin administration (or 72 hours post IM Rh D immunoglobulin administration)	Rh D negative women following FMH ≥ 6 mL of fetal red cells (equivalent to 12 mL of whole fetal blood)	6.2.3

ABO: ABO blood group system; FMH: fetomaternal haemorrhage; IM: intramuscular; IV: intravenous; NIPT: non-invasive prenatal testing;

RHD: refers to genotype; Rh D immunoglobulin: refers to the product; Rh D positive/negative: refers to blood type.

* The magnitude of FMH should be assessed by a method capable of quantifying a haemorrhage of ≥ 6 mL of fetal red cells (equivalent to 12 mL of whole blood). Flow cytometry is accepted as the most accurate quantitative test for FMH and is the method of choice for quantitation if readily available (Refer to EOP12).

Summary of guidance on the use and timing of Rh D immunoglobulin for routine immunoprophylaxis

Clinical Indication	Rh D immunoglobulin dose and timing	Target group	Section
Routine antenatal immunoprophylaxis	625 IU At 28 and 34 weeks of pregnancy	Rh D negative pregnant women with no preformed anti-D antibodies (unless NIPT for fetal *RHD* has predicted that they are not carrying an Rh D positive fetus)	6.1
Routine postnatal immunoprophylaxis	625 IU After giving birth	All Rh D negative women with no preformed anti-D antibodies after giving birth to an Rh D positive baby (based on cord blood or neonatal Rh D typing*). If the baby is Rh D positive, administer Rh D immunoglobulin even if the NIPT predicted an Rh D negative baby. If the baby is Rh D positive and is born preterm, give the postnatal dose even if the birth is within 72 hours of a dose given for routine antenatal immunoprophylaxis or for a sensitising event.	6.2.1

* Cord blood or neonatal testing should be performed regardless of NIPT for fetal *RHD results*.

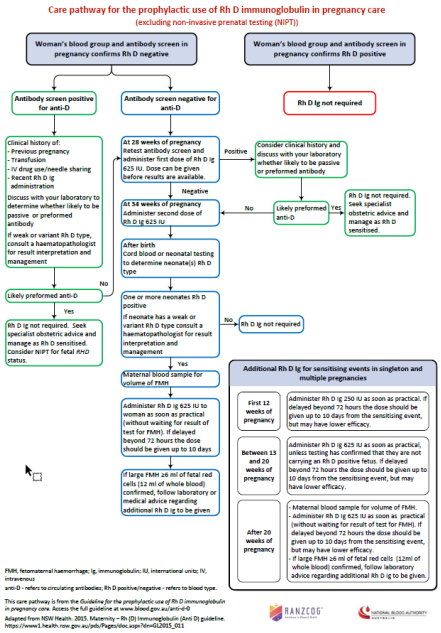
IU: international units; NIPT: non-invasive prenatal testing

anti-D - refers to circulating antibodies; *RHD* - refers to genotype; Rh D immunoglobulin - refers to the product; Rh D positive/negative - refers to blood type.

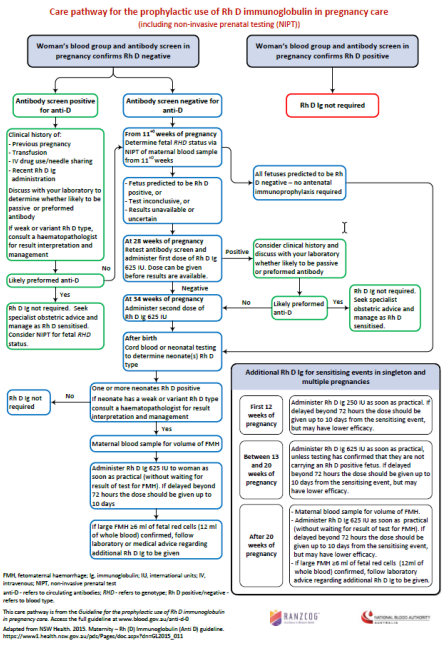
3. Care pathway

The ERG developed two flow charts that outline the care pathway for timing of pathology testing for all pregnant women and administration of Rh D immunoglobulin in Rh D negative pregnant women.

Care pathway for the prophylactic use of Rh D immunoglobulin **excluding non-invasive prenatal testing**



Care pathway for the prophylactic use of Rh D immunoglobulin **including non-invasive prenatal testing**



4. Introduction

Background

Maternal Rh D antibodies may develop during pregnancy when an Rh D negative pregnant woman carries an Rh D positive fetus. Development of antibodies occurs when fetal red blood cells (RBCs) enter the maternal circulation, and antibodies are produced towards the fetal Rh D antigen. The most common sources of fetal RBCs entering the maternal circulation are thought to be small fetomaternal haemorrhages (FMHs) at birth and silent transplacental haemorrhages in the antenatal period *[9][10][11]*. The maternal response to the fetal RBCs is known as 'sensitisation' or alloimmunisation. No apparent adverse health outcomes occur in the mother as a result of this sensitisation; however, haemolytic disease of the fetus and newborn (HDFN) can arise in an Rh D positive fetus (usually in subsequent pregnancies).

HDFN occurs when maternal antibodies cross the placenta into the baby's circulation and mediate destruction of the baby's RBCs. This destruction causes fetal anaemia (a shortage of RBCs, which are required to carry oxygen), and can lead to hyperbilirubinaemia (elevated levels of bilirubin, a waste product of the degraded RBCs) and jaundice (yellowing of the skin and whites of the eyes). In severe cases, the HDFN causes hydrops fetalis (gross oedema or accumulation of fluid leading to fetal death) or kernicterus (a form of brain damage) *[9][11][12]*. In the absence of intervention, HDFN affects 1% of neonates, and is a significant cause of perinatal mortality and morbidity, and long-term disability *[9][10]*.

Rh D immunoglobulin is manufactured from plasma of Rh D negative blood donors who are stimulated to produce elevated levels of anti-D antibodies. It is given to Rh D negative women with no preformed anti-D antibodies (during pregnancy and immediately postpartum) to prevent Rh D alloimmunisation. In Australia, about 17% of blood donors are Rh D negative *[19]*. This blood type is highest in those who are of European origin (16%), less common in those of African origin (7%), and rare in Indigenous peoples and those of East Asian origin (< 1%). In the United Kingdom, it is estimated that 10% of live births are Rh D positive babies delivered to Rh D negative women *[10]*; however, this number may be higher in the Australian setting *[67]*.

Variant (weak and partial) D phenotypes are fairly common (e.g., 0.2% to 1% of populations of European ancestry) and are caused by *RHD* coding region alterations that affect the number and antibody binding characteristics of Rh D sites per cell *[49][112]*. Depending on the test used, *RHD* variants can be responsible for discrepancies between Rh D phenotyping results using serologic reagents and genotyping assays.

Individuals with some *RHD* variants may appear Rh D positive but may still become alloimmunised to Rh D so pregnant women with these variants would benefit from antenatal Rh D immunoprophylaxis (e.g. the DVI found in Caucasians) while individuals with some other *RHD* variants appear Rh D negative but can safely avoid Rh D immunoprophylaxis as they are not at risk for being alloimmunised (eg Asian DEL variant (*RHD*DEL1*) which accounts for approximately 20 to 30% of all Asian individuals who appear to phenotype as Rh D negative *[143]*.

Furthermore, different genotyping assays have variable ability to detect *RHD* variants depending on the method and the genetic target used. Consequently, maternal and fetal *RHD* variants may have implications for the need for Rh D immunoprophylaxis and laboratories offering *RHD* genotyping should be aware of the *RHD* variants found in their population and the impact of these variants on the interpretation of fetal *RHD* genotyping results.

Before Rh D immunoprophylaxis became available in the late 1960s, approximately 16% of women who had given birth to an Rh D positive, ABO compatible baby developed alloantibodies in their first susceptible pregnancy *[9]*. The risk of alloimmunisation increased with the number of susceptible pregnancies. Alloimmunisation can still occur, albeit at a lower rate, if the mother and baby are ABO incompatible and it can still result in severe HDFN *[73]*. Without immunoprophylaxis, the overall risk when considering both ABO compatible and incompatible mother-baby pairs was estimated at about 13%. As a result, in the first two thirds of the 20th century, HDFN was estimated to affect as many as 1 in 100 women, causing death of the fetus or newborn in 20% of first affected and 40% of subsequently affected pregnancies *[9]*.

Clinical trials demonstrated that Rh D immunoprophylaxis given immediately after birth decreases the risk about 10-fold to approximately 1% *[43]*, results supported by observational studies *[38][53]*. Adding antenatal immunoprophylaxis may reduce the risk to about 0.2% *[11]*. As a result of programs of immunoprophylaxis, HDFN has gone from being a leading cause of fetal and neonatal illness and death *[54]* to a very uncommon one.

Although, in the remaining affected pregnancies, life-threatening and disabling consequences of HDFN can usually be prevented by skilled contemporary clinical care *[9][78]*, the burdens of increased diagnostic testing in pregnancy are significant, even if the HDFN is mild. In moderate or severe HDFN the maternal and neonatal burden of investigation and management are substantial, indicating that there is high value in continuing successful programs of prevention.

When anti-D is identified in a positive routine prenatal antibody screening test, it is essential to determine whether this anti-D is preformed (by a maternal immune response to previous exposure to the Rh D antigen) or passive (through the recent administration of Rh D immunoglobulin). This differentiation is important for the appropriate management of the pregnant woman and requires consideration of clinical history and laboratory findings. The clinician responsible for management of the pregnant woman should discuss the antibody screen results with the laboratory if necessary. Routine Rh D immunoprophylaxis should be recommended unless it is certain that the anti-D is preformed *[29]*.

The national prophylaxis program

The National Health and Medical Research Council's (NHMRC's) 1999 *Guidelines on the prophylactic use of Rh D immunoglobulin (anti-D) in obstetrics [95]* were updated by the National Blood Authority (NBA) in 2003 *[1]* with the aim of informing clinicians, other health professionals and policy makers of new recommendations for the staged implementation of full antenatal prophylaxis with Rh D immunoglobulin in Australia. The 2003 guideline *[1]* also included a strategy to enable the staged introduction of antenatal prophylaxis in the short term, while working towards self-sufficiency in the longer term.

Stage 1 of the national program for prophylaxis commenced in November 2002; it covered routine antenatal prophylaxis at 28 and 34 weeks gestation for Rh D negative women without preformed anti-D antibodies having their first baby, and sensitising event prophylaxis for Rh D negative women without preformed anti-D antibodies. During this stage, an imported Rh D immunoglobulin product was used for postnatal prophylaxis.

Stage 2 commenced in January 2005, with routine antenatal prophylaxis at 28 and 34 weeks gestation being extended to all Rh D negative women without preformed anti-D antibodies. During this stage, an imported Rh D immunoglobulin product was still required for postnatal prophylaxis.

Stage 3 commenced in March 2006, with both antenatal and postnatal Rh D prophylaxis being fully supported by Australian-sourced Rh D immunoglobulin.

Clinical need for this guideline

Key Australian guidance has been published since 2003, including two publications from 2015: *Guidelines for the use of Rh (D) immunoglobulin (anti-D) in obstetrics in Australia [124]* and *Expert panel consensus position statement regarding the use of Rh(D) immunoglobulin in patients with a body mass index ≥30 [14]*.

In September 2016, the NBA commenced a scoping exercise to identify clinical guidance published since the release of the 2003 guideline *[1]*. The aim was to ensure that Australia's clinical guidance and antenatal prophylaxis program still reflect current evidence and best clinical practice.

The scoping exercise found a number of international guidelines on the prophylactic use of Rh D immunoglobulin that had been published since 2003 *[8][34][41][44][45][51][55][99][97][126][136]*. However, the recommendations for application and administration of Rh D immunoglobulin within this guidance was not consistent. This is discussed in Appendix 1 of the technical report *[16]*.

The exercise also found that the 2003 guideline *[1]* did not address a number of issues that have emerged since

publication; for example, alternative dosage regimens, NIPT for fetal *RHD* and the use of Rh D immunoglobulin in women with high BMI.

These findings were shared with the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG), and it was agreed that the NBA and RANZCOG should collaborate to develop a new evidence-based guideline.

A multidisciplinary ERG with expertise from a range of clinical settings was established to identify the key issues that should be investigated for a new evidence-based guideline on the prophylactic use of Rh D immunoglobulin in pregnancy care. The following key issues were identified *[16]*:

1. Does the available evidence still support universal routine antenatal prophylaxis?
2. Should universal routine antenatal prophylaxis be moved from a two-dose regimen to a one-dose regimen?
3. Should the list of sensitising events in the first 12 weeks of pregnancy be amended to include additional events?
4. To reduce unnecessary use of Rh D immunoglobulin, should non-invasive prenatal screening be used in the first trimester so that prophylaxis can be targeted?
5. Does increasing BMI impact on the efficacy of Rh D immunoglobulin?

Intent of the guideline

The intent of the guideline is to provide updated clinical guidance on the prophylactic use of Rh D immunoglobulin in pregnancy care in accordance with current evidence and consensus among clinical experts. It is targeted at health care professionals involved in the management of pregnant Rh D negative women.

Structure of the guideline

This guideline contains:

- a summary of the clinical guidance, in the form of recommendations (Rs) and expert opinion points (EOPs) (Summary of clinical guidance)
- flow charts illustrating the alternative care pathways (Care pathway)
- the background to the current antenatal prophylaxis program, the clinical need for this document and guidance transferred from the 2003 guidelines*[1]* (Introduction)
- a summary of the systematic review methodology and the process used to translate evidence into clinical guidance (Methodology)
- the clinical guidance developed by the ERG (Clinical guidance)
- cost, supply and safety considerations and challenges
- monitoring the use of Rh D immunoglobulin, implementing the guideline, governance arrangements and terminology

The clinical guidance consists of two layers:

1. Recommendations and expert opinion points

The process of developing recommendations and expert opinion points is described in Methodology

2. Supporting information

Under each recommendation there are several tabs which contain information that supports the recommendation. These are outlined below:

Research evidence tab: Contains a summary of the evidence used to make the recommendation. Each recommendation may have a different number of options depending on the number of comparators assessed in the systematic review. The evidence for the intervention versus each comparator is presented in outcomes, graphical view and summary.

- **Outcomes:** a tabular view of the overall effect estimates for each outcome assessed in the systematic review. For further information or a detailed description of the outcome, study results of certainty of the evidence, click on the eye icon in the top right-hand corner of the relevant cell.
- **Graphical view:** graphical representation of the effect of the intervention versus comparator for each outcome.
- **Summary:** overview and brief review of the underlying evidence.

Evidence to decision tab: Gives a summary of the factors that the ERG considered relevant under each GRADE domain:

- benefits and harms
- certainty of the evidence
- values and preferences
- resources and other considerations

Rationale tab: Describes the question and rationale for the literature search.

Practical information tab: Provides information for health professionals to implement the recommendation including recommended doses, timing and monitoring.

Feedback tab: If you are logged into MAGIC as a user, you can comment here on specific recommendations. Your feedback will be entered into a feedback register maintained by the NBA.

References tab: Lists the studies used to develop the recommendation.

Related material

The technical report that underpins this document is available from the NBA website in three volumes:

- Volume 1 contains background information and the results of the systematic reviews pertaining to the clinical questions posed within this guideline *[16]*
- Volume 2 contains appendixes that document the literature searches and critical appraisal of the studies *[17]*
- Volume 3 presents additional literature published and identified after the initial systematic literature review *[18]*

Disclaimer

This document is a general guide to appropriate practice, to be followed subject to the circumstances, clinician's judgement and a woman's preference in each individual case. It is designed to provide information to assist decision making. Recommendations contained herein are based on the best available evidence published up to 27-28 September 2021. The relevance and appropriateness of the information and recommendations in this document depend on the individual circumstances.

Moreover, the recommendations and guidelines are subject to change over time. Each of the parties involved in developing this document expressly disclaims and accepts no responsibility for any undesirable consequences arising from relying on the information or recommendations contained herein.

Acknowledgements and endorsements

This guideline was developed by a multidisciplinary Expert Reference Group (ERG) with expertise from a range of clinical settings. Membership of the ERG included representation of the following clinical colleges and organisations:

- Australian and New Zealand Society of Blood Transfusion
- Australian College of Midwives

- Australian Red Cross Lifeblood
- Royal Australian College of General Practitioners
- Royal Australasian College of Physicians
- Royal Australian and New Zealand College of Obstetricians and Gynaecologists
- Royal College of Pathologists of Australasia

The National Blood Authority gratefully acknowledges the expertise and clinical input provided by the ERG. Membership of the ERG is provided at Governance.

Endorsement of this guideline from clinical colleges and societies can be found at www.blood.gov.au.

The development of the *Guideline for the prophylactic use of Rh D immunoglobulin in pregnancy care* was a joint project between the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) and the National Blood Authority, Australia (NBA). The NBA provided project management oversight and funded all goods and services associated with the development of this guideline. The development of the clinical guidance was not influenced by the views or interests of the funding body.

5. Methodology

These evidence-based clinical practice guidelines were developed by following the principles proposed by the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) Working Group. The process involved developing a set of research questions, systematically reviewing the scientific literature for evidence related to those questions, and then developing and grading recommendations based on a structured assessment of the evidence. The methods used to apply this process are outlined in this section and are given in full in the accompanying technical reports, which present in detail the methodology used to identify the evidence base (clinical questions addressed, systematic literature search undertaken, and inclusion and exclusion criteria described), the characteristics and quality of the evidence base (data extraction and risk of bias forms), and detailed results presented by outcome (evidence summary tables and GRADE profiles) *[17][16][18]*.

The systematic review process was based on that described in the *Cochrane handbook for systematic reviews of interventions [123]*. Covidence, a web-based platform for producing systematic reviews was used to store data that are compatible with the Cochrane data collection tools. RevMan *[26]* was used for the main analyses, and GRADEpro GDT software was used to record decisions and derive an overall GRADE (high, moderate, low or very low) for the certainty of evidence for each outcome.

Question development

Between September 2016 and October 2017, relevant clinical research questions for these guidelines were identified, developed and prioritised by a multidisciplinary ERG, working with an independent systematic review expert and the NBA *[31]*. The four main clinical questions (and two subquestions) chosen for evidence review are listed below, and were structured according to PICO (population, intervention, comparator and outcome) criteria.

Systematic review questions

Question 1 – In Rh D negative pregnant women with no preformed anti-D, does universal routine antenatal prophylaxis with Rh D immunoglobulin (one or two doses) prevent Rh D alloimmunisation?

Question 1 (subquestion) – In Rh D negative pregnant women with no preformed anti-D, is universal routine antenatal prophylaxis with one dose of Rh D immunoglobulin as effective at preventing Rh D alloimmunisation as universal routine prophylaxis with two doses of Rh D immunoglobulin?

Question 2 – In Rh D negative women with no preformed anti-D who have experienced one of the following first trimester sensitising events – abdominal trauma, molar pregnancy, ectopic pregnancy, spontaneous miscarriage, threatened miscarriage or medical termination of pregnancy (with or without a curette), does universal first trimester sensitising event prophylaxis with Rh D immunoglobulin prevent Rh D alloimmunisation?

Question 3 – In Rh D negative pregnant women with no preformed anti-D, does targeted routine antenatal or sensitising event prophylaxis to women with an Rh D positive fetus increase the incidence of Rh D alloimmunisation compared with universal routine antenatal or sensitising event prophylaxis?

Question 3 (subquestion) – In Rh D negative pregnant women with no preformed anti-D, what is the diagnostic accuracy of non-invasive prenatal screening to identify fetal Rh D status?

Question 4 – In Rh D negative pregnant or postpartum women with no preformed anti-D, does increasing BMI increase the risk of failure of anti-D administration?

A research protocol was then developed that described the methodology used to source the clinical evidence (a systematic search of the literature), select the best available evidence, critically appraise and present the evidence, and determine the quality of the evidence base for each question, using a structured assessment of the body of evidence in accordance with GRADE methodology *[116]*.

Systematic review process

To identify the evidence base for the four clinical questions, a systematic search of published medical literature was conducted. Characteristics of the ideal evidence base specific to each question were based on guidance from the NHMRC *[96]*. A systematic review of randomised controlled trials (RCTs) was considered the highest level of evidence for all question types. The review considered peer-reviewed, unpublished and grey literature. Ongoing trials and studies published as abstracts only were also included if they provided sufficient information for the outcome of interest.

The systematic review was conducted using a stepped process in which the highest level of evidence was assessed before lower levels of evidence were considered. Further assessment down to noncomparative interventional studies or case series was not conducted for any research question, irrespective of whether sufficient higher-level evidence was found to address all critical and important outcomes for that question. This is because it is difficult (if not impossible) to attribute observed changes in outcomes at this level.

Literature search

The medical literature was searched on 19-20 July 2018 and again on 27-28 September 2021.

The search strategy was developed in Ovid (for Embase and Medline), based on key elements provided in the research questions. The primary databases searched were Embase, Medline, CINAHL Plus, the Cochrane Library and PubMed (limited to in-process citations and citations not indexed in Medline). Additional searches were conducted on clinical trial registries, health technology assessment and guideline websites (e.g. the National Institutes of Health and Care Excellence), and literature sources recommended by expert members of the ERG. Details of the systematic literature search are provided in Volumes 2 and 3 of the technical report *[17][18]*.

The search strategy was not limited by language; however, publications in languages other than English were only considered where a full text translation into English was available. No date or geographic limitations were applied when conducting the search. A literature search start date of 2002, defined by the ERG for Question 1, was applied once citations had been imported into the bibliographic management database.

Study selection

All potentially relevant studies were identified after applying prespecified inclusion and exclusion criteria, as outlined in Volume 1 of the technical report *[16]*. The study selection process was completed by one systematic reviewer, with a second reviewer crosschecking the screening process to ensure adherence to the prespecified exclusion criteria. Any differences were resolved by discussion with a third reviewer (with advice sought from the ERG as necessary) to confirm study eligibility.

Briefly, Questions 1-3 included *pregnant* women who were Rh D negative and did not have preformed anti-D antibodies. The focus of these questions was *antenatal prophylaxis* (i.e. during pregnancy) with Rh D immunoglobulin. Question 4 included women who were Rh D negative with no preformed anti-D antibodies receiving prophylaxis either *during pregnancy* or *postpartum* (after the birth of an Rh D positive baby). There were no restrictions on the product type, mode of administration, number of doses or dosage.

There were no limits to age, race or nationality, but studies were to be set in countries with health systems broadly comparable to those in Australia,* especially in terms of the health care facilities and resourcing. Studies set in low or middle-income countries were identified for consideration by the ERG; however, unless there was additional information demonstrating that the population or setting was comparable to Australia, these studies were excluded.

For Question 3, to provide *targeted* prophylaxis, identification of an Rh D positive fetus is required. The prenatal tests were to be non-invasive (i.e. a simple blood test that uses maternal blood to determine the fetal Rh D status), but there were no restrictions on the timing, product type or testing methodology.

The critical outcome measure for all questions was the incidence of Rh D alloimmunisation. Additional data to be extracted related to timing of the event (i.e. during pregnancy, postpartum or in subsequent pregnancies). Other outcome measures included the incidence of a positive test for FMH (any test that detected fetal cells in the maternal blood), utilisation rates of Rh D immunoglobulin and any adverse event (mild, moderate or severe).

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* For example, Canada, Europe, New Zealand, the United Kingdom and the United States of America.

Strengths and limitations of the evidence

The methodological quality of included studies was assessed, and relevant data were extracted into data extraction tables by one systematic reviewer. For each study, the most appropriate risk of bias assessment tool (based on

study design) was used *[64][117][116]*, with a summary judgement provided in relation to the clarity and completeness of reporting, methods and processes, as well as the underlying assumptions and limitations. Available effect estimates (95% confidence intervals [CI], *p*-values) were presented in tables structured by PICO criteria and study design. These data were then crosschecked by a second reviewer and summarised into appropriate categories or subquestions, according to the key research question.

GRADE evidence profiles were then developed for each comparison and outcome, with relevance to the Australian context considered at the time. As per GRADE guidance *[116]*, the body of evidence was consolidated and rated across five key domains:

- *risk of bias* – based on the summary assessment across studies for each outcome reported for a comparison
- *inconsistency* – based on heterogeneity in the observed intervention effects across studies that suggests important differences in the effect of the intervention, and whether this can be explained
- *imprecision* – based on interpretation of the upper and lower confidence limits, and whether the intervention has a clinically important effect
- *indirectness* – based on important differences between the review questions and the characteristics of included studies that may lead to important differences in the intervention effects
- *publication bias* – based on the extent to which the evidence is available; such bias would be suspected when the evidence is limited to a small number of small trials.

For each domain, a judgement was made about whether there were *serious, very serious* or *no concerns*, resulting in an overall grade (high, moderate, low or very low) for the certainty of evidence for each outcome.

GRADE certainty of evidence
High certainty (⊕⊕⊕⊕): We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty (⊕⊕⊕⊖): We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty (⊕⊕⊖⊖): Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low certainty (⊕⊖⊖⊖): We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

Scoring of the certainty of the evidence began as 'high' for randomised trials (score=4) and was downgraded by –1 for each domain with serious concerns, or –2 for very serious concerns, with observational studies being a 'low'. Footnotes were used to record judgements made by the ERG about downgrading (or upgrading) of the evidence. Further information is detailed in Volume 2 of the technical report *[17]*.

Formulating recommendations

A consensus process (see Process report) was used to ensure that the clinical guidance was consistent with the evidence presented. GRADE evidence profiles and summaries of findings were used to inform translation of the evidence into recommendations for use in Clinical guidance. Evidence-to-decision tables provided in the GRADEpro GDT software were used to guide this process *[66]*.

Recommendations were based on four key concepts: balance of benefits and risks, values and preferences, resource use and quality of evidence. Recommendations were carefully worded to ensure that the recommended action was clear. Definitions of the strength and direction of recommendations are set out below.

Definition of the strength of recommendations

Strong recommendation (for an action) – the ERG is confident that the desirable effects of an intervention outweigh its undesirable effects.

Weak recommendation (for an action) – the desirable effects *probably* outweigh the undesirable effects (for an intervention) but appreciable uncertainty exists. Recommendation is influenced by a woman's values, resources available and/or setting.

Weak recommendation (against an action) – the undesirable effects *probably* outweigh the desirable effects, but appreciable uncertainty exists. Recommendation is influenced by a woman's values, resources available and/or setting.

Discretionary (weak) recommendation – the desirable effects *probably* outweigh the undesirable effects (for an intervention), but appreciable uncertainty exists. Action may be discretionary based on opinion of a woman or practitioner.

Qualified (weak) recommendation – the desirable effects *probably* outweigh the undesirable effects (for an intervention) but appreciable uncertainty exists. An explanation regarding the issues that would lead to different decisions is offered.

Expert Opinion Points – developed by the ERG through a consensus process where there was insufficient quantity or certainty of evidence to develop evidence-based recommendations, or in areas not subject to a systematic review, but where it was considered important to offer guidance.

The recommendations and EOPs were reviewed by the ERG in December 2021 following an update of the literature searches in September 2021. The updated evidence base did not result in any material changes to the recommendations or EOPs.

6. Clinical guidance

6.1 Routine antenatal Rh D immunoprophylaxis

Expert opinion point

EOP1: All women should have an ABO / Rh D type and antibody screen performed no later than 10 weeks gestation *[2]*. Rh D positive pregnant women do not require Rh D immunoglobulin.*

**If the mother has a weak or variant Rh D type, consult a haematopathologist in regard to interpretation of results and management.*

Strong recommendation , Low certainty evidence

R1: The ERG **recommends** access to antenatal Rh D immunoglobulin for the prevention of Rh D alloimmunisation in Rh D negative pregnant women with no preformed anti-D antibodies.*

**See R6 (recommendation for targeted immunoprophylaxis)*

Evidence to decision

Benefits and harms

Reducing the incidence of Rh D alloimmunisation is important because it is the most critical intermediate step for reducing the incidence of HDFN (and the consequent risk of serious fetal or neonatal morbidity or death). This also protects the woman from the need for invasive treatments that are needed if HDFN causes significant anaemia in an Rh D positive fetus as well as potential clinical complications that affect her own health. The intervention has an excellent safety record, with most errors associated with Rh D immunoglobulin related to omission or late administration *[28][37][58]*.

A two-dose regimen may offer compliance benefits in comparison to single-dose regimen. A potential secondary benefit is that an Rh D negative pregnant woman may, because of the need for a second dose at 34 weeks of pregnancy, have an increased incentive to attend antenatal appointments later in her pregnancy.

See Safety of Rh D immunoglobulin

Certainty of the Evidence

Low

Although the comparative evidence for third trimester RAADP (one or two doses) is of low to very low certainty, large population studies on the incidence of Rh D alloimmunisation show a reduction in risk following the introduction of this intervention.

There is evidence that the incidence of FMH of sufficient size to cause Rh D alloimmunisation is higher in the third trimester than earlier in pregnancy *[130]*. Antenatal immunoprophylaxis reduces the incidence of a subsequent positive test for FMH (moderate certainty of evidence), suggesting a reduced risk of Rh D alloimmunisation through effective removal of fetal red cells by the passive anti-D antibodies.

There was no conclusive evidence to suggest that a single dose of Rh D immunoglobulin (1500 IU) given

at 28 weeks of pregnancy is superior or inferior to a two-dose regimen (500 IU to 625 IU) given at 28 and 34 weeks of pregnancy in terms of efficacy or safety.

Values and preferences

Recent literature and international guidelines support the indications for, and the dosing of, Rh D immunoprophylaxis. However, maintenance of supply of Rh D immunoglobulin is a global issue. Boosting donors to maintain the supply of Rh D immunoglobulin poses potential clinical risks that raise ethical concerns, it also places a considerable burden on those donors.

A single injection at 28 weeks of pregnancy would reduce the burden on women and their caregivers by removing the need for a second injection at 34 weeks of pregnancy. However, the transition from two Rh D immunoglobulin doses of 625 IU (totalling 1250 IU) to a single Rh D immunoglobulin dose of 1500 IU would require an additional 250 IU of Rh D immunoglobulin per Rh D negative pregnancy. The requirement for additional product would place an increased burden on the donor pool, particularly on the small number of donors with high levels of anti-D antibodies.

See Challenges - Consent and the choice to decline Rh D immunoglobulin

Resources and other considerations

Costs associated with caring for Rh D alloimmunised women and their babies can be avoided with prophylactic administration of antenatal Rh D immunoglobulin. Routine antenatal immunoprophylaxis with Rh D immunoglobulin in Rh D negative women with no preformed anti-D antibodies has been available in Australia since the staged introduction of the national prophylaxis program started in 2003. The resources and costs associated with this program are considered reasonable *[95]*. The logistics of implementing a single dose of Rh D immunoglobulin 1500IU would require the supplier to manufacture and license a new product suitable for Australia. Any increased dose of Rh D immunoglobulin could potentially place an increased burden on the donor panel.

See Cost considerations

See Supply considerations

Rationale

Question 1 – (Intervention)

In Rh D negative pregnant women with no preformed anti-D, does universal routine antenatal prophylaxis with Rh D immunoglobulin (one or two doses) prevent Rh D alloimmunisation?

Subquestion 1 – (Intervention)

In Rh D negative pregnant women with no preformed anti-D, is universal routine antenatal prophylaxis with one dose of Rh D immunoglobulin as effective at preventing Rh D alloimmunisation as universal routine prophylaxis with two doses of Rh D immunoglobulin?

Rh D immunoglobulin is given antenatally and immediately postpartum to prevent Rh D alloimmunisation in Rh D negative pregnant women with no preformed anti-D antibodies. The literature search for this question aimed to establish whether administration of Rh D immunoglobulin should be routine in the third trimester of pregnancy, and whether one dose at 28 weeks of pregnancy is as effective as two smaller doses at 28 and 34 weeks of pregnancy. The review examined third trimester routine antenatal anti-D prophylaxis (RAADP) in

either one or two doses, looking at the effect on detectable FMHs, HDFN and Rh D alloimmunisation during pregnancy, after birth or in a subsequent pregnancy.

Clinical question/ PICO

Population: Rh D negative pregnant women with no preformed anti-D
Intervention: Universal routine antenatal Rh D immunoprophylaxis (one or two doses)
Comparator: no universal routine antenatal Rh D immunoprophylaxis

Summary

One or two doses versus placebo or no routine antenatal Rh D immunoprophylaxis

Five systematic reviews *[10][11][108][129][140]* were included that evaluated the effectiveness of RAADP in Rh D negative women. The reviews identified two RCTs *[65][76]* and nine nonrandomised studies *[39][48][40][63][85][88][105][127][128]* meeting the search criteria. One additional nonrandomised study *[74]* was identified in this review.

The primary studies used to inform on the effectiveness of routine antenatal immunoprophylaxis each varied with regards to the total dose of Rh D immunoglobulin administered (ranging from 500 IU to 3000 IU) and the timing of outcome measurement; therefore, several analyses were conducted to assess the implications for effectiveness. Many of the included studies had problems with study design, with concerns in relation to the comparability of treatment groups and missing data, and thus may overestimate the degree of protection provided by RAADP.

One-dose versus two-dose routine antenatal Rh D immunoprophylaxis

Three of the included systematic reviews *[11][108][129]* searched for head-to-head comparisons of one-dose versus two-dose RAADP regimes. None of the reviews identified any published evidence. In the absence of evidence, Pilgrim et al. (2009) *[108]* and Turner et al. (2012) *[129]* provided assessments based on expert opinion. McBain et al. (2015) *[11]* noted an ongoing RCT that compared a one-dose versus two-dose regime of RAADP, with the primary outcomes being detectable anti-D antibodies at birth and compliance. Preliminary results *[106]* of this study were considered by the ERG; with the published results *[134]* included in the 2021 update.

Incidence of Rh D alloimmunisation

One or two doses, any timepoint

The meta-analyses of the two available RCTs *[65][76]* demonstrated a nonsignificant effect favouring third trimester routine antenatal administration of Rh D immunoprophylaxis *[11]*. The study by Lee and Rawlinson (1995) *[76]* used a lower dose (250 IU at 28 and 34 weeks gestation) than is currently used in the Australian context (625 IU at 28 and 34 weeks gestation). The meta-analyses reported by Xie et al. (2020) *[140]*, Turner et al. (2012) *[129]*, Pilgrim et al. (2009) *[108]* and Chilcott et al. (2003) *[10]* each showed an effect favouring RAADP, regardless of dose or timing of outcome measurement when compared with no RAADP. Turner et al. (2012) *[129]*, estimated the odds of Rh D alloimmunisation (during pregnancy, at birth or in a subsequent pregnancy) to be 0.31 (95% CI 0.17, 0.56), after adjusting for internal biases related to study design (e.g. women selection, performance, attrition and outcome measurement) and external biases related to Rh D immunoprophylaxis (as rated by four assessors).

A meta-analysis of the eight nonrandomised studies and the two RCTs revealed a significant effect favouring RAADP (any dose, any timepoint) compared with no RAADP for the incidence of Rh D alloimmunisation (RR 0.33; 95% CI 0.20, 0.53; $p < 0.00001$), but significant heterogeneity between

studies was noted (I^2 = 70%).

One or two doses, timing of outcome measurement

The included primary studies measured the incidence of Rh D alloimmunisation at varying timepoints including those detected in a subsequent pregnancy, during pregnancy, at birth or within 3 days of delivery, or at postnatal follow-up. When assessed in a subsequent pregnancy (up to the first 12 weeks of pregnancy), a significant effect favouring RAADP (RR 0.43; 95% CI 0.31, 0.59; p < 0.00001; I^2 = 0%) was observed. In contrast, when Rh D alloimmunisation was detected during pregnancy, the effect was nonsignificant (RR 0.33; 95% CI 0.08, 1.37; p = 0.13; I^2 = 78%). The risk reduction associated with RAADP decreased over time, in a large part because fewer women in the control group were sensitised in the later studies. Explanations for the observed decrease are conjectural but may reflect changes in pregnancy care over time not directly related to Rh D management.

An effect favouring RAADP was also observed among the eight studies that assessed the incidence of Rh D alloimmunisation at birth or within three days of delivery (RR 0.19; 95% CI 0.08, 0.45; p = 0.0001; I^2 = 57%), and in the seven studies that assessed the incidence of Rh D alloimmunisation at postnatal follow-up (RR 0.19; 95% CI 0.13, 0.29; p < 0.00001; I^2 = 0%).

Incidence of a positive test for FMH

One RCT *[65]* found that a positive Kleihauer result was reported less often in women who received RAADP both during pregnancy (4.2% vs 7.0%; RR 0.60; 95% CI 0.41, 0.88; p = 0.0094) and at birth of an Rh D positive baby (12.2% vs 20.2%; RR 0.60; 95% CI 0.46, 0.79; p = 0.00023) when compared with women who did not receive RAADP. No between-group difference was observed for the number of women with a Kleihauer result of greater than one fetal red cell in 10 000 maternal red cells (5.2% vs 5.4%; RR 0.95; 95% CI 0.89, 1.54; p = 0.85).

Adverse neonatal events

One RCT *[65]* and three observational studies *[40][127][74]* provided limited data on adverse neonatal events relating to RAADP. Huchet et al. (1987) *[65]* reported one case of neonatal jaundice among neonates born to Rh D negative women who had received RAADP, compared with four cases among neonates born to women who had not received RAADP (0.11% vs 0.42%; RR 0.26; 95% CI 0.03, 2.30; p = 0.22).

Both Tovey et al. (1983) *[127]* and Bowman and Pollock (1987) *[40]* reported several cases of treatment related to HDFN (either in a first or subsequent pregnancy) among Rh D negative women who had not received RAADP, but data relating to this outcome among the women who received RAADP were not reported.

Using case-finding from comprehensive laboratory records of women with Rh D alloantibodies, Koelewijn et al. (2008) *[74]* calculated the prevalence of severe HDFN in their second ongoing pregnancies among Rh D negative women whose first pregnancy was after 1999 (when routine RAADP [intervention] was offered) compared with those whose first pregnancy was before 1999 (before the introduction of RAADP in 1998). The study reported an incidence of severe HDFN of 0.1% if the first pregnancy had occurred in the epoch when RAADP was routinely available compared with 0.23% among the historical controls, correlating to a nonsignificant risk reduction of 0.55% (RR 0.45; 95% CI 0.10, 1.08, p = NR). However, when they excluded cases in which the history of postnatal and antenatal immunoprophylaxis was unknown, an effect favouring RAADP was observed (RR 0.51, 95% CI 0.9, 0.92; p = NR). No HDFN perinatal mortality was reported in either group. Unsurprisingly, once Rh D alloimmunisation had occurred, the risk of developing HDFN was the same in the intervention and control groups (19% vs 25%; RR 0.76; 95% CI 0.41, 1.42, p = NR).

Adverse maternal events attributed to Rh D immunoglobulin administration

None of the identified studies reported any adverse maternal events that could be attributed to administration of Rh D immunoglobulin.

Outcome Timeframe	**Study results and measurements**	**Comparator** risk with placebo or no universal RAADP	**Intervention** risk with universal RAADP (one or two doses)	**Certainty of the Evidence** (Quality of evidence)	**Summary**
Incidence of Rh D alloimmunisation (RCTs) [1] any timepoint 9 Critical	Relative risk 0.39 (CI 95% 0.09 — 1.63) Based on data from 2,297 participants in 2 studies. [2] (Randomized controlled) Follow up: any timepoint.	14 per 1000 Difference:	5 per 1000 **9 fewer per 1000** (CI 95% 13 fewer — 9 more)	Low Due to serious risk of bias, Due to serious inconsistency, Due to serious imprecision. [3]	In Rh D negative women with no preformed anti-D, universal RAADP may reduce the incidence of Rh D alloimmunisation (1 or 2 doses, any timepoint) but we are uncertain about the size of the effect.
Incidence of Rh D alloimmunsation [4] any timepoint 9 Critical	Relative risk 0.31 (CI 95% 0.18 — 0.54) Based on data from 51,987 participants in 8 studies. [5] (Observational (non-randomized))	11 per 1000 Difference:	3 per 1000 **8 fewer per 1000** (CI 95% 9 fewer — 5 fewer)	Very low Due to serious risk of bias, Due to serious inconsistency, Upgraded due to clear dose-response gradient [6]	In Rh D negative women with no preformed anti-D, universal RAADP may reduce the incidence of Rh D alloimmunisation (1 or 2 doses, any timepoint) but we are uncertain about the size of the effect.
Incidence of Rh D alloimmunisation in subsequent pregnancy 6 Important	Relative risk 0.43 (CI 95% 0.31 — 0.59) Based on data from 31,826 participants in 6 studies. [7] (Observational (non-randomized))	8 per 1000 Difference:	3 per 1000 **5 fewer per 1000** (CI 95% 6 fewer — 3 fewer)	Low Due to serious risk of bias, Upgraded due to clear dose-response gradient [8]	In Rh D negative women with no preformed anti-D, universal RAADP may reduce the incidence of Rh D alloimmunisation (in a subsequent pregnancy) but we are uncertain about the size of the effect.
Incidence of Rh D alloimmunisation during pregnancy 6 Important	Relative risk 0.33 (CI 95% 0.08 — 1.37) Based on data from 28,357 participants in 4 studies. [9] (Observational (non-randomized))	6 per 1000 Difference:	2 per 1000 **4 fewer per 1000** (CI 95% 6 fewer — 2 more)	Very low Due to serious risk of bias, Due to serious inconsistency, Due to serious imprecision [10]	In Rh D negative pregnant women with no preformed anti-D, universal RAADP may reduce the incidence of Rh D alloimmunisation (during pregnancy) but we are very uncertain about the size of the effect.
Incidence of Rh D	Relative risk 0.19 (CI 95% 0.08 — 0.45)	14	3	Very low Due to serious	In Rh D negative pregnant women with

Outcome Timeframe	**Study results and measurements**	**Comparator** risk with placebo or no universal RAADP	**Intervention** risk with universal RAADP (one or two doses)	**Certainty of the Evidence** (Quality of evidence)	**Summary**
alloimmunisation at birth of Rh D positive newborn or within 3 days of delivery 6 Important	Based on data from 24,622 participants in 8 studies. [11] (Observational (non-randomized))	per 1000 Difference:	per 1000 **11 fewer per 1000** (CI 95% 13 fewer — 8 fewer)	risk of bias, Due to serious inconsistency, Upgraded due to clear dose-response gradient [12]	no preformed anti-D, universal RAADP may reduce the incidence of Rh D alloimmunisation (at birth or within three days of delivery of an Rh D positive newborn) but we are very uncertain about the size of the effect
Incidence of Rh D alloimmunisation up to 12 months postnatal follow-up 6 Important	Relative risk 0.19 (CI 95% 0.13 — 0.29) Based on data from 17,372 participants in 7 studies. [13] (Observational (non-randomized))	15 per 1000 Difference:	3 per 1000 **12 fewer per 1000** (CI 95% 13 fewer — 11 fewer)	Low Due to serious risk of bias, Upgraded due to clear dose-response gradient [14]	In Rh D negative pregnant women with no preformed anti-D, universal RAADP may reduce the incidence of Rh D alloimmunisation (up to 12 months after the birth of an Rh D positive newborn) but we are uncertain about the size of the effect.
Incidence of a positive test for FMH [15] assessed with: Kleihauer test at 32 to 35 weeks of pregnancy 3 Not Important	Relative risk 0.6 (CI 95% 0.41 — 0.88) Based on data from 1,884 participants in 1 studies. [16] (Randomized controlled)	70 per 1000 Difference:	42 per 1000 **28 fewer per 1000** (CI 95% 41 fewer — 8 fewer)	Moderate Due to serious risk of bias [17]	in Rh D negative pregnant women with no preformed anti-D, universal RAADP (1 or 2 doses) likely reduces the incidence of a positive test for FMH (assessed at 32-35 weeks of pregnancy).
Incidence of a positive test for FMH [18] assessed with: Kleihauer test at birth of Rh D positive newborn 3 Not Important	Relative risk 0.6 (CI 95% 0.46 — 0.79) Based on data from 1,189 participants in 1 studies. [19] (Randomized controlled)	202 per 1000 Difference:	121 per 1000 **81 fewer per 1000** (CI 95% 109 fewer — 42 fewer)	Moderate Due to serious risk of bias [20]	In Rh D negative women with no preformed anti-D, universal RAADP (1 or 2 doses) likely reduces the incidence of a positive test for FMH (assessed at birth of an Rh D positive newborn)
Adverse neonatal events (jaundice) 3 Not Important	Relative risk 0.26 (CI 95% 0.03 — 2.3) Based on data from 1,882 participants in 1 studies. [21] (Randomized controlled)	4 per 1000 Difference:	1 per 1000 **3 fewer per 1000** (CI 95% 4 fewer — 5 more)	Low Due to serious risk of bias, Due to serious imprecision [22]	In Rh D negative women with no preformed anti-D, the effect of universal RAADP (1 or 2 doses) on neonatal jaundice is uncertain.

Outcome Timeframe	**Study results and measurements**	**Comparator** risk with placebo or no universal RAADP	**Intervention** risk with universal RAADP (one or two doses)	**Certainty of the Evidence** (Quality of evidence)	**Summary**
Adverse neonatal events (prevalence of severe HDFN) [23] (perinatal mortality, need for IUT and/or exchange transfusion) 6 Important	Relative risk 0.51 (CI 95% 0.09 — 0.92) Based on data from 21,221 participants in 1 studies. [24] (Observational (non-randomized)) Follow up: detected at GW 12 or 30.	2 per 1000 Difference:	1 per 1000 **1 fewer per 1000** (CI 95% 2 fewer — 0 fewer)	Very low Due to serious risk of bias [25]	In Rh D negative women with no preformed anti-D, the effect of universal RAADP (1 or 2 doses) on severe adverse neonatal events is very uncertain.
Adverse maternal events attributed to Rh D immunoprophylaxis 6 Important	[26] (Observational (non-randomized))	None of the identified studies reported any serious adverse events. A few cases of mild pain, soreness, and itching at the injection site noted. One study reported marked flushing and mild chest pain that was attributed to a specific batch study drug.			In Rh D negative women with no preformed anti-D, the effect of universal RAADP (1 or 2 doses) on adverse maternal events is unknown.

1. varying timepoints including those detected in a subsequent pregnancy, during pregnancy, at birth or within 3 days after delivery, or at postnatal followup.
2. Systematic review [92] with included studies: Hutchet 1987 (2x500 IUat GW 28&34), Lee 1995 (2x250 IUat GW 28&34). **Baseline/comparator:** Control arm of reference used for intervention.
3. **Risk of Bias: serious.** One or more randomised studies with plausible bias that raises serious doubts about the results. Missing data and exclusion of some women may overestimate the clinical effectiveness of RAADP. Includes one quasi-randomised trial with high risk of selection bias.. **Inconsistency: no serious.** No significant heterogeneity, with variability in effect estimates assessed as moderate (I2 statistic between 25% and 50%). Does not reduce confidence in results to inform decision making.. **Indirectness: no serious.** Obstetric practice and the baseline characteristics of the population may not be reflective of current practice; however, this was considered to not seriously affect the confidence in the observed effect and could be sensibly applied.. **Imprecision: serious.** Low event rate and/or wide confidence intervals that cross the line of no effect. Confidence in the results is weak.. **Publication bias: no serious.**
4. varying timepoints including those detected in a subsequent pregnancy, during pregnancy, at birth or within 3 days after delivery, or at postnatal followup.
5. Systematic review [92] with included studies: Mayne 1997 (2x500 IUat GW 28&34), Bowman and Pollock 1978 (1x1500 IUat GW 28), MacKenzie 1999 (2x500 IUat GW 28&34), Koelewijn 2008 (1x1000 IUat GW 30), Tovey 1983 (2x500 IUat GW 28&34), Combined Bowman (3 studies), Trolle 1989 (1x1500 IUat GW 28), Bowman 1978 (2x1500 IUat GW 28&34), Bowman 1987 (1x1500 IUat GW 28). Data reported by Bowman (3 studies) combined to avoid double counting of the controls.. **Baseline/comparator:** Control arm of reference used for intervention. **Supporting references:** [16], [18], [17],
6. **Risk of Bias: serious.** One or more comparative observational studies with some important problems that seriously weaken the confidence in the results. Missing data and exclusion of some women may overestimate the clinical effectiveness of RAADP. Studies include historical and/or geographic controls,

and it is not clear whether intervention and control groups are comparable at baseline.. **Inconsistency: serious.** Significant heterogeneity with substantial variability in effect estimates (I2 statistic > 50%). Reduces confidence in the results to inform decision making.. **Indirectness: no serious.** Obstetric practice and the baseline characteristics of the population may not be reflective of current practice; however, this was considered to not seriously affect the confidence in the observed effect and could be sensibly applied.. **Imprecision: no serious. Publication bias: no serious. Upgrade: clear dose-response gradient.**

7. Systematic review [92] with included studies: [63], Mayne 1997 (2x500 IUat GW 28&34), Tovey 1983 (2x500 IUat GW 28&34), MacKenzie 1999 (2x500 IUat GW 28&34), Koelewijn 2008 (1x1000 IUat GW 30), Bowman 1978 (2x1500 IUat GW 28&34). **Baseline/comparator:** Control arm of reference used for intervention.
8. **Risk of Bias: serious.** One or more comparative observational studies with some important problems that seriously weaken the confidence in the results. Missing data and exclusion of some women may overestimate the clinical effectiveness of RAADP. Studies include historical and/or geographic controls and it is not clear whether intervention and control groups are comparable at baseline.. **Inconsistency: no serious.** No significant heterogeneity (I2 statistic = 0%).. **Indirectness: no serious.** Obstetric practice and the baseline characteristics of the population may not be reflective of current practice; however, this was considered to not seriously affect the confidence in the observed effect and could be sensibly applied.. **Imprecision: no serious. Publication bias: no serious. Upgrade: clear dose-response gradient.**
9. Systematic review [92] with included studies: Lee 1995 (2x250 IUat GW 28&34), Koelewijn 2008 (1x1000 IUat GW 30), Bowman 1978 (2x1500 IUat GW 28&34), Hutchet 1987 (2x500 IUat GW 28&34). Includes one RCT and one quasi-RCT.. **Baseline/comparator:** Control arm of reference used for intervention.
10. **Risk of Bias: serious.** One or more comparative observational studies with some important problems that seriously weaken the confidence in the results. Includes one quasi-randomised trial with high risk of selection bias. Missing data and exclusion of some women may overestimate the clinical effectiveness of RAADP. Studies include historical and/or geographic controls, and it is not clear whether intervention and control groups are comparable at baseline. One or more randomised studies with plausible bias that raises serious doubts about the results. Includes one RCT and one quasi-RCT.. **Inconsistency: serious.** Significant heterogeneity with substantial variability in effect estimates (I2 statistic > 50%). Reduces confidence in the results to inform decision making.. **Indirectness: no serious.** Obstetric practice and the baseline characteristics of the population may not be reflective of current practice; however, this was considered to not seriously affect the confidence in the observed effect and could be sensibly applied.. **Imprecision: serious.** Low event rate and/or wide confidence intervals that cross the line of no effect. Confidence in the results is weak.. **Publication bias: no serious. Upgrade: clear dose-response gradient.**
11. Systematic review [92] with included studies: Trolle 1989 (1x1500 IUat GW 28), Tovey 1983 (2x500 IUat GW 28&34), Lee 1995 (2x250 IUat GW 28&34), Bowman 1987 (1x1500 IUat GW 28), Bowman and Pollock 1978 (1x1500 IUat GW 28), Bowman 1978 (2x1500 IUat GW 28&34), Hermann 1984 (1x1250 IUat GW 32-34), Combined Bowman (3 studies), Hutchet 1987 (2x500 IUat GW 28&34). Includes one RCT, one quasi-RCT and six observational studies. One observational study does not contribute any data.. **Baseline/comparator:** Control arm of reference used for intervention.
12. **Risk of Bias: serious.** One or more randomised studies with plausible bias that raises serious doubts about the results. One or more comparative observational studies with some important problems that seriously weaken the confidence in the results. Includes one quasi-randomised trial with high risk of selection bias. Missing data and exclusion of some women may overestimate the clinical effectiveness of RAADP. Studies include historical and/or geographic controls, and it is not clear whether intervention and control groups are comparable at baseline. Includes one RCT, one quasi-RCT and six observational

studies. One observational study does not contribute any data. . **Inconsistency: serious.** Significant heterogeneity with substantial variability in effect estimates (I2 statistic >50%). Reduces confidence in the results to inform decision making. . **Indirectness: no serious.** Obstetric practice and the baseline characteristics of the population may not be reflective of current practice; however, this was considered to not seriously affect the confidence in the observed effect and could be sensibly applied. . **Imprecision: no serious. Publication bias: no serious. Upgrade: clear dose-response gradient.**

13. Systematic review [92] with included studies: Lee 1995 (2x250 IUat GW 28&34), Tovey 1983 (2x500 IUat GW 28&34), Bowman 1987 (1x1500 IUat GW 28), Hermann 1984 (1x1250 IUat GW 32-34), Trolle 1989 (1x1500 IUat GW 28), Hutchet 1987 (2x500 IUat GW 28&34), Combined Bowman (1978, 1987), Bowman 1978 (2x1500 IUat GW 28&34). Includes one RCT, one quasi-RCT and five observational studies. One observational study does not contribute any data.. **Baseline/comparator:** Control arm of reference used for intervention.
14. **Risk of Bias: serious.** Includes one RCT, one quasi-RCT and six observational studies. Two observational studies do not contribute any data. One or more randomised studies with plausible bias that raises serious doubts about the results. Missing data and exclusion of some women may overestimate the clinical effectiveness of RAADP. Includes one quasi-randomised trial with high risk of selection bias. Studies include historical and/or geographic controls, and it is not clear whether intervention and control groups are comparable at baseline. One or more comparative observational studies with some important problems that seriously weaken the confidence in the results. . **Inconsistency: no serious.** No significant heterogeneity (I2 statistic = 0%). . **Indirectness: no serious.** Obstetric practice and the baseline characteristics of the population may not be reflective of current practice; however, this was considered to not seriously affect the confidence in the observed effect and could be sensibly applied. . **Imprecision: no serious. Publication bias: no serious. Upgrade: clear dose-response gradient.**
15. Assessed with Kleihauer test
16. Systematic review [92] with included studies: [65]. Hutchet 1987 (2x 500 IU at GW 28 and 34). **Baseline/comparator:** Control arm of reference used for intervention.
17. **Risk of Bias: serious.** One or more randomised studies with plausible bias that raises serious doubts about the results. Missing data and exclusion of some women may overestimate the clinical effectiveness of RAADP.. **Inconsistency: no serious.** One study only. Heterogeneity not assessed.. **Indirectness: no serious.** Obstetric practice and the baseline characteristics of the population may not be reflective of current practice; however, this was considered to not seriously affect the confidence in the observed effect and could be sensibly applied.. **Imprecision: no serious. Publication bias: no serious.**
18. Assessed with Kleihauer test
19. Systematic review [92] with included studies: [65]. Hutchet 1987 (2x 500 IU at GW 28 and 34). **Baseline/comparator:** Control arm of reference used for intervention.
20. **Risk of Bias: serious.** One or more randomised studies with plausible bias that raises serious doubts about the results. Includes one quasi-randomised trial with high risk of selection bias. Missing data and exclusion of some patients may over-estimate the clinical effectiveness of RAADP.. **Inconsistency: no serious.** One study only. Heterogeneity not assessed.. **Indirectness: no serious.** Obstetric practice and the baseline characteristics of the population may not be reflective of current practice; however, this was not considered to seriously affect the confidence in the observed effect and could be sensibly applied.. **Imprecision: no serious. Publication bias: no serious.**
21. Systematic review [92] with included studies: [65]. **Baseline/comparator:** Control arm of reference used for intervention.
22. **Risk of Bias: serious.** One or more randomised studies with plausible bias that raises serious doubts about the results. Includes one quasi-randomised trial with high risk of selection bias. Missing data and exclusion of some women may overestimate the clinical effectiveness of RAADP. . **Inconsistency: no serious.** One study only. Heterogeneity not assessed.. **Indirectness: no serious.** Obstetric practice and

the baseline characteristics of the population may not be reflective of current practice; however, this was considered to not seriously affect the confidence in the observed effect and could be sensibly applied.. **Imprecision: serious.** Low event rate and/or wide CIs that cross the line of no effect. Confidence in the results is weak.. **Publication bias: no serious.**

23. Koelewijn 2008 calculated the prevalence of severe HDFN using case-finding from records of women with Rh D alloantibodies detected at Week 12 or Week 30 among Rh D negative parae-1 women in their second ongoing pregnancies (whose first pregnancy was after 1999 when routine RAADP [intervention] was offered) compared with those whose first pregnancy was before the introduction of RAADP in 1998 (and had not received RAADP [control]).
24. Systematic reviewwith included studies: [74]. **Baseline/comparator:** Control arm of reference used for intervention.
25. **Risk of Bias: serious.** One or two comparative observational studies that appear to provide sound evidence for a nonrandomised study but cannot be considered comparable to a well-performed RCT. Some concerns with reporting bias and missing data. **Inconsistency: no serious.** One study only. Heterogeneity not assessed.. **Indirectness: no serious. Imprecision: no serious. Publication bias: no serious.**
26. Systematic review [92]. There were too few who experienced serious adverse maternal events to be attributed to Rh D immunoprophylaxis (1 or 2 doses). **Supporting references:** [40], [84], [11], [108],

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108. Pilgrim H., Lloyd-Jones M., Rees A.. Routine antenatal anti-D prophylaxis for RhD-negative women: a systematic review and economic evaluation. Health Technology Assessment 2009;13(37):1-126 Journal

129. Turner RM, Lloyd-Jones M., Anumba DOC, Smith GCS, Spiegelhalter DJ, Squires H., et al. Routine antenatal anti-D prophylaxis in women who are Rh(D) negative: Meta-analyses adjusted for differences in study design and quality. PLoS ONE 2012;7(2):e30711 Journal

Clinical question/ PICO

Population: Rh D negative pregnant women with no preformed anti-D
Intervention: Universal routine antenatal Rh D immunoprophylaxis (one or two doses)
Comparator: no universal routine antenatal Rh D immunoprophylaxis

Summary

One-dose versus two-dose routine antenatal Rh D immunoprophylaxis

Three of in the included systematic reviews *[11][108][129]* searched for head-to-head comparisons of one-dose versus two-dose RAADP regimes. None of the reviews identified any published evidence. In the absence of evidence, Pilgrim et al. (2009) *[108]* and Turner et al. (2012) *[129]* provided assessments based on expert opinion. McBain et al. (2015) *[11]* noted an ongoing RCT that compared a one-dose versus two-dose regime of RAADP, with the primary outcomes being detectable anti-D antibodies at birth and compliance. Preliminary results *[106]* of this study were considered by the ERG; with the published results *[134]* included in the 2021 update.

Incidence of Rh D alloimmunisation

One or two doses, any timepoint

Both Turner et al. (2012) *[129]* and Pilgrim et al. (2009) *[108]* assessed whether the different dosing regimens influenced the effectiveness of Rh D immunoglobulin, but found no evidence to suggest whether one or two doses was superior. Turner et al. (2012) *[129]* used a multidisciplinary panel of experts to first analyse risk of bias in ten studies of RAADP using various dose sizes and either one or two doses, then conducted a bias-adjusted meta-regression analysis to assess their relative effectiveness compared to no RAADP. Pilgrim et al. (2009) *[108]* calculated unadjusted odds ratios for the risk of alloimmunisation. Both studies suggested similar effectiveness of a single dose (1500 IU) and a two-dose regimen (500 IU per dose), and that both regimens were superior to no RAADP, though methodological issues with the studies included in both analyses limit the certainty of the effect sizes.

Based on data from a network meta-analysis, Xie et al. (2020) *[140]* suggested that two doses of Rh D immunoglobulin (1500 IU at 28 and 34 weeks gestation) is better than other dosing regimens; with the second alternative being a single dose (1500 IU) given at 28 weeks gestation, followed by two doses (500 IU) given between 28 and 34 weeks gestation. Given a lack of transparency of data included in the network meta-analysis, and the known methodological issues associated with the studies assessing RAADP (including the variability of the interventions, controls and outcomes reported), further assessment or interpretation of the results presented by Xie et al. (2020) *[140]* was too problematic to be useful.

In general agreement with Turner et al. (2012) *[129]* and Pilgrim et al. (2009), *[108]* pooled data from studies included in this review revealed a significant effect favouring RAADP (any timepoint) compared with no RAADP for the incidence of Rh D alloimmunisation regardless of whether the regimen used a single dose (RR 0.31; 95% CI 0.12, 0.80; $p = 0.02$) or a two-dose regimen (RR 0.32; 95% CI 0.20, 0.51; $p < 0.00001$). When pooled data were assessed based on the total administered dose, an effect favouring a higher dose was observed. However, given the heterogeneity and quality of the included studies and the variability of the interventions, controls and outcomes reported, caution should be taken when interpreting these results.

Outcome Timeframe	**Study results and measurements**	**Comparator** Risk with no RAADP	**Intervention** Risk with RAADP (one or two doses)	**Certainty of the Evidence** (Quality of evidence)	**Summary**
Incidence of Rh D alloimmunisation (one dose) [1] any timepoint 9 Critical	Relative risk 0.31 (CI 95% 0.12 — 0.8) Based on data from 36,555 participants in 4 studies. [2] (Observational (non-randomized))	12 per 1000 Difference:	4 per 1000 **8 fewer per 1000** (CI 95% 11 fewer — 2 fewer)	Very low Due to serious risk of bias, Due to serious inconsistency [3]	In Rh D negative women with no preformed anti-D, universal RAADP (1 dose) may reduce the incidence of Rh D alloimmunisation (any timepoint) but we are uncertain about the size of the effect
Incidence of Rh D alloimmunisation (two doses) [4] any timepoint 9 Critical	Relative risk 0.32 (CI 95% 0.2 — 0.51) Based on data from 15,264 participants in 6 studies. [5] (Observational (non-randomized))	10 per 1000 Difference:	3 per 1000 **7 fewer per 1000** (CI 95% 8 fewer — 5 fewer)	Very low Due to serious risk of bias, Due to serious imprecision [6]	In Rh D negative women with no preformed anti-D, universal RAADP (2 doses) may reduce the incidence of Rh D alloimmunisation (any timepoint) but we are uncertain about the size of the effect.
Incidence of Rh D allommunisation (one dose, estimated) 9 Critical	Based on data from participants in 10 studies. [7] (Observational (non-randomized))	In a meta-regression model, one study estimated an OR of 0.42 (95% CI 0.17, 0.73) for a single dose based on the relative effectiveness observed in published studies adjusted for bias and expert opinion. Using studies relevant to the UK health system one study estimated the risk of sensitisation using a single dose to be		Low Due to very serious risk of bias [8]	In Rh D negative women with no preformed anti-D, universal RAADP (1 dose) may reduce the incidence of Rh D alloimmunisation (any timepoint) but we are uncertain about the size of the effect

Outcome Timeframe	**Study results and measurements**	**Comparator** Risk with no RAADP	**Intervention** Risk with RAADP (one or two doses)	**Certainty of the Evidence** (Quality of evidence)	**Summary**
		0.34% (95% CI 0.28, 0.40).			
Incidence of Rh D allommunisation (two doses, estimated) 9 Critical	Based on data from participants in 10 studies. [9] (Observational (non-randomized))	In a meta-regression model, one study estimated an OR of 0.31 (95% CI 0.09, 0.65) for two doses based on the relative effectiveness observed in published studies adjusted for bias and expert opinion. Using only studies relevant to the UK health system, one study estimated the risk of sensitisation using two doses to be 0.30% (95% CI 0.22, 0.38).		Low Due to very serious risk of bias [10]	In Rh D negative women with no preformed anti-D, universal RAADP (2 doses) may reduce the incidence of Rh D alloimmunisation (any timepoint) but we are uncertain about the size of the effect.

1. 1000 or 1500 IU given at GW28 or GW30
2. Systematic review [92] with included studies: Combined Bowman (1978, 1987), Bowman and Pollock 1978 (1x1500 IUat GW 28), Bowman 1987 (1x1500 IUat GW 28), Trolle 1989 (1x1500 IUat GW 28), Koelewijn 2008 (1x1000 IUat GW 30). To avoid double counting of the controls in the studies reported by Bowman (1987, 1978) data for the intervention group were combined. It is not clear if some of the women included in the intervention group were reported in one, two (or all three) studies.. **Baseline/comparator:** Control arm of reference used for intervention. **Supporting references:** [129], In a meta-regression model, Turner et al. (2012) estimated an OR of 0.42 (95% CI 0.17, 0.73) for a single dose based on the relative effectiveness observed in published studies adjusted for bias and expert opinion. . [108], Using studies relevant to the UK health system Pilgrim et al.(2009) estimated the risk of sensitisation using a single dose to be 0.34% (95% CI 0.28, 0.40)..
3. **Risk of Bias: serious.** Several comparative observational studies with some important problems that seriously weaken the confidence in the results. Studies include historical and/or geographic controls and it is not clear if intervention and control groups are comparable at baseline. Missing data and exclusion of some women may overestimate the clinical effectiveness of RAADP. . **Inconsistency: serious.** Substantial variability in effect estimates (I2 statistic > 50%). Reduces confidence in the results to inform decision making.. **Indirectness: no serious.** Obstetric practice and the baseline characteristics of the population may not be reflective of current practice however this was considered to not seriously alter the confidence in the effect.. **Imprecision: no serious. Publication bias: no serious.**
4. 250, 500 or 1500 IU given at GW28 and GW34
5. Systematic review [92] with included studies: Lee 1995 (2x250 IUat GW 28&34), MacKenzie 1999 (2x500 IUat GW 28&34), Hutchet 1987 (2x500 IUat GW 28&34), Bowman 1978 (2x1500 IUat GW 28&34), Tovey 1983 (2x500 IUat GW 28&34), Mayne 1997 (2x500 IUat GW 28&34). Includes one RCT and one quasi-RCT.. **Baseline/comparator:** Control arm of reference used for intervention. **Supporting references:** [129], In a meta-regression model, Turner et al.(2012) estimated an OR of 0.31 (95% CI 0.09, 0.65) for two doses based on the relative effectiveness observed in published studies adjusted for bias and expert opinion. . [108], Using only studies relevant to the UK health system, Pilgrim et al.(2009) estimated the risk of sensitisation using two doses to be 0.30% (95% CI 0.22, 0.38)..
6. **Risk of Bias: serious.** Two randomised studies with plausible bias that raise some doubts about the results. Several comparative observational studies with some important problems that seriously weaken the confidence in the results. Studies include historical and/or geographic controls and it is not clear whether intervention and control groups are comparable at baseline. Missing data and exclusion of some

women may overestimate the clinical effectiveness of RAADP. . **Inconsistency: no serious.** No heterogeneity (I2 statistic = 0%). Does not reduce confidence in results to inform decision making. . **Indirectness: no serious.** Obstetric practice and the baseline characteristics of the population may not be reflective of current practice; however, this was considered to not seriously alter the confidence in the effect.. **Imprecision: serious.** Low event rate and wide confidence intervals. Confidence in the results is weak.. **Publication bias: no serious.**

7. Systematic review Turner et al. (2012) elicited expert opinion to estimate association between the relative and observed effectiveness for different dosing regimens.. **Supporting references:** [140], Based on data from a network meta-analysis, Xie et al. (2020) suggested that two doses of Rh D immunoglobulin (1500 IU at 28 and 34 gestational weeks) is better than other dosing regimens; with the second alternative being a single dose (1500 IU) given at 28 gestational weeks, followed by two doses (500 IU) given between 28 and 34 gestational weeks. Given a lack of transparency of data included in the network meta-analysis, and the known methodological issues associated with the studies assessing RAADP (including the variability of the interventions, controls and outcomes reported), further assessment or interpretation of the results presented by Xie et al. (2020)48 was too problematic to be useful.. [129], Turner et al. (2012) used a multidisciplinary panel of experts to first analyse risk of bias in ten studies of RAADP using various dose sizes and either one or two doses, then conducted a bias-adjusted meta-regression analysis to assess their relative effectiveness compared to no RAADP. . [108], Pilgrim et al. (2009) calculated unadjusted odds ratios for the risk of alloimmunisation. .
8. **Risk of Bias: very serious.** Two randomised studies with plausible bias that raise some doubts about the results. Several comparative observational studies with some important problems that seriously weaken the confidence in the results. Missing data and exclusion of some women may overestimate the clinical effectiveness of RAADP. Studies include historical or geographic controls, and it is not clear whether intervention and control groups are comparable at baseline. .
9. Systematic review Turner et al. (2012) elicited expert opinion to estimate association between the relative and observed effectiveness for different dosing regimens.. **Supporting references:** [129], Turner et al. (2012) used a multidisciplinary panel of experts to first analyse risk of bias in ten studies of RAADP using various dose sizes and either one or two doses, then conducted a bias-adjusted meta-regression analysis to assess their relative effectiveness compared to no RAADP. . [108], Pilgrim et al. (2009) calculated unadjusted odds ratios for the risk of alloimmunisation. .
10. **Risk of Bias: very serious.** Two randomised studies with plausible bias that raise some doubts about the results. Several comparative observational studies with some important problems that seriously weaken the confidence in the results. Studies include historical or geographic controls, and it is not clear whether intervention and control groups are comparable at baseline. Missing data and exclusion of some women may overestimate the clinical effectiveness of RAADP..

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92. Jorgensen M, Allerdice S. Routine antenatal prophylaxis with RhD IgG for prevention of haemolytic disease of the fetus and newborn in Rh D negative pregnant women. RevMan 5.4 2021.

108. Pilgrim H., Lloyd-Jones M., Rees A.. Routine antenatal anti-D prophylaxis for RhD-negative women: a systematic review and economic evaluation. Health Technology Assessment 2009;13(37):1-126 Journal

129. Turner RM, Lloyd-Jones M., Anumba DOC, Smith GCS, Spiegelhalter DJ, Squires H., et al. Routine antenatal anti-D prophylaxis in women who are Rh(D) negative: Meta-analyses adjusted for differences in study design and quality. PLoS ONE 2012;7(2):e30711 Journal

134. White SW, Cheng JC, Penova-Veselinovic B., Wang C., White M., Ingleby B., et al. Single dose v two-dose antenatal anti-D prophylaxis: a randomised controlled trial. Med J Aust 2019;211(6):261-265 Journal

140. Xie X, Zhou D, Fu Q, Bao Z, Zhang YI. Clinical value of different anti-D immunoglobulin strategies for preventing Rh hemolytic disease of the fetus and newborn: A network meta-analysis. PLoS ONE 2020;15(3):e0230073 Pubmed Journal

Weak recommendation , Low certainty evidence

R2: The ERG **recommends** that administration of Rh D immunoglobulin 625 IU at 28 and 34 weeks of pregnancy* continue in Rh D negative pregnant women with no preformed anti-D antibodies unless NIPT for fetal *RHD*** has predicted that they are not carrying an Rh D positive fetus.

The ERG does not currently suggest changing to a single dose of Rh D immunoglobulin 1500 IU.

* *A woman's pregnancy care schedule and clinical discretion may warrant the administration of Rh D immunoglobulin within 2 weeks before or after the recommended 28 and 34 weeks of pregnancy. However, if the second dose of Rh D immunoglobulin is given before 34 weeks and the pregnancy goes beyond the due date, the risk of inadequate anti-D coverage at birth increases.*

** *All women should have an ABO/Rh D type and antibody screen performed no later than 10 weeks gestation. Women who are Rh D negative should be retested at 28 weeks unless NIPT for fetal RHD has predicted that they are not carrying an Rh D positive fetus. The specimen should be collected before giving prophylactic Rh D immunoglobulin; however, the immunoglobulin can be given before the results are available [2].*

Practical info

See Supply considerations

See Safety of Rh D immunoglobulin

Evidence to decision

Benefits and harms

See Benefits and harms R1

Certainty of the Evidence

Low

See Certainty of the Evidence R1

Values and preferences

See Values and preferences R1

See Challenges - Consent and the choice to decline Rh D immunoglobulin

Resources and other considerations

See Resources and other considerations R1

See Cost considerations

See Supply considerations

Rationale

See Rationale R1.

Clinical question/ PICO

Population: Rh D negative pregnant women with no preformed anti-D
Intervention: Universal routine antenatal Rh D immunoprophylaxis (one dose)
Comparator: Universal routine antenatal Rh D immunoprophylaxis (two doses)

Summary

Additional outcomes

One RCT provided limited data relating to serum anti-D antibody levels in Rh D negative pregnant women. White et al. (2019) *[106][134]* observed that the number of women with anti-D antibodies present at birth was higher in women who received the two-dose regimen compared with women who received the one-dose regimen (86% vs 56%; OR 4.91; 95% CI 2.67, 9.02; $p < 0.001$); however the association between the two-dose regimen and detectability was not significant after adjusting for maternal body weight and the interval between final dose and birth (adjusted OR 1.55; 95% CI 0.62, 3.87; $p = 0.35$).

The relationship between a lack of detectable circulating anti-D antibody following Rh D immunoprophylaxis and risk of alloimmunisation detected in a subsequent pregnancy is not known. However, meta-analyses of effectiveness of RAADP (total dose) suggests a dose-response *[16][108][129][140]*, which could have been mediated through longer duration of detectable passive anti-D.

Outcome Timeframe	**Study results and measurements**	**Comparator** Risk with RAADP (two doses)	**Intervention** Risk with RAADP (one dose)	**Certainty of the Evidence** (Quality of evidence)	**Summary**
Undetectable serum anti-D antibodies at birth 3 Not Important	Odds ratio 4.85 (CI 95% 2.63 — 8.92) Based on data from 254 participants in 1 studies. [1] (Randomized controlled)	140 per 1000 Difference:	440 per 1000 **301 more per 1000** (CI 95% 160 more — 369 more)	Low Due to serious risk of bias, Due to serious imprecision [2]	In Rh D negative women with no preformed anti-D, universal RAADP (1 or 2 doses) may have little or no difference on undetectable serum anti-D antibodies
Incidence of Rh D		No evidence found.			No studies were found that looked at incidence

Outcome Timeframe	**Study results and measurements**	**Comparator** Risk with RAADP (two doses)	**Intervention** Risk with RAADP (one dose)	**Certainty of the Evidence** (Quality of evidence)	**Summary**
alloimmunisation					of Rh D alloimmunisation
Incidence of a positive test for FMH		No evidence found.			No studies were found that looked at incidence of a positive test for FMH
Adverse neonatal events		No evidence found.			No studies were found that looked at adverse neonatal events
Adverse maternal events		No evidence found.			No studies were found that looked at adverse maternal events

1. Systematic review [92] with included studies: White 2019. **Baseline/comparator:** Control arm of reference used for intervention. **Supporting references:** [134],
2. **Risk of Bias: serious.** Study has plausible bias that raises some doubts about the results. Concerns relate to missing data and knowledge of the intervention received that affected the conduct of the study. . **Inconsistency: no serious. Indirectness: no serious. Imprecision: serious.** In a multivariate analysis adjusted for maternal body weight and the interval between final dose and birth, the association between two-dose administration and detection of anti-D antibodies was not significant (adjusted OR 1.55; 95% CI 0.62, 3.87). . **Publication bias: no serious.**

References

92. Jorgensen M, Allerdice S. Routine antenatal prophylaxis with RhD IgG for prevention of haemolytic disease of the fetus and newborn in Rh D negative pregnant women. RevMan 5.4 2021.

134. White SW, Cheng JC, Penova-Veselinovic B., Wang C., White M., Ingleby B., et al. Single dose v two-dose antenatal anti-D prophylaxis: a randomised controlled trial. Med J Aust 2019;211(6):261-265 Journal

Expert opinion point

EOP2: If antibody screening identifies anti-D in an Rh D negative pregnant woman, consideration of clinical history and laboratory findings is required to determine whether the anti-D is likely to be preformed (due to sensitisation) or passive (due to administration of Rh D immunoglobulin in the past 12 weeks).*

In cases of likely preformed anti-D antibodies, seek specialist obstetric advice, manage as Rh D sensitised and consider NIPT for fetal Rh D status.

**See EOP3*

Expert opinion point

EOP3: Rh D immunoglobulin should not be given to Rh D negative pregnant women with preformed anti-D antibodies. However, if it is unclear whether the anti-D detected in the mother's blood is preformed (due to sensitisation) or passive (due to administration of Rh D immunoglobulin in the past 12 weeks), the treating clinician should be consulted. If there is continuing doubt, Rh D immunoglobulin should be administered.

6.2 Universal sensitising event immunoprophylaxis

6.2.1 Summary of guidance on the use and timing of Rh D immunoglobulin for sensitising event immunoprophylaxis

Info Box

Clinical indication	Rh D immunoglobulin		Target group	Section
	Dose	Timing		
First 12 weeks of pregnancy: • miscarriage • termination of pregnancy (after 10 weeks gestation) • ectopic pregnancy • molar pregnancy • chorionic villus sampling	250 IU	As soon as practical within 72 hours. If delayed beyond 72 hours, the dose should be given up to 10 days from the sensitising event, but may have lower efficacy	All Rh D negative women with no preformed anti-D antibodies	6.2.2
• ongoing uterine bleeding alone	250 IU	Where bleeding is repeated, or heavy, a repeat dose may be appropriate after an interval of 6 weeks		
After 12^{+6} weeks of pregnancy: • genetic studies (chorionic villus sampling, amniocentesis and cordocentesis) • abdominal trauma considered sufficient to cause FMH, even if FMH testing is negative • each occasion of revealed or concealed antepartum haemorrhage. Where the woman suffers unexplained uterine pain the possibility of concealed antepartum haemorrhage (and the need for immunoprophylaxis) should be considered • external cephalic version (successful or attempted) • miscarriage or termination of pregnancy	625 IU	As soon as practical within 72 hours. If delayed beyond 72 hours, the dose should be given up to 10 days from the sensitising event, but may have lower efficacy	All Rh D negative women with no preformed anti-D antibodies (unless NIPT for fetal *RHD* has predicted the fetus to be Rh D negative)	6.2.3
• ongoing uterine bleeding alone	625 IU	Where bleeding is repeated, or heavy, a repeat dose may be appropriate at 6 weekly intervals		
Large FMH ≥ 6mL of fetal red cells (equivalent to 12mL of whole blood): • antepartum • postpartum	625 IU as initial dose with follow up dose according to FMH quantitation	As soon as possible. Follow laboratory or specialist obstetric advice for additional doses of IM Rh D immunoglobulin or IV Rh D immunoglobulin, and for follow-up testing	All Rh D negative women with no preformed anti-D antibodies (unless NIPT for fetal *RHD* has predicted the fetus to be Rh D negative)	6.2.3 6.5

FMH: fetomaternal haemorrhage; IM: intramuscular; IU: international units; IV: intravenous; NIPT: non-invasive prenatal testing

anti-D - refers to circulating antibodies; *RHD* - refers to genotype; Rh D immunoglobulin - refers to the product; Rh D positive/negative - refers to blood type.

6.2.2 Universal sensitising event immunoprophylaxis in the first 12 weeks of pregnancy

Certain events can lead to maternal exposure to fetal antigens during pregnancy or when giving birth. In the first 12 weeks of pregnancy such events include abdominal trauma, molar pregnancy, ectopic pregnancy, spontaneous miscarriage, threatened miscarriage or medical termination of pregnancy (with or without a curette).

Strong recommendation , Very low certainty evidence

R3: After the following sensitising events in the first 12 weeks of singleton or multiple pregnancy: miscarriage, termination of pregnancy (after 10 weeks gestation), ectopic pregnancy, molar pregnancy and chorionic villus sampling, the ERG **recommends** that a dose of Rh D immunoglobulin 250 IU be given to all Rh D negative women with no preformed anti-D antibodies to prevent Rh D alloimmunisation.

Practical info

For dosing see Summary of guidance on the use and timing of Rh D immunoglobulin for sensitising event immunoprophylaxis

Evidence to decision

Benefits and harms

There is a clear health benefit in avoiding sensitisation if possible. Reducing the incidence of Rh D alloimmunisation is important because it is the most critical intermediate step for reducing the incidence of HDFN (and the consequent risk of serious fetal or neonatal morbidity or death). This also protects the woman from the need for invasive treatments that are needed if HDFN causes significant anaemia in an Rh D positive fetus as well as potential clinical complications that affect her own health. The intervention has an excellent safety record *[37]*.

Taken as a whole, the risk of sensitisation for Rh D negative women if they do not receive Rh D immunoprophylaxis following a sensitising event in the first 12 weeks of pregnancy outweighs the risk of harm. The risk of sensitisation increases when there is a greater likelihood of maternal tissues being exposed to fetal blood; surgical intervention greatly increases the risk of this happening.

Certainty of the Evidence

Very low

The evidence is very uncertain about the effect of sensitising event immunoprophylaxis compared with placebo or no sensitising event immunoprophylaxis on the incidence of Rh D alloimmunisation after spontaneous miscarriage, incomplete miscarriage, therapeutic evacuation or induced abortion in Rh D negative women. The small size of the studies meant that detecting any benefit was unlikely. Horvath et al. (2022) suggest that a close reading of studies indicate that forgoing Rh immunoglobulin administration before 12 weeks gestation is highly unlikely to increase risk of Rh (D) antibody development, and more recent studies indicate that fetal RBC exposure during aspiration abortion < 12 weeks gestation is below the calculated threshold to cause maternal Rh sensitisation *[147]*.

The effectiveness of sensitising event immunoprophylaxis compared with placebo or no sensitising event immunoprophylaxis on the incidence of Rh D alloimmunisation after abdominal trauma, molar pregnancy or ectopic pregnancy is not known. The available evidence does not justify changes to the 2003 guideline *[1]*. However, the ERG has clarified the wording around threatened miscarriage and has

added guidance related to molar pregnancy. This guidance is consistent with international guidelines.

A recommendation was made because these events are known, or likely to cause FMH and any event that leads to maternal exposure to fetal red cells could cause alloimmunisation. The risks of Rh D immunoprophylaxis are very low and are likely to be outweighed by potential benefit.

Values and preferences

Recent literature and international guidelines support the indications for, and dosing of, Rh D sensitising event immunoprophylaxis.

See Challenges - Consent and the choice to decline Rh D immunoglobulin

See Safety of Rh D immunoglobulin

Resources and other considerations

Costs associated with caring for Rh D alloimmunised women and their babies can be avoided with prophylactic administration of antenatal Rh D immunoglobulin. Recommendations about sensitising event immunoprophylaxis with Rh D immunoglobulin in Rh D negative women with no preformed anti-D antibodies remain unchanged since the staged introduction of the national immunoprophylaxis program started in 2003. The resources and costs associated with this program are considered reasonable *[95]*.

Rationale

Although the Rh D antigen is expressed on fetal RBCs from about 6 weeks of pregnancy (which would make alloimmunisation possible in the second half of the first trimester) the volume of fetal RBCs is very small at this gestation, so a low dose of Rh D immunoglobulin is justified for immunoprophylaxis.

Question 2 – (Intervention)

In Rh D negative women with no preformed anti-D who have experienced one of the following first trimester sensitising events – abdominal trauma, molar pregnancy, ectopic pregnancy, spontaneous miscarriage, threatened miscarriage or medical termination of pregnancy (with or without a curette) – does universal first trimester sensitising event prophylaxis with Rh D immunoglobulin prevent Rh D alloimmunisation?

The literature search for this question aimed to establish whether administration of sensitising event immunoprophylaxis in the first 12 weeks of pregnancy should be recommended in the presence of any of the following events: abdominal trauma, molar pregnancy, ectopic pregnancy, spontaneous miscarriage, threatened miscarriage and medical termination of pregnancy.

Clinical question/ PICO

Population: Rh D negative pregnant women with no preformed anti-D with a first 12 weeks of pregnancy sensitising event

Intervention: Routine sensitising event immunoprophylaxis

Comparator: Placebo or no sensitising event immunoprophylaxis

Summary

Three systematic reviews *[94][71][115]* were identified that evaluated the effectiveness of prophylactic Rh D immunoglobulin in response to a sensitising event in the first 12 weeks of pregnancy. The reviews included one RCT *[132]* and two nonrandomised studies *[56][118]* meeting the PICO criteria. All three studies were published before the previous 2003 guideline *[1]*. The systematic review by Schmidt-Hansen et al. (2020) *[115]* was used to inform the 2019 NICE guidelines on abortion care *[3]* and specifically searched for evidence relating to sensitising events in women undergoing either medical abortion with mifepristone and misoprostol or surgical abortion using vacuum aspiration of a pregnancy up to 13+6 weeks gestation. No studies evaluating the use of prophylactic Rh D immunoglobulin in women with first trimester ectopic pregnancy, threatened miscarriage or molar pregnancy were identified.

The 2012 guidelines from the UK's National Institute of Health and Care Excellence (NICE) *[94]* also included five noncomparative, descriptive studies *[72][90][93][119][133]* of the incidence of alloimmunisation in women who did not receive Rh D immunoprophylaxis following first trimester obstetric events. These studies did not meet the PICO criteria for this review.

Incidence of Rh D alloimmunisation

One RCT *[132]* and two nonrandomised studies *[56][118]* assessed whether immunoprophylaxis with Rh D immunoglobulin prevented Rh D alloimmunisation after a sensitising event in the first 12 weeks of pregnancy. All three studies reported data on women who had either a miscarriage or therapeutic abortion, but no evidence was presented for women with a threatened miscarriage, ectopic pregnancy or molar pregnancy, or after abdominal trauma.

There were large variations within the included studies, with different doses of Rh D immunoglobulin used (1500 IU, 250 IU or not reported), different methods used to measure potential Rh D alloimmunisation (Enzyme-Coombs or Indirect Coombs), and different criteria with regards to the included sensitising events (spontaneous miscarriage or therapeutic evacuation). All included studies were small and were unlikely to be sufficiently powered to detect meaningful differences between comparator groups.

Incidence 4–6 months after sensitising event

Two studies *[132][56]* reported no increased risk of Rh D alloimmunisation between 4 and 6 months after miscarriage (spontaneous or incomplete) or therapeutic abortion. The RCT by Visscher and Visscher (1972) *[132]* found no cases of Rh D alloimmunisation (Enzyme-Coombs test; 0/19 in the intervention group compared with 0/29 in the placebo group). The cohort study by Gavin (1972) *[56]* also reported no significant increase in Rh D alloimmunisation (Indirect Coombs test; 0/21 in the intervention group compared with 2/36 in the placebo group). This did not reach statistical significance (RR 0.34; 95% CI 0.02, 6.69, p = 0.48).

Incidence in a subsequent pregnancy

Two studies *[132][118]* reported the incidence of alloimmunisation in a subsequent pregnancy after miscarriage (spontaneous or incomplete) or therapeutic abortion.

The study by Visscher and Visscher (1972) *[132]* reported no Rh D alloimmunisation in nine subsequent Rh D positive pregnancies (6/19 from the intervention group, and 3/29 from the placebo group). It was not reported whether any of the other participants had given birth to an Rh D positive neonate beyond the follow-up period.

Simonovits et al. (1974) *[118]* recorded three instances of Rh D alloimmunisation among 241 Rh D negative women after therapeutic abortion (1 in the intervention group). No significant difference

between treatment groups was observed (1.0% vs 1.4%; RR 0.76; 95% CI 0.0, 8.21, p = 0.82).

Incidence of a positive Kleihauer test

No studies were identified.

Adverse neonatal events

No studies were identified.

Adverse maternal events

No studies were identified.

Outcome Timeframe	**Study results and measurements**	**Comparator** Risk with placebo or no sensitising event immunoprophylaxis	**Intervention** Risk with sensitising event immunoprophylaxis	**Certainty of the Evidence** (Quality of evidence)	**Summary**
Incidence of Rh D alloimmunisation [1] (4-6 months after spontaneous miscarriage and/or therapeutic evacuation) assessed with: Enzyme-Coombs screening 9 Critical	Relative risk 0 (CI 95% 0 — 0) Based on data from 48 participants in 1 studies. [2] (Randomized controlled)	0 per 1000 Difference:	0 per 1000 **0 fewer per 1000** (CI 95% 0 fewer — 0 fewer)	Very low Due to very serious risk of bias, Due to serious indirectness, Due to serious imprecision, Due to serious publication bias [3]	The evidence is very uncertain about the effect of sensitising event immunoprophylaxis on the incidence of Rh D alloimmunisation 4–6 months after spontaneous miscarriage or therapeutic evacuation in Rh D negative women.
Incidence of Rh D alloimmunsation [4] (4-6 months after incomplete miscarriage or therapeutic abortion) assessed with: Indirect Coombs 9 Critical	Relative risk 0.34 (CI 95% 0.02 — 6.69) Based on data from 57 participants in 1 studies. [5] (Observational (non-randomized))	56 per 1000 Difference:	19 per 1000 **37 fewer per 1000** (CI 95% 55 fewer — 319 more)	Very low Due to very serious risk of bias, Due to serious indirectness, Due to serious imprecision, Due to serious publication bias [6]	The evidence is very uncertain about the effect of sensitising event immunoprophylaxis on the incidence of Rh D alloimmunisation 4–6 months after incomplete miscarriage or therapeutic abortion in Rh D negative women.
Incidence of Rh D alloimmunisatio	Relative risk 0 (CI 95% 0 — 0) Based on data from 9	0 per 1000	0 per 1000	Very low Due to very serious risk of	The evidence is very uncertain about the effect of sensitising

Outcome Timeframe	**Study results and measurements**	**Comparator** Risk with placebo or no sensitising event immunoprophylaxis	**Intervention** Risk with sensitising event immunoprophylaxis	**Certainty of the Evidence** (Quality of evidence)	**Summary**
n [7] (at subsequent pregnancy after spontaneous miscarriage and/or therapeutic evacuation) assessed with: Enzyme-Coombs screening 9 Critical	participants in 1 studies. [8] (Randomized controlled)	Difference:	**0 fewer per 1000** (CI 95% 0 fewer — 0 fewer)	bias, Due to serious indirectness, Due to serious imprecision, Due to serious publication bias [9]	event immunoprophylaxis on the incidence of Rh D alloimmunisation 4–6 months after incomplete miscarriage or therapeutic abortion in Rh D negative women.
Incidence of Rh D alloimmunisation [10] (at subsequent pregnancy after induced abortion) assessed with: Papain-treated cells or Indirect Coombs 9 Critical	Relative risk 0.76 (CI 95% 0.07 — 8.21) Based on data from 241 participants in 1 studies. [11] (Observational (non-randomized))	14 per 1000 Difference:	10 per 1000 **3 fewer per 1000** (CI 95% 13 fewer — 101 more)	Very low Due to very serious risk of bias, Due to serious imprecision, Due to serious publication bias [12]	The evidence is very uncertain about the effect of sensitising event immunoprophylaxis on the incidence of Rh D alloimmunisation in a subsequent pregnancy after induced abortion in Rh D negative pregnant women.
Incidence of Rh D alloimmunsation after abdominal trauma, molar pregnancy, ectopic pregnancy 9 Critical	[13]	No comparative evidence found.			The effect of sensitising event immunoprophylaxis on the incidence of Rh D alloimmunisation after abdominal trauma, molar pregnancy, or ectopic pregnancy in Rh D negative women is unknown.
Incidence of a positive test for FMH 6 Important	[14]	No comparative evidence found.			The effect of sensitising event immunoprophylaxis on the incidence of a positive test for FMH after abdominal trauma, molar pregnancy, or ectopic pregnancy in Rh D negative women is

Outcome Timeframe	**Study results and measurements**	**Comparator** Risk with placebo or no sensitising event immunoprophylaxis	**Intervention** Risk with sensitising event immunoprophylaxis	**Certainty of the Evidence** (Quality of evidence)	**Summary**
					unknown
Adverse neonatal events [15] (e.g. jaundice) 6 Important	[16]	No comparative evidence found.			The effect of sensitising event immunoprophylaxis on the incidence of adverse neonatal events after abdominal trauma, molar pregnancy, or ectopic pregnancy in Rh D negative women is unknown.
Adverse maternal events attributed to Rh D immunoprophylaxis 6 Important	[17]	No comparative evidence found.			The effect of sensitising event immunoprophylaxis on the incidence of adverse maternal events after abdominal trauma, molar pregnancy, or ectopic pregnancy in Rh D negative women is unknown.

1. assessed with Enzyme-Coombs screening
2. Systematic review [92] with included studies: Visscher 1972. **Baseline/comparator:** Control arm of reference used for intervention. **Supporting references:** [132],
3. **Risk of Bias: very serious.** One randomised study with plausible bias that raises serious doubts about the results. Method of randomisation not reported and unclear whether treatment allocation concealed. Some concerns with reporting bias and missing data.. **Inconsistency: no serious.** Single study. Heterogeneity not assessed. **Indirectness: serious.** The evidence is not directly applicable to the target population or the Australian healthcare context, and it is difficult to judge whether it could be sensibly applied. Obstetric practice and the baseline characteristics of the population may not be reflective of current practice. The study was conducted in the United States among Rh D negative women with complete miscarriage (n = 9) or incomplete miscarriage with curettage (n = 48). An unknown proportion of women had miscarriage outside the first 12 weeks of pregnancy and the intervention was administered at a dose higher than recommended in Australia (1500 IU vs 625 IU). . **Imprecision: serious.** Small study not sufficiently powered to detect a statistically significant difference. . **Publication bias: serious.** Single study. Publication bias suspected. .
4. assessed with Indirect Coombs
5. Systematic review [92] with included studies: Gavin 1972. There were too few who experienced Rh D alloimmunsation (4-6 months after after incomplete miscarriage or therapeutic abortion) to determine whether routine sensitising event immunoprophylaxis made a difference.. **Baseline/ comparator:** Control arm of reference used for intervention. **Supporting references:** [56],
6. **Risk of Bias: very serious.** Comparative study with some important problems that seriously weakens the confidence in the results. Method of treatment allocation or blinding not reported. Some

concerns with reporting bias and missing data.. **Inconsistency: no serious.** Single study. Heterogeneity not assessed. . **Indirectness: serious.** The evidence is not directly applicable to the target population or the Australian healthcare context, and it is difficult to judge whether it could be sensibly applied. Obstetric practice and the baseline characteristics of the population may not be reflective of current practice. The study was conducted in the United States among Rh D negative women who had therapeutic abortion (n = 33) or were treated for incomplete miscarriage (n = 24). Thirteen (22.8%) women were treated outside the first 13 weeks of pregnancy and the dose of intervention (Rhogam) was not stated. . **Imprecision: serious.** Low event rate or wide CIs that cross the line of no effect. Confidence in the results is weak. . **Publication bias: serious.** Single study. Publication bias suspected..

7. assessed with: Enzyme-Coombs screening
8. Systematic review [92] with included studies: Visscher 1972. There were too few who experienced Rh D alloimmunisation (at subsequent pregnancy after spontenous miscarriage or therapeutic evacuation) to determine whether routine sensitising event immunoprophylaxis made a difference.. **Baseline/comparator:** Control arm of reference used for intervention. **Supporting references:** [132],
9. **Risk of Bias: very serious.** One randomised study with plausible bias that raises serious doubts about the results. Method of randomisation not reported and unclear whether treatment allocation concealed. Some concerns with reporting bias and missing data. . **Inconsistency: no serious.** Single study. Heterogeneity not assessed.. **Indirectness: serious.** The evidence is not directly applicable to the target population or the Australian health care context, and it is difficult to judge whether it could be sensibly applied. Obstetric practice and the baseline characteristics of the population may not be reflective of current practice. The study was conducted in the United States among Rh D negative women with complete miscarriage (n = 9) or incomplete miscarriage with curettage (n = 48). An unknown proportion of women had miscarriage outside the first 12 weeks of pregnancy and the intervention was administered at a dose higher than recommended in Australia (1500 IU vs 625 IU). . **Imprecision: serious.** Small study not sufficiently powered to detect a statistically significant difference.. **Publication bias: serious.** Single study. Publication bias suspected. .
10. assessed with: Papain-treated cells or Indirect Coombs
11. Systematic review [92] with included studies: Simonovitis 1974. There were too few who experienced Rh D alloimmunisation at subsequent pregnancy after induced abortion to determine whether routine sensitising event immunoprophylaxis made a difference.. **Baseline/comparator:** Control arm of reference used for intervention. **Supporting references:** [118],
12. **Risk of Bias: very serious.** Comparative study with some important problems that seriously weakens the confidence in the results. Method of treatment allocation or blinding not reported. Some concerns with reporting bias and missing data.. **Inconsistency: no serious.** Single study. Heterogeneity not assessed. . **Indirectness: no serious.** The evidence is probably applicable to the Australian population and healthcare context with some caveats. The study was conducted in Hungary among Rh D negative women in their second pregnancy, whose first pregnancy was terminated in the first trimester by induced abortion (method of termination not clear). The intervention was administered at the same dose as recommended in Australia (250 IU). . **Imprecision: serious.** Low event rate or wide CIs that cross the line of no effect. Confidence in the results is weak. . **Publication bias: serious.** Single study. Publication bias suspected. .
13. Systematic review No studies were found that looked at the effect of sensitising event immunoprophylaxis on the incidence of Rh D alloimmunisation after abdominal trauma, molar pregnancy, or ectopic pregnancy.. **Supporting references:** [94],
14. Systematic review No studies were found that looked at the effect of sensitising event immunoprophylaxis on the incidence of a positive test for FMH after abdominal trauma, molar pregnancy, or ectopic pregnancy.. **Supporting references:** [71],
15. (e.g., jaundice)

16. Systematic review **Supporting references:** [71],
17. Systematic review **Supporting references:** [71],

References

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132. Visscher RD, Visscher HC. Do Rh-negative women with an early spontaneous abortion need Rh immune prophylaxis?. Am J Obstet Gynecol 1972;113(2):158-65 Journal

Weak recommendation against , Very low certainty evidence

R4: *(discretionary)* In the setting of termination of pregnancy before 10 weeks of gestation there is insufficient evidence to suggest the routine use of Rh D immunoglobulin *[3][4][147]*.

**See R3 for Research evidence and additional References*

Practical info

See Clinical-Guideline-for-Abortion-Care.pdf (ranzcog.edu.au)

Evidence to decision

Benefits and harms

See Benefits and harms R3

Certainty of the Evidence

Very low

See Certainty of the Evidence R3

Values and preferences

See Vales and preferences R3

Resources and other considerations

See Resources and other considerations R3

Rationale

For further information refer to tabs for R3.

Expert opinion point

EOP4: For sensitising events in the first 12 weeks of pregnancy, where there is any uncertainty with gestational age, consider offering Rh D immunoglobulin. Consider ultrasound to confirm gestational age.

Practical info

For dosing see Summary of guidance on the use and timing of Rh D immunoglobulin for sensitising event immunoprophylaxis

Weak recommendation against , Very low certainty evidence

R5: *(qualified)* In Rh D negative women with an ongoing pregnancy who have uterine bleeding in the first 12 weeks of pregnancy there is insufficient evidence to support the routine use of Rh D immunoglobulin. However, where the bleeding is repeated, heavy or associated with abdominal pain or significant pelvic trauma, immunoprophylaxis may be considered in women with no preformed anti-D antibodies.

Evidence to decision

Benefits and harms

See Benefits and harms R3

Certainty of the Evidence

Very low

See Certainty of the Evidence R3

Values and preferences

See Values and preferences R3

Resources and other considerations

See Resources and other considerations R3

Clinical question/ PICO

Population: Rh D negative pregnant women with no preformed anti-D with a first 12 weeks of pregnancy sensitising event

Intervention: Routine sensitising event immunoprophylaxis

Comparator: Placebo or no sensitising event immunoprophylaxis

Summary

Three systematic reviews *[94][71][115]* were identified that evaluated the effectiveness of prophylactic Rh D immunoglobulin in response to a sensitising event in the first 12 weeks of pregnancy. The reviews included one RCT *[132]* and two nonrandomised studies *[56][118]* meeting the PICO criteria. All three studies were published before the previous 2003 guideline *[1]*. The systematic review by Schmidt-Hansen et al. (2020) *[115]* was used to inform the 2019 NICE guidelines on abortion care *[3]* and specifically searched for evidence relating to sensitising events in women undergoing either medical abortion with mifepristone and misoprostol or surgical abortion using vacuum aspiration of a pregnancy up to 13+6 weeks gestation. No studies evaluating the use of prophylactic Rh D immunoglobulin in women with first trimester ectopic pregnancy, threatened miscarriage or molar pregnancy were identified.

The 2012 guidelines from the UK's National Institute of Health and Care Excellence (NICE) *[94]* also included five noncomparative, descriptive studies *[72][90][93][119][133]* of the incidence of alloimmunisation in women who did not receive Rh D immunoprophylaxis following first trimester obstetric events. These studies did not meet the PICO criteria for this review.

Incidence of Rh D alloimmunisation

One RCT *[132]* and two nonrandomised studies *[56][118]* assessed whether immunoprophylaxis with Rh D immunoglobulin prevented Rh D alloimmunisation after a sensitising event in the first 12 weeks of pregnancy. All three studies reported data on women who had either a miscarriage or therapeutic abortion, but no evidence was presented for women with a threatened miscarriage, ectopic pregnancy or molar pregnancy, or after abdominal trauma.

There were large variations within the included studies, with different doses of Rh D immunoglobulin used (1500 IU, 250 IU or not reported), different methods used to measure potential Rh D alloimmunisation (Enzyme-Coombs or Indirect Coombs), and different criteria with regards to the included sensitising events (spontaneous miscarriage or therapeutic evacuation). All included studies were small and were unlikely to be sufficiently powered to detect meaningful differences between comparator groups.

Incidence 4–6 months after sensitising event

Two studies *[132][56]* reported no increased risk of Rh D alloimmunisation between 4 and 6 months after miscarriage (spontaneous or incomplete) or therapeutic abortion. The RCT by Visscher and Visscher (1972) *[132]* found no cases of Rh D alloimmunisation (Enzyme-Coombs test; 0/19 in the intervention group compared with 0/29 in the placebo group). The cohort study by Gavin (1972) *[56]* also reported no significant increase in Rh D alloimmunisation (Indirect Coombs test; 0/21 in the intervention group compared with 2/36 in the placebo group). This did not reach statistical significance (RR 0.34; 95% CI 0.02, 6.69, $p = 0.48$).

Incidence in a subsequent pregnancy

Two studies *[132][118]* reported the incidence of alloimmunisation in a subsequent pregnancy after miscarriage (spontaneous or incomplete) or therapeutic abortion.

The study by Visscher and Visscher (1972) *[132]* reported no Rh D alloimmunisation in nine subsequent Rh D positive pregnancies (6/19 from the intervention group, and 3/29 from the placebo group). It was not reported whether any of the other participants had given birth to an Rh D positive neonate beyond the follow-up period.

Simonovits et al. (1974) *[118]* recorded three instances of Rh D alloimmunisation among 241 Rh D negative women after therapeutic abortion (1 in the intervention group). No significant difference between treatment groups was observed (1.0% vs 1.4%; RR 0.76; 95% CI 0.0, 8.21, p = 0.82).

Incidence of a positive Kleihauer test

No studies were identified.

Adverse neonatal events

No studies were identified.

Adverse maternal events

No studies were identified.

Outcome Timeframe	**Study results and measurements**	**Comparator** Risk with placebo or no sensitising event immunoprophylaxis	**Intervention** Risk with sensitising event immunoprophylaxis	**Certainty of the Evidence** (Quality of evidence)	**Summary**
Incidence of Rh D alloimmunisation [1] (4-6 months after spontaneous miscarriage and/or therapeutic evacuation) assessed with: Enzyme-Coombs screening 9 Critical	Relative risk 0 (CI 95% 0 — 0) Based on data from 48 participants in 1 studies. [2] (Randomized controlled)	0 per 1000 Difference:	0 per 1000 **0 fewer per 1000** (CI 95% 0 fewer — 0 fewer)	Very low Due to very serious risk of bias, Due to serious indirectness, Due to serious imprecision, Due to serious publication bias [3]	The evidence is very uncertain about the effect of sensitising event immunoprophylaxis on the incidence of Rh D alloimmunisation 4–6 months after spontaneous miscarriage or therapeutic evacuation in Rh D negative women.
Incidence of Rh D alloimmunsation [4] (4-6 months after incomplete miscarriage or therapeutic abortion) assessed with: Indirect Coombs	Relative risk 0.34 (CI 95% 0.02 — 6.69) Based on data from 57 participants in 1 studies. [5] (Observational (non-randomized))	56 per 1000 Difference:	19 per 1000 **37 fewer per 1000** (CI 95% 55 fewer — 319 more)	Very low Due to very serious risk of bias, Due to serious indirectness, Due to serious imprecision, Due to serious publication bias [6]	The evidence is very uncertain about the effect of sensitising event immunoprophylaxis on the incidence of Rh D alloimmunisation 4–6 months after incomplete miscarriage or therapeutic abortion in Rh D negative

Outcome Timeframe	**Study results and measurements**	**Comparator** Risk with placebo or no sensitising event immunoprophylaxis	**Intervention** Risk with sensitising event immunoprophylaxis	**Certainty of the Evidence** (Quality of evidence)	**Summary**
9 Critical					women.
Incidence of Rh D alloimmunisation [7] (at subsequent pregnancy after spontaneous miscarriage and/or therapeutic evacuation) assessed with: Enzyme-Coombs screening 9 Critical	Relative risk 0 (CI 95% 0 — 0) Based on data from 9 participants in 1 studies. [8] (Randomized controlled)	0 per 1000 Difference:	0 per 1000 **0 fewer per 1000** (CI 95% 0 fewer — 0 fewer)	Very low Due to very serious risk of bias, Due to serious indirectness, Due to serious imprecision, Due to serious publication bias [9]	The evidence is very uncertain about the effect of sensitising event immunoprophylaxis on the incidence of Rh D alloimmunisation 4–6 months after incomplete miscarriage or therapeutic abortion in Rh D negative women.
Incidence of Rh D alloimmunisation [10] (at subsequent pregnancy after induced abortion) assessed with: Papain-treated cells or Indirect Coombs 9 Critical	Relative risk 0.76 (CI 95% 0.07 — 8.21) Based on data from 241 participants in 1 studies. [11] (Observational (non-randomized))	14 per 1000 Difference:	10 per 1000 **3 fewer per 1000** (CI 95% 13 fewer — 101 more)	Very low Due to very serious risk of bias, Due to serious imprecision, Due to serious publication bias [12]	The evidence is very uncertain about the effect of sensitising event immunoprophylaxis on the incidence of Rh D alloimmunisation in a subsequent pregnancy after induced abortion in Rh D negative pregnant women.
Incidence of Rh D alloimmunsation after abdominal trauma, molar pregnancy, ectopic pregnancy 9 Critical	[13]	No comparative evidence found.			The effect of sensitising event immunoprophylaxis on the incidence of Rh D alloimmunisation after abdominal trauma, molar pregnancy, or ectopic pregnancy in Rh D negative women is unknown.

Outcome Timeframe	**Study results and measurements**	**Comparator** Risk with placebo or no sensitising event immunoprophylaxis	**Intervention** Risk with sensitising event immunoprophylaxis	**Certainty of the Evidence** (Quality of evidence)	**Summary**
Incidence of a positive test for FMH 6 Important	14	No comparative evidence found.			The effect of sensitising event immunoprophylaxis on the incidence of a positive test for FMH after abdominal trauma, molar pregnancy, or ectopic pregnancy in Rh D negative women is unknown
Adverse neonatal events [15] (e.g. jaundice) 6 Important	16	No comparative evidence found.			The effect of sensitising event immunoprophylaxis on the incidence of adverse neonatal events after abdominal trauma, molar pregnancy, or ectopic pregnancy in Rh D negative women is unknown.
Adverse maternal events attributed to Rh D immunoprophylaxis 6 Important	17	No comparative evidence found.			The effect of sensitising event immunoprophylaxis on the incidence of adverse maternal events after abdominal trauma, molar pregnancy, or ectopic pregnancy in Rh D negative women is unknown.

1. assessed with Enzyme-Coombs screening
2. Systematic review [92] with included studies: Visscher 1972. **Baseline/comparator:** Control arm of reference used for intervention. **Supporting references:** [132],
3. **Risk of Bias: very serious.** One randomised study with plausible bias that raises serious doubts about the results. Method of randomisation not reported and unclear whether treatment allocation concealed. Some concerns with reporting bias and missing data.. **Inconsistency: no serious.** Single study. Heterogeneity not assessed. **Indirectness: serious.** The evidence is not directly applicable to the target population or the Australian healthcare context, and it is difficult to judge whether it could be sensibly applied. Obstetric practice and the baseline characteristics of the population may not be reflective of current practice. The study was conducted in the United States among Rh D negative women with complete miscarriage (n = 9) or incomplete miscarriage with curettage (n = 48). An unknown proportion of women had miscarriage outside the first 12 weeks of pregnancy and the intervention was administered at a dose higher than recommended in Australia (1500 IU vs 625 IU). . **Imprecision: serious.** Small study not sufficiently powered to detect a statistically significant difference. . **Publication bias: serious.** Single study. Publication bias suspected. .

4. assessed with Indirect Coombs
5. Systematic review [92] with included studies: Gavin 1972. There were too few who experienced Rh D alloimmunsation (4-6 months after after incomplete miscarriage or therapeutic abortion) to determine whether routine sensitising event immunoprophylaxis made a difference.. **Baseline/ comparator:** Control arm of reference used for intervention. **Supporting references:** [56],
6. **Risk of Bias: very serious.** Comparative study with some important problems that seriously weakens the confidence in the results. Method of treatment allocation or blinding not reported. Some concerns with reporting bias and missing data.. **Inconsistency: no serious.** Single study. Heterogeneity not assessed. . **Indirectness: serious.** The evidence is not directly applicable to the target population or the Australian healthcare context, and it is difficult to judge whether it could be sensibly applied. Obstetric practice and the baseline characteristics of the population may not be reflective of current practice. The study was conducted in the United States among Rh D negative women who had therapeutic abortion (n = 33) or were treated for incomplete miscarriage (n = 24). Thirteen (22.8%) women were treated outside the first 13 weeks of pregnancy and the dose of intervention (Rhogam) was not stated. . **Imprecision: serious.** Low event rate or wide CIs that cross the line of no effect. Confidence in the results is weak. . **Publication bias: serious.** Single study. Publication bias suspected..
7. assessed with: Enzyme-Coombs screening
8. Systematic review [92] with included studies: Visscher 1972. There were too few who experienced Rh D alloimmunisation (at subsequent pregnancy after spontenous miscarriage or therapeutic evacuation) to determine whether routine sensitising event immunoprophylaxis made a difference.. **Baseline/comparator:** Control arm of reference used for intervention. **Supporting references:** [132],
9. **Risk of Bias: very serious.** One randomised study with plausible bias that raises serious doubts about the results. Method of randomisation not reported and unclear whether treatment allocation concealed. Some concerns with reporting bias and missing data. . **Inconsistency: no serious.** Single study. Heterogeneity not assessed.. **Indirectness: serious.** The evidence is not directly applicable to the target population or the Australian health care context, and it is difficult to judge whether it could be sensibly applied. Obstetric practice and the baseline characteristics of the population may not be reflective of current practice. The study was conducted in the United States among Rh D negative women with complete miscarriage (n = 9) or incomplete miscarriage with curettage (n = 48). An unknown proportion of women had miscarriage outside the first 12 weeks of pregnancy and the intervention was administered at a dose higher than recommended in Australia (1500 IU vs 625 IU). . **Imprecision: serious.** Small study not sufficiently powered to detect a statistically significant difference.. **Publication bias: serious.** Single study. Publication bias suspected. .
10. assessed with: Papain-treated cells or Indirect Coombs
11. Systematic review [92] with included studies: Simonovitis 1974. There were too few who experienced Rh D alloimmunisation at subsequent pregnancy after induced abortion to determine whether routine sensitising event immunoprophylaxis made a difference.. **Baseline/comparator:** Control arm of reference used for intervention. **Supporting references:** [118],
12. **Risk of Bias: very serious.** Comparative study with some important problems that seriously weakens the confidence in the results. Method of treatment allocation or blinding not reported. Some concerns with reporting bias and missing data.. **Inconsistency: no serious.** Single study. Heterogeneity not assessed. . **Indirectness: no serious.** The evidence is probably applicable to the Australian population and healthcare context with some caveats. The study was conducted in Hungary among Rh D negative women in their second pregnancy, whose first pregnancy was terminated in the first trimester by induced abortion (method of termination not clear). The intervention was administered at the same dose as recommended in Australia (250 IU). . **Imprecision: serious.** Low event rate or wide CIs that cross the line of no effect. Confidence in the results is weak. . **Publication bias: serious.** Single study. Publication bias suspected. .

13. Systematic review No studies were found that looked at the effect of sensitising event immunoprophylaxis on the incidence of Rh D alloimmunisation after abdominal trauma, molar pregnancy, or ectopic pregnancy.. **Supporting references:** [94],
14. Systematic review No studies were found that looked at the effect of sensitising event immunoprophylaxis on the incidence of a positive test for FMH after abdominal trauma, molar pregnancy, or ectopic pregnancy.. **Supporting references:** [71],
15. (e.g., jaundice)
16. Systematic review **Supporting references:** [71],
17. Systematic review **Supporting references:** [71],

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Expert opinion point

EOP5: At all times when Rh D immunoglobulin is being administered for a sensitising event, it should be given as soon as practical within 72 hours. If delayed beyond 72 hours, the dose should be given up to 10 days from the sensitising event, but may have lower efficacy.

Practical info

For dosing see Summary of guidance on the use and timing of Rh D immunoglobulin for sensitising event immunoprophylaxis

Expert opinion point

EOP6: For repeated sensitising events in the first 12 weeks of pregnancy, there is no evidence to guide practice. Specialist obstetric consultation is advised regarding further administration of Rh D immunoprophylaxis.

For new sensitising events a repeated dose of Rh D immunoglobulin may be indicated.

For ongoing uterine bleeding alone, a repeat dose of Rh D immunoglobulin (250 IU if during the first 12 weeks and 625 IU if after) may be appropriate after an interval of 6 weeks *[5][6]*.

Practical info

For dosing see Summary of guidance on the use and timing of Rh D immunoglobulin for sensitising event immunoprophylaxis

6.2.3 Universal sensitising event immunoprophylaxis beyond the first 12 weeks of pregnancy and postpartum

In addition to considering key areas of concern for a new evidence-based guideline, the ERG also considered the currency and relevance of guidance in the 2003 guideline *[1]*. The ERG agreed that the clinical guidance on sensitising event immunoprophylaxis beyond the first 12 weeks of pregnancy and postpartum immunoprophylaxis is still current, and therefore a review of the evidence is not required at this time. The existing guidance for both of these issues is presented below. The changes that have been made are based on consensus among the ERG.

Expert opinion point

EOP8: A dose of Rh D immunoglobulin 625 IU should be offered to every Rh D negative woman with no preformed anti-D antibodies, unless NIPT for fetal *RHD* has predicted the fetus to be Rh D negative, to ensure adequate protection against alloimmunisation for the following indications *after* 12^{+6} weeks of pregnancy:

- genetic studies (chorionic villus sampling, amniocentesis and cordocentesis)
- abdominal trauma considered sufficient to cause FMH, even if FMH testing is negative
- each occasion of revealed or concealed antepartum haemorrhage. Where the woman suffers unexplained uterine pain the possibility of concealed antepartum haemorrhage (and the need for immunoprophylaxis) should be considered
- external cephalic version (successful or attempted)
- miscarriage or termination of pregnancy.

Expert opinion point

EOP9: For sensitising events after 20 weeks of pregnancy, the magnitude of FMH should be assessed, and further doses of Rh D immunoglobulin administered if required.*

**The first dose of the Rh D immunoglobulin should be given without waiting for the result of the test for FMH.*

**See Point 4.3 of the BCSH Guidelines for the estimation of fetomaternal haemorrhage [8].*

**See Dosing of Rh D immunoglobulin following fetomaternal haemorrhage quantitation*

Expert opinion point

EOP10: For ongoing uterine bleeding alone beyond 12 weeks gestation a further dose of Rh D immunoglobulin (625 IU) may be appropriate at 6 weekly intervals *[8]*.

New sensitising events should be managed with a further dose of Rh D immunoglobulin (625 IU) and assessment of FMH (after 20 weeks or where otherwise indicated) with additional dosing to cover large volume FMH if required (100 IU for each mL of fetal red cells beyond 6 mL).*

**See Dosing of Rh D immunoglobulin following fetomaternal haemorrhage quantitation*

Expert opinion point

EOP11: In reference to antenatal sensitising events after 20 weeks of pregnancy and after giving birth, a maternal sample to assess the volume of FMH should be taken before administration of Rh D immunoglobulin.

At no time should Rh D immunoglobulin be delayed based on, or pending, the results of testing to quantitate FMH. Between 13 and 20 weeks of pregnancy, the magnitude of FMH may be assessed at clinical discretion.

Expert opinion point

EOP12: The magnitude of the FMH should be assessed by a method capable of quantifying a haemorrhage of ≥ 6 mL of fetal red cells (equivalent to 12 mL of whole blood).

Flow cytometry is accepted as the most accurate quantitative test for FMH and is the method of choice for quantitation if readily available. Where FMH quantitation shows that FMH greater than that covered by the dose already administered has occurred, an additional dose or doses of Rh D immunoglobulin sufficient to provide immunoprophylaxis must be administered as soon as practical within 72 hours.*

If delayed beyond 72 hours, the dose should be given up to 10 days from the sensitising event, but may have lower efficacy.

**See Dosing of Rh D immunoglobulin following fetomaternal haemorrhage quantitation*

Expert opinion point

EOP13: For large bleeds ≥ 6 mL of fetal red cells (equivalent to 12 mL of whole blood), follow-up testing should be performed on a sample collected 48 hours post intravenous Rh D immunoglobulin administration or 72 hours post intramuscular Rh D immunoglobulin administration, to determine whether further dosing is required.

Supplemental Rh D immunoglobulin should be administered if the test for FMH is still positive.*

If testing for fetal cells is negative on a follow-up sample, no further testing is required.

**See Dosing of Rh D immunoglobulin following fetomaternal haemorrhage quantitation*

6.3 Targeted routine antenatal or sensitising event immunoprophylaxis

There are questions over the efficacy of targeted routine antenatal or sensitising event immunoprophylaxis in Rh D negative pregnant women, and about the diagnostic accuracy of NIPT to identify fetal Rh D status. NIPT for

fetal *RHD* is a molecular blood group genotyping assay used to predict the Rh D status of the fetus in pregnancies where the mother is Rh D negative and the fetus is at risk of being affected by HDFN because of anti-D antibodies. It uses a maternal peripheral whole blood sample for the extraction of cell-free DNA (cfDNA),* which is analysed for the presence of the *RHD* gene.

Various terms are used to describe the test for determining the *RHD* genotype of a fetus, including non-invasive prenatal screening, non-invasive prenatal assessment, non-invasive prenatal testing (NIPT) and non-invasive fetal *RHD* genotype testing. The term NIPT for fetal *RHD* is used in the recommendations and expert opinion points. The terminology used in the discussion of evidence reflects the terminology in the literature.

** Cell-free DNA is colloquially known as cell-free fetal DNA (cffDNA).

Strong recommendation , Low certainty evidence

R6: The ERG **recommends** that antenatal Rh D immunoprophylaxis in Rh D negative pregnant women with no preformed anti-D antibodies be targeted to those predicted to be carrying an Rh D positive fetus, based on NIPT for fetal *RHD*.

This applies to both routine and sensitising event immunoprophylaxis, if the result of fetal *RHD* genotyping is available.*

**See EOP3 and EOP8*

Practical info

See Challenges - Consent and the choice to decline Rh D immunoglobulin

Evidence to decision

Benefits and harms

It is estimated that the use of NIPT for fetal *RHD* will result in about 33% to 38% of Rh D negative women avoiding unnecessary exposure to blood products and receiving fewer injections during pregnancy. This will be balanced by the very small increased risk of Rh D alloimmunisation among women with false-negative results, leading to a theoretical increase in the incidence of HDFN and associated complications. This is in line with international guidelines *[8][34][41][44][45][51][55][97][99][126][136]*. Also, the knowledge that an Rh D negative woman is carrying an Rh D positive fetus may improve uptake and adherence to the recommended Rh D immunoprophylaxis regimen.

Potential issues include those surrounding the collection of DNA. Some pregnant women may be aware that their partner is Rh D negative and therefore decline testing. There is a need for counselling in relation to NIPT for fetal *RHD* to address these and other issues, such as the accuracy of the test and the benefits of confirming the Rh D status of the fetus. Counselling should include reassurance that the testing is only for the presence or absence of a single gene, and that no other genetic profile or information will be sought or obtained.

See Safety of Rh D immunoglobulin

Certainty of the Evidence

Low

NIPT for fetal Rh D status is considered highly accurate, with no apparent adverse effects. The test is less accurate when maternal blood is sampled earlier than 11 weeks of pregnancy, and evidence of the performance of the test in multiple pregnancies is very uncertain. The advice of including multiple

pregnancies is in concordance with guidelines for similar programs internationally.

High-throughput testing methodology will need to be validated for the Australian context, with accreditation and standardisation consistent with international standards. Laboratory standardisation would also assist with the collection of data to monitor and track any change in the incidence of sensitisation associated with the introduction of NIPT for fetal *RHD*.

Test thresholds should be set to a minimum of 99% sensitivity, to lessen the number of women with a false-negative test result. These women would be at risk of sensitisation, because they would not be offered antenatal Rh D immunoprophylaxis. It is expected that women with inconclusive test results would either need a repeat test, or would be treated as test positive (in which case, they would receive antenatal Rh D immunoprophylaxis, both routine and for sensitising events if required).

NIPT may be unable to predict the fetal *RHD* type when the mother has a weak or variant D type. Further investigation of the maternal D type by a reference laboratory can provide some guidance for the management of antenatal Rh D immunoglobulin prophylaxis. However, in most cases the pregnant woman should receive antenatal Rh D immunoglobulin as though the maternal blood type is Rh D negative and the fetus assumed to be positive. The blood group of the newborn should be confirmed at birth and postpartum Rh D immunoglobulin administered to women who have delivered an Rh D positive baby.

Given that cfDNA in maternal blood increases throughout the pregnancy, NIPT for fetal *RHD* can be undertaken at any time after 11^{+0} weeks. However, to determine fetal Rh D status before a sensitising event such as an episode of haemorrhage or an amniocentesis in the second trimester, NIPT for fetal *RHD* should be undertaken as soon as possible after 11^{+0} weeks.

There was no comparative evidence examining the clinical effectiveness of targeted Rh D immunoprophylaxis compared with routine universal Rh D immunoprophylaxis.

Certain knowledge of Rh D negativity in the biologic father of the fetus can obviate the need for antenatal prophylaxis, however, paternal testing is not routinely recommended.

Values and preferences

Many pregnant women would prefer to minimise their exposure to blood products where clinically reasonable to do so. NIPT for fetal *RHD* offers the opportunity to avoid the unnecessary administration of Rh D immunoglobulin in about one-third of Rh D negative pregnant women.

The ERG suggests a national program of targeted Rh D immunoprophylaxis needs to maintain universal access to achieve the calculated reductions in requirement for antenatal immunoprophylaxis; that is, all Rh D negative women must have equity of access to NIPT for fetal *RHD*. Policy relating to universal access to NIPT for fetal *RHD* is outside the scope of this guideline.

See Challenges - Consent and the choice to decline Rh D immunoglobulin

Resources and other considerations

Currently, the number of donors for the Rh D immunoglobulin program to maintain an adequate supply of Rh D immunoglobulin is limited. NIPT for fetal *RHD* offers the opportunity to reduce the need for Rh D immunoglobulin.

It is expected that all neonates born to Rh D negative women would continue to have postpartum blood typing of a sample of neonatal or cord blood, and the women would have postnatal Rh D immunoprophylaxis, as required.

Rationale

Question 3 – (Screening intervention)

In Rh D negative pregnant women with no preformed anti-D, does targeted routine antenatal or sensitising event prophylaxis to women with an Rh D positive fetus increase the incidence of Rh D alloimmunisation compared with universal routine antenatal or sensitising event prophylaxis?

Subquestion 3 – (Diagnostic accuracy)

In Rh D negative pregnant women with no preformed anti-D, what is the diagnostic accuracy of non-invasive prenatal screening to identify fetal Rh D status?

The literature search for this question aimed to establish whether targeted Rh D immunoprophylaxis can replace universal immunoprophylaxis during pregnancy, thereby reducing the number of women who need to receive Rh D immunoglobulin. It also considered the diagnostic accuracy of NIPT to identify fetal Rh D status in Rh D negative pregnant women with no preformed anti-D antibodies. This technique replaces the requirement for invasive direct sampling methods for fetal DNA, such as amniocentesis or chorionic villus sampling (CVS) sampling *[32]*.

Clinical question/ PICO

Population: Rh D negative pregnant women with no preformed anti-D
Intervention: Targeted antenatal Rh D Immunoprophylaxis (based on NIPT)
Comparator: Universal antenatal Rh D immunoprophylaxis

Summary

Targeted antenatal Rh D immunoprophylaxis versus universal antenatal Rh D immunoprophylaxis

Three systematic reviews *[101][109][114]* were identified that searched for evidence regarding the comparative effectiveness of targeted antenatal Rh D immunoprophylaxis against universal routine immunoprophylaxis. The reports did not identify any head-to-head studies of targeted versus routine antenatal immunoprophylaxis regimes that met the criteria for this review.

Saramago et al. (2018) *[114]* was a published health technology assessment report conducted for the National Health Service (NHS) in the UK. The study examined the diagnostic accuracy of high-throughput NIPT and the clinical impacts of implementation of targeted antenatal immunoprophylaxis, to underpin an economic assessment. Seven observational studies were identified in the review of clinical effectiveness. Two studies *[33][126]* assessed the incidence of Rh D alloimmunisation in women receiving NIPT compared with controls (i.e. women who did not receive RAADP). The remaining five studies were single-armed, noncomparative cohort studies for women receiving NIPT only *[41][47][60][61][120]*.

The Ontario Health (2020) *[101]* report was a health technology assessment used to inform the Canadian Agency for Drugs and Technologies in Health on the use of NIPT for fetal Rh D blood group genotyping. The authors conducted a systematic search of the literature to create an overview of reviews for test accuracy, clinical utility and cost-effectiveness compared with usual care. Similarly,

Runkel et al. (2020) *[109]* was a published summary of a health technology assessment report used to inform the German Institute for Quality and Efficiency in Health Care. In the absence of RCT evidence, the authors used a linked evidence approach to inform reimbursement decisions regarding Rh D testing in non-sensitised Rh D negative pregnant women.

Assuming that any relevant primary studies had been identified in Saramago et al. (2018) *[114]*, the systematic screen for RCTs and nonrandomised studies was conducted in studies published 6 months after the literature search date of that review (2015 onwards). No additional studies were identified.

Clinical effectiveness

None of the identified studies provided sufficient information to assess clinical effectiveness; therefore, Saramago et al. (2018) *[114]* conducted a Monte Carlo simulation relevant to the UK health system, based on data presented in each of the studies. The model was populated using results from the diagnostic accuracy of high-throughput NIPT to identify fetal Rh D status and other relevant parameters required to provide a link between the diagnostic accuracy, the impact of subsequent treatment decisions, and the ultimate effect on health outcomes and costs. The sensitivity of NIPT used in the model was 99.79% (95% CI 99.52, 99.01) and the specificity was 95.42% (95% CI 95.42, 92.84).

The following clinical scenarios were considered:*

- no antenatal Rh D immunoglobulin; postpartum Rh D immunoglobulin based on cord blood serology only (control)
- antenatal Rh D immunoglobulin offered to all Rh D negative women; postpartum Rh D immunoglobulin based on cord blood serology (current practice)
- antenatal Rh D immunoglobulin offered based on NIPT; postpartum Rh D immunoglobulin based on cord blood test for all Rh D negative women
- both antenatal and postpartum Rh D immunoglobulin based on NIPT only; no cord blood testing.

No additional studies to those identified by Saramago et al. (2018) *[114]* were identified in this review; therefore, the results of the model were considered.

The authors noted that the determination of the Rh D status of the fetus through NIPT may affect the administration of Rh D immunoglobulin in three situations: following potentially sensitising events, before routine third trimester administration and at birth. In addition, NIPT results may affect postpartum maternal screening for alloimmunisation, screening for FMH and cord blood testing. The test is not perfect; thus, some women with an Rh D negative fetus will still receive Rh D immunoglobulin (e.g. those with an Rh D negative fetus who screen as 'inconclusive', those who fail to undertake the screening test and those with a false-positive test result).

The model from Saramago et al. (2018) *[114]* estimated that targeted RAADP increased the risk of Rh D alloimmunisation from 281 per 100 000 pregnant women with universal RAADP to 284 (base case scenario) or 309 (worst case scenario) per 100 000. That is, the use of NIPT to determine whether women would receive Rh D immunoglobulin would increase the number of Rh D sensitisations by between 3 and 15 in 100 000 pregnancies if postpartum cord blood testing were continued, or between 15 and 28 per 100 000 women if postpartum cord blood testing were withdrawn (and postnatal Rh D immunoglobulin was given or withheld on the basis of the NIPT result).The range in numbers is due to different assumptions as to whether women who do not receive NIPT would still be offered RAADP.

The Ontario Health (2020) *[101]* report estimated the risk of Rh D alloimmunisation was 45% lower in the cohort that received NIPT compared with the historic reference cohort that received postnatal and

antenatal Rh D immunoglobulin prophylaxis following any potentially sensitising event. These data are based on results reported by Tiblad et al. (2013) *[126]* who estimated the incidence of Rh D alloimmunisation in Stockholm had decreased from 0.46% (95% CI 0.37%, 0.56%) in 2003 to 2008 to 0.26% (95% CI 0.15%, 0.36%) in 2009 (after the introduction of NIPT).

Use of Rh D immunoglobulin

Based on an assumed compliance of 99%, the simulation model *[114]* estimated that the use of NIPT to determine RAADP would reduce the number of Rh D negative women receiving Rh D immunoglobulin to between 62.7% and 65.9%. This corresponds to an estimated reduction in the use of Rh D immunoglobulin of between 33.1% and 36.9%. These results were sensitive to compliance, with the range in numbers being due to different assumptions as to whether women who do not receive NIPT would still be offered RAADP.

In this model, the number of women who would avoid unnecessary Rh D immunoprophylaxis would be reduced from 38.9% to 4.5–5.7%, and the number of women who would fail to receive needed immunoprophylaxis would increase from an estimated 0.6% to 1.2–3.2%. The estimated one-third reduction in the use of Rh D immunoglobulin corresponds with the observed numbers reported by Soothill et al. (2015) *[120]* (29%) and Banch Clausen et al. (2014) *[33]* (37.1%), which were used to inform the simulation model. It also corresponds with the reduction reported by Macher et al. (2012), *[81]* who observed a 38% reduction in the use of Rh D immunoglobulin in a single centre in Spain .

Similar data from eight studies were presented in the Ontario Health (2020) *[101]* report which indicated between 25.3% to 39% of all Rh D negative pregnant women avoided unnecessary Rh D immunoprophylaxis after the introduction of noninvasive fetal Rh D blood group genotyping. Among the Rh D negative women carrying an Rh D negative fetus (i.e. not at risk for alloimmunisation), an estimated 93% avoided unnecessary Rh D immunoprophylaxis.

Incidence of a positive test for FMH

No studies were identified.

Adverse neonatal events

No studies were identified.

Adverse maternal events attributed to Rh D immunoglobulin administration

No studies were identified.

*Assumptions that feed into the model are provided in Saramago et al. (2018) *[114]*.

Outcome Timeframe	**Study results and measurements**	**Comparator** Risk with universal routine antenatal Rh D immunoprophylaxis	**Intervention** Risk with targeted antenatal Rh D immunoprophylaxis	**Certainty of the Evidence** (Quality of evidence)	**Summary**
Incidence of Rh D alloimmunisatio	1	One study conducted a simulation based on diagnostic accuracy of the test and expected management in			No studies were found that directly assessed the effect of targeted routine antenatal or sensitising

Outcome Timeframe	**Study results and measurements**	**Comparator** Risk with universal routine antenatal Rh D immunoprophylaxis	**Intervention** Risk with targeted antenatal Rh D immunoprophylaxis	**Certainty of the Evidence** (Quality of evidence)	**Summary**
n 9 Critical		women with positive and negative test results. The authors estimated targeted RAADP increased the risk of Rh D alloimmunisation from 281 per 100 000 pregnancies with universal RAADP to 284 (base case scenario) or 309 (worst case scenario) per 100 000.			event immunoprophylaxis on the incidence of Rh D alloimmunisation
Utilisation of Rh D immunoglobulin 9 Critical	2	One study conducted a simulation based on data from three noncomparative studies, and estimated utilisation of anti-D would decrease by between 33.1% and 36.9%.			No comparative studies were found that directly assessed the effect of targeted routine antenatal or sensitising event immunoprophylaxis on utilisation of anti-D.
Incidence of a positive test for FMH 6 Important	3	No evidence			No studies were found that directly assessed the effect of targeted routine antenatal or sensitising event immunoprophylaxis on the incidence of a positive test for FMH.
Adverse neonatal events		No evidence			No studies were found that reported any data on adverse neonatal events relating to NIPT or antenatal anti-D administration.
Adverse maternal events attributed to Rh D immunoprophylaxis		No evidence			No studies were found that reported any data on adverse maternal events relating to NIPT or antenatal anti-D administration.

1. Systematic review [114]. **Supporting references:** [70], [101],
2. Systematic review [114]. **Supporting references:** [120], [61], [33],
3. Systematic review [16].

References

16. National Blood Authority (NBA). Update of the 2003 guidelines on the prophylactic use of Rh D immunoglobulin (anti-D) in obstetrics: Technical report volume 1 Evidence review. Canberra: The Commonwealth of Australia as represented by the NBA 2019. Website

33. Banch Clausen F., Steffensen R., Christiansen M, Rudby M, Jakobsen MA, Jakobsen TR, et al. Routine noninvasive prenatal screening for fetal RHD in plasma of RhD negative pregnant women - 2 years of screening experience from Denmark. Prenatal diagnosis 2014;34(10):1000-1005 Journal

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101. Ontario Health. Noninvasive fetal rhd blood group genotyping: A health technology assessment. Ontario Health Technology Assessment Series 2020;20(15):1-160 Website

120. Soothill PW, Finning K., Latham T., Wreford-Bush T., Ford J., Daniels G.. Use of cffDNA to avoid administration of anti-D to pregnant women when the fetus is RhD-negative: implementation in the NHS. BJOG: An International Journal of Obstetrics & Gynaecology 2015;122(12):1682-1686 Pubmed Journal

Clinical question/ PICO

Population: Rh D negative pregnant women with no preformed anti-D (for routine or sensitising event immunoprophylaxis)

Intervention: NIP screening for fetal Rh D status

Comparator: Postnatal cord blood testing (or other neonatal sample) for fetal Rh D status or other noninvasive prenatal test for fetal Rh D status

Summary

Diagnostic accuracy of NIPT for fetal Rh D status

Six systematic reviews were identified that examined the diagnostic accuracy of NIPT to identify fetal Rh D status *[24][57][86][109][114][141]*. The reviews included over 90 studies meeting their search criteria. Assuming that relevant primary studies had been identified, the screening for RCTs and diagnostic accuracy studies was limited to those published after the literature search date of Saramago et al. (2018) *[114]*. Studies excluded by the included reviews were also scrutinised for inclusion.

Seven additional diagnostic accuracy studies (with consecutive pregnant women) *[27][62][81][87][89][104][107]* and seven additional diagnostic accuracy studies (with non-consecutive pregnant women) *[68][69][102][103][111][121][77]* were identified and subsequently included in this review.

Studies that were excluded were those of small sample size (N<200), conference abstracts that did not provide sufficient data, and those in which the NIPT was not conducted in a context considered similar to Australia (see Appendix B of Volume 2 *[17]* and Volume 3 *[18]* of the technical report).

Alsheri et al. (2021) *[24]* was a systematic review focused on the diagnostic accuracy of NIPT to identify fetal Rh D status. The authors identified 16 studies, 11 of which were included in a bivariate meta-analysis. Runkel et al. (2020) *[109]* also conducted a bivariate meta-analysis of diagnostic accuracy studies involving 12 studies across 10 countries. The authors had identified over 70 studies but noted the size of 58 studies was small (between 2 and 467 participants) so were not included in meta-analysis.

Saramago et al. (2018) *[114]* only considered studies that used high-throughput NIPT, defined by the authors as any NIPT that was conducted using an automatic robotic platform (including automated DNA extraction and liquid handling) able to process large numbers of samples rapidly for large-scale screening purposes. Studies in which the test was used to determine fetal genotype in women who had already been sensitised were excluded. There were no restrictions on gestational age or exclusion of tests conducted in multiple pregnancies. The literature search was conducted from database inception to February 2016, with eight studies meeting these inclusion criteria.

Mackie et al. (2017) *[86]* looked at cfDNA NIPT in singleton pregnancies for various conditions including Rh D status. The meta-analysis was restricted to cohort studies that used outcome at birth for the reference standard, but it was noted that 12 of the included studies used CVS or amniocentesis results as the reference standard. Thirty studies (10 290 tests) were identified that had been conducted in various countries, including Australia. Key concerns related to subject selection bias and index test bias, with only 13 of 30 studies reporting inconclusive test results. The diagnostic accuracy of different test platforms – real-time quantitative polymerase chain reaction (PCR), conventional PCR and mass spectrometry – was explored.

Zhu et al. (2014) *[141]* identified 41 publications (11 129 tests) that assessed NIPT for fetal Rh D status using cfDNA in maternal whole blood only. No details regarding the included studies or assessment of bias were provided. It is unclear whether any effort was made to ensure that duplicate sample results were not included. The diagnostic accuracy of testing was assessed by gestational age at time of sampling.

Geifman-Holtzman et al. (2006) *[57]* identified 37 publications performing 44 protocols and involving 3261 samples. The meta-analysis was restricted to studies that used outcome at birth for the reference standard. Descriptions of the risk of bias assessment for the included studies were not presented, but the authors noted that 16 included studies reported 100% diagnostic accuracy in their fetal *RHD* genotyping, and many authors excluded samples because of the absence of detectable DNA or the inability to verify fetal or neonatal blood type, suggesting possible reporting biases. The diagnostic accuracy of testing was assessed by gestational age at time of sampling.

The additional diagnostic accuracy studies (with consecutive pregnant women) were performed in a variety of countries including Canada, Czech Republic, Finland, India, Italy, Spain and the United States, and used NIPT of cfDNA in maternal plasma targeting exons 5 and 7 of the *RHD* gene *[62][81][107]*, exons 5 and 10 *[104]*, exons 5, 7 and 10 *[27][87]*, or exons 4, 5 and 7, as well as probes for the 37-base pair insertion in exon 4 (*RHD* pseudogene) *[89]*. The additional diagnostic accuracy studies (with non-consecutive pregnant women) were conducted in Austria, Australia, Cyprus, Denmark, Ireland, Norway and Poland, and used NIPT of cfDNA in maternal plasma targeting exons 5 and 7 of the *RHD* gene *[102]*, exons 5 and 10 *[68][69][111][121]* or exons 5, 7 and 10 *[77][103]*.

The reference standard used in all studies was serological testing at birth, except for one study in which it was not stated *[111]*. The studies enrolled Rh D negative pregnant women with gestational

ages ranging between 5 and 39 weeks. Participants were predominantly Caucasian. None of the studies reported whether any procedural complications were attributed to either test.

Some included studies were at risk of selection bias. Women who were Rh D alloimmunised were explicitly excluded in two studies *[62][89]*, and one study only included women with suspected red cell alloimmunisation *[102]*. Multiple gestation pregnancies may pose an issue for NIPT (e.g. if twin fetuses have discordant Rh D status); thus, exclusion of multiple pregnancies may also introduce selection bias. Multiple pregnancies were included in five studies *[62][69][81][87][89]*, whereas their inclusion or exclusion was not stated in the other studies.

Manfroi et al. (2018) *[87]* recruited women who had partners known to be Rh D positive, or partners of unknown Rh D phenotype (while excluding those who had partners known to be Rh D negative). The study by Papasavva et al. (2016) *[103]* was conducted in a Cypriot population, where the prevalence of Rh D negative serology was estimated to be 7.2% (95% CI 5, 10). In addition, the study enrolled pregnant women with Rh D positive partners. For these reasons, in both studies a higher proportion of Rh D positive neonates would be expected than among all Australian neonates born to Rh D negative women.

Inconclusive results were reported in only seven studies *[62][68][69][87][89][103][121]*. Exclusion of inconclusive results would introduce bias in favour of the index test.

The sex-determining region Y (*SRY*) gene was used as an internal control for male fetal DNA in three studies, which may also have introduced bias *[81][102][103]*. Other studies used internal controls to account for the total genomic DNA *[69][111][121]*. In the nationwide screening program, no internal control was used *[62]*.

NIPT for fetal Rh D status sensitivity and specificity

Each of the included studies varied in their inclusion criteria (e.g. exclusion of multiple pregnancies), how inconclusive test results were handled (e.g. counted as test positive or investigated further), gestational age at sampling and the conduct of the test (e.g. number and location of exons used, type of platform and source of fetal DNA sample). Therefore, several analyses were conducted to assess the implications for diagnostic performance (see subgroup analyses, below).

Saramago et al. (2018) *[114]* conducted a bivariate meta-analysis of eight studies that were considered most applicable to the UK health care system. Sensitivity was estimated to be 99.66 (95% CI 99.24, 99.85) and specificity was 96.14 (95% CI 94.18, 97.46). The I^2 statistic for heterogeneity was 75% for sensitivity and 99% for specificity. The authors noted that the high heterogeneities are, in part, a consequence of the high accuracy of the test and the large size of the studies (and consequently small within-study variance), rather than being indicative of any clinically meaningful differences between studies, because I^2 increases as the average within-study variance declines.

Similar data were reported in the bivariate analyses by Runkel et al. (2020) *[109]* and Alshehri et al. (2021) *[24]* with pooled data showing high sensitivity and high specificity, respectively (12 studies, 60 011 participants: 99.9%; 95% CI 99.5, 100 and 99.2%; 95% CI 98.5, 99.5) and (16 studies, number of participants not reported: 99.3%; 95% CI 98.7, 99.7 and 98.4%; 95% CI 97.4, 99).

Saramago et al. (2018) *[114]* also conducted sensitivity analyses to adjust for potential bias associated with two of the studies *[61] [125]* that did not report inconclusive results (resulting in a potential overestimate of diagnostic accuracy). In this analysis, sensitivity was 99.62 (95% CI 99.06, 99.85) and specificity was 95.63 (95% CI 93.22, 97.21).

The bivariate meta-analysis reported by Mackie et al. (2017) *[86]* provided a sensitivity of 99.3 (95% CI 98.2, 99.7) and a specificity of 98.4 (95% CI 96.4, 99.3). Seventeen of the 30 studies included in the

meta-analysis did not report inconclusive results, which may result in an overestimation of test accuracy. The authors noted that the most common reasons given for inconclusive results (in order of frequency) were: no reason given, *RHD* gene variant, insufficient number of markers present from prespecified cut-off, test failure or low fetal fraction (of free DNA detected in maternal blood). The most common reasons for false-positive results were: presumed low fetal fraction (not quantified by authors), no reason given, presumed *RHD* gene variant (not confirmed), confirmed *RHD* gene variant, test failure, possible contamination, DNA degradation, pipetting error or incorrect neonatal blood testing.

The meta-analysis by Zhu et al. (2014) *[141]* (random effects) included 44 studies, many of which probably overlapped with those included by Mackie et al. (2017) *[86]*, but full details regarding the included studies were not provided. It is likely that inconclusive results were not included in the analysis. Here, sensitivity was estimated to be 99 (95% CI 99, 99) and specificity was 98 (95% CI 97, 98). The I^2 statistic for heterogeneity was 80.5% for sensitivity and 78% for specificity; this is probably due to small within-study variance rather than representing clinically meaningful differences between studies.

Geifman-Holtzman et al. (2006) *[57]* conducted two meta-analyses involving up to 44 protocols, with the random effects model estimating a sensitivity of 95.4 (95% CI 90.6, 97.8) and a specificity of 98.6 (95% CI 96.4, 99.5), and the Bayesian model estimating a sensitivity of 96.7 (95% CI 92.5, 98.9) and a specificity of 98.9 (95% CI 96.7, 99.9). Details of the included studies were not provided, but it is likely that inconclusive results and substandard samples were not included in the analysis.

For the Australian context, it was assumed women with inconclusive results would be treated as test positive (without further testing); therefore, for the purposes of analysis in this review, all reported inconclusive results were treated as test positive.

Among the 13 protocols (10 studies) identified in this review, 12 showed a sensitivity of 100%, meaning that all women with an Rh D positive fetus would be correctly identified. Picchiassi et al. (2015) *[107]* reported a sensitivity of 92.8 (95% CI 86.9, 96.2), which is notably lower than the other studies and is probably due to the small sample size and the early gestational age (10–15 weeks of pregnancy) at which sampling for fetal DNA occurred (see subgroup analyses below).

The widest 95% confidence interval for sensitivity (95% CI 93 to 100) was observed in a small study conducted in Cyprus *[103]* that involved 73 women with Rh D positive partners. This means that, potentially, up to 7% of women with an Rh D positive fetus would be incorrectly identified. The single reverse transcriptase PCR (RT-PCR) protocol reported by Macher et al. (2012) *[81]* also had a wide confidence interval (95% CI 95, 100), which was improved with the transition to multiplex RT-PCR (95% CI 99, 100).

For diagnostic specificity, the protocols ranged between 91.60 (95% CI 89, 94) *[69]* and 100 (95% CI 81, 100) *[103]* meaning that up to 8.4% (between 6% and 11%) of women with an Rh D negative fetus would be incorrectly identified. The heterogeneity in specificity is likely to be a consequence of differences in reporting and handling of inconclusive tests.

A bivariate meta-analysis of included studies revealed a sensitivity of 0.997 (95% CI 0.994, 0.999) and specificity of 0.983 (95% CI 0.974, 0.989) (random effects correlation 0.412).

Subgroup analyses of sensitivity and specificity

Method of detection

Mackie et al. (2017) *[86]* performed a subgroup analysis to assess whether different technologies or techniques used to detect Rh D status include diagnostic performance. Here, better diagnostic

performance was observed with RT-PCR (sensitivity of 99.7; specificity of 98.9) than with conventional PCR (sensitivity of 92.4; specificity of 95.4). Saramago et al. (2018) *[114]* noted that, because each country used a different machine to perform NIPT, a subgroup analysis by type of NIPT method was not feasible because it would be confounded by study location.

Sample source

Geifman-Holtzman et al. (2006) *[57]* demonstrated a significant improvement in diagnostic performance using free fetal DNA from maternal serum, plasma or blood (diagnostic accuracy between 91.8% and 96.5%) compared with using DNA or RNA from fetal cells within maternal blood (diagnostic accuracy between 67.7% and 76.3%).

Alloimmunised women

Geifman-Holtzman et al. (2006) *[57]* also performed a subgroup analysis of the diagnostic performance of NIPT in Rh D negative pregnant women who were alloimmunised. The analysis showed diagnostic accuracy to be 91.8% in this group.

Gestational age

Saramago et al. (2018) *[114]* performed a subgroup analysis to determine the significance of gestational age on false-negative rate (FNR), false-positive rate (FPR) and number of inconclusive results in the included studies. This analysis was undertaken because of concerns that diagnostic sensitivity and specificity is worse in samples collected before 11 weeks of pregnancy (due to the lower amount of cfDNA in maternal blood). The study authors plotted FNR against gestational age of the included studies, and found that FNRs were higher before 11 weeks of pregnancy but were consistent after 11 weeks of pregnancy. No obvious relationship between gestational age and FPR or number of inconclusive results was observed.

Ethnicity

Saramago et al. (2018) *[114]* intended to assess whether ethnicity affected diagnostic performance of NIPT for fetal Rh D status, but found the relevant data were not reported in any publication. All studies were conducted in Europe; hence, numbers of participants of non-white ethnicity were likely to be few.

Supplementary data provided in the study reported by de Haas 2016 *[52]** revealed 100% sensitivity regardless of ethnicity (95% CI ranged from 93 to 100 in Asian and Hindustani populations). However, women of Creole ethnicity had noticeably lower specificity (71; 95% CI 57, 83) than women of European ethnicity (98; 95% CI 98, 98).

*This study population overlaps with the population reported by Thurik et al. (2015) *[125]* and De Haas et al. (2012) *[60]* that was included in Saramago et al. (2018) *[114]*.

Outcome Timeframe	**Study results and measurements**	**Comparator** Reference standard	**Intervention** NIPT for fetal Rh D status	**Certainty of the Evidence** (Quality of evidence)	**Summary**
True positives [1] 9 Critical	Based on data from 76,349 participants in 48 studies. [2]	575 to 620 per 1000 patients tested (95% CI) with assumed pre-test prevalence of 62% (likely estimate for Australia).		High [3]	Around 57.5% to 62.0% of Rh D negative women would receive Rh D immunoglobulin.

Outcome Timeframe	**Study results and measurements**	**Comparator** Reference standard	**Intervention** NIPT for fetal Rh D status	**Certainty of the Evidence** (Quality of evidence)	**Summary**
False negatives [4] 9 Critical	Based on data from 76,349 participants in 48 studies. [5]	0 to 45 per 1000 patients tested (95% CI) with assumed pre-test prevalence of 62% (likely estimate for Australia).		High [6]	Around 0% to 4.5% of Rh D negative women with an Rh D positive fetus would not receive Rh D immunoglobulin.
True negatives [7] 9 Critical	Based on data from 76,349 participants in 48 studies.	348 to 380 per 1000 patients tested (95% CI) with assumed pre-test prevalence of 62% (likely estimate in Australia)		High [8]	Around 34.8% to 38.0% of Rh D negative women would avoid unnecessary Rh D immunoglobulin. These women would avoid two injections of Rh D immunoglobulin (current recommendation is two doses at 28 and 34 weeks of pregnancy). This assumes the sampling is derived from bloods already taken, and that they would also not receive postnatal Rh D immunoglobulin after cord serology.
False positives [9] 9 Critical	Based on data from 76,349 participants in 48 studies.	0 to 32 per 1000 patients tested (95% CI) with assumed pre-test prevalence of 62% (likely estimate in Australia)		High [10]	Around 0% to 3.2% of women would unnecessarily receive Rh D immunoglobulin. This is much smaller than the current rate of 35% to 40%, which occurs with universal RAADP. No adverse effects are anticipated to occur in these women.
Inconclusive		Where possible, inconclusive results were treated as test positive.			Approximately 6.7% of results are estimated to be inconclusive.

1. (patients with positive fetal Rh D status)
2. Systematic review [145]. The prevalence of Rh D positive babies born to Rh D negative women in Australia is not known, but it was considered reasonable to assume a similar prevalence as estimated for the UK (62% estimated by Saramago et al. (2018)84). This is based on the prevalence of Rh D negative status in the donor population in Australia (15%), which is comparable with the UK. Range of sensitivities: 0.93 to 1.00 | Range of specificities: 0.92 to 1.00. **Supporting references:** [16], The prevalence of Rh D positive babies born to Rh D negative women in Australia is not known, but it was considered reasonable to assume a similar prevalence as estimated for the UK (62% estimated by Saramago et al. (2018)). This is based on the prevalence of Rh D negative status in the donor population in Australia (15%), which is comparable with the UK.. [86], [114],

3. **Risk of Bias: no serious.** Despite some gaps in reporting, most included studies were judged to be at low risk of bias. Concerns relating to selection bias (e.g. exclusion of multiple pregnancies or exclusion of sensitised women) or conduct of the index test (e.g. number of exons amplified and controls used) were small, and are not considered to have substantially altered the test results. Cord blood serology was the reference standard in all studies and was usually conducted independent of the index test. . **Inconsistency: no serious.** Almost all studies were consistent, and inconsistencies could be explained. Samples taken before 12 weeks of pregnancy would reduce confidence in the specificity of the test. Some studies did not report inconclusive results, which would favour the index test; however, this was not considered to substantially reduce the confidence in the overall quality of the evidence.. **Indirectness: no serious.** The evidence was considered applicable to the Australian health care context with some caveats. Much of the evidence is from Northern European countries with a predominantly Caucasian majority. This was considered comparable to the Australian context in which the prevalence of the Rh D negative phenotype among donors is around 15%. The prevalence of Rh D negative babies born to Rh D negative women is estimated to be 38%, but the prevalence of specific RHD genotypes is not known. The meta-analyses by Zhu et al. (2014) and Geifman-Holtzman et al. (2006) were not included, because changes and improvements have occurred in how the test is conducted. It is expected that the screening test would, at a minimum, include primers for two exons (4, 5, 7 or 10), involve real-time quantitative polymerase chain reaction (RT-qPCR) and be conducted in duplicate. Diagnostic performance may by overestimated if only high-throughput studies are considered (as reported in Saramago et al. (2018)); therefore the inclusion of Mackie et al. (2017) and smaller studies was considered appropriate for the Australian context. Care should be taken when interpreting test results in women with multiple pregnancies, because this subgroup was excluded from the meta-analysis by Mackie et al. (2017) and other studies. . **Imprecision: no serious.** Many studies were included. Smaller CIs were observed in the large studies with central reference laboratories and those that used thresholds to maintain an acceptable level of sensitivity, and thus confidence in the evidence from those studies is high. In small, single-centre studies, a wider confidence interval would suggest a lower certainty of evidence. . **Publication bias: no serious.**
4. (patients incorrectly classified as not having positive fetal Rh D status)
5. Systematic review Assuming that routine postnatal Rh D immunoprophylaxis continues, the likelihood of a woman with a false-negative result experiencing a sensitising event is approximately 0.3%.91 Of these events, the likelihood that sensitisation causes mild HDFN is 90% and that it causes severe morbidity is 10%. Among those with severe morbidity, fetal death is estimated to occur in 5%. Range of sensitivities: 0.93 to 1.00 | Range of specificities: 0.92 to 1.00. **Supporting references:** [16], Assuming that routine postnatal Rh D immunoprophylaxis continues, the likelihood of a woman with a false-negative result experiencing a sensitising event is approximately 0.3%. Of these events, the likelihood that sensitisation causes mild HDFN is 90% and that it causes severe morbidity is 10%. Among those with severe morbidity, fetal death is estimated to occur in 5%.. [86], [114],
6. **Risk of Bias: no serious.** Despite some gaps in reporting, most included studies were judged to be at low risk of bias. Concerns relating to selection bias (e.g. exclusion of multiple pregnancies or exclusion of sensitised women) or conduct of the index test (e.g. number of exons amplified and controls used) were small, and are not considered to have substantially altered the test results. Cord blood serology was the reference standard in all studies and was usually conducted independent of the index test. . **Inconsistency: no serious.** Almost all studies were consistent, and inconsistencies could be explained. Samples taken before 12 weeks of pregnancy would reduce confidence in the specificity of the test. Some studies did not report inconclusive results, which would favour the index test; however, this was not considered to substantially reduce the confidence in the overall quality of the evidence.. **Indirectness: no serious.** The evidence was considered applicable to the Australian health care context with some caveats. Much of the evidence is from Northern European countries with a predominantly Caucasian majority. This was considered comparable to the Australian context in which the prevalence of the Rh D negative phenotype among donors is around 15%. The prevalence of Rh D negative babies born to Rh D negative

women is estimated to be 38%, but the prevalence of specific RHD genotypes is not known. The meta-analyses by Zhu et al. (2014) and Geifman-Holtzman et al. (2006) were not included, because changes and improvements have occurred in how the test is conducted. It is expected that the screening test would, at a minimum, include primers for two exons (4, 5, 7 or 10), involve real-time quantitative polymerase chain reaction (RT-qPCR) and be conducted in duplicate. Diagnostic performance may by overestimated if only high-throughput studies are considered (as reported in Saramago et al. (2018)); therefore the inclusion of Mackie et al. (2017) and smaller studies was considered appropriate for the Australian context. Care should be taken when interpreting test results in women with multiple pregnancies, because this subgroup was excluded from the meta-analysis by Mackie et al. (2017) and other studies. . **Imprecision: no serious.** Many studies were included. Smaller CIs were observed in the large studies with central reference laboratories and those that used thresholds to maintain an acceptable level of sensitivity, and thus confidence in the evidence from those studies is high. In small, single-centre studies, a wider confidence interval would suggest a lower certainty of evidence. . **Publication bias: no serious.**

7. (patients without positive fetal Rh D status)
8. **Risk of Bias: no serious.** Despite some gaps in reporting, most included studies were judged to be at low risk of bias. Concerns relating to selection bias (e.g. exclusion of multiple pregnancies or exclusion of sensitised women) or conduct of the index test (e.g. number of exons amplified and controls used) were small, and are not considered to have substantially altered the test results. Cord blood serology was the reference standard in all studies and was usually conducted independent of the index test. . **Inconsistency: no serious.** Almost all studies were consistent, and inconsistencies could be explained. Samples taken before 12 weeks of pregnancy would reduce confidence in the specificity of the test. Some studies did not report inconclusive results, which would favour the index test; however, this was not considered to substantially reduce the confidence in the overall quality of the evidence.. **Indirectness: no serious.** The evidence was considered applicable to the Australian health care context with some caveats. Much of the evidence is from Northern European countries with a predominantly Caucasian majority. This was considered comparable to the Australian context in which the prevalence of the Rh D negative phenotype among donors is around 15%. The prevalence of Rh D negative babies born to Rh D negative women is estimated to be 38%, but the prevalence of specific RHD genotypes is not known. The meta-analyses by Zhu et al. (2014) and Geifman-Holtzman et al. (2006) were not included, because changes and improvements have occurred in how the test is conducted. It is expected that the screening test would, at a minimum, include primers for two exons (4, 5, 7 or 10), involve real-time quantitative polymerase chain reaction (RT-qPCR) and be conducted in duplicate. Diagnostic performance may by overestimated if only high-throughput studies are considered (as reported in Saramago et al. (2018)); therefore the inclusion of Mackie et al. (2017) and smaller studies was considered appropriate for the Australian context. Care should be taken when interpreting test results in women with multiple pregnancies, because this subgroup was excluded from the meta-analysis by Mackie et al. (2017) and other studies. . **Imprecision: no serious.** Many studies were included. Smaller CIs were observed in the large studies with central reference laboratories and those that used thresholds to maintain an acceptable level of sensitivity, and thus confidence in the evidence from those studies is high. In small, single-centre studies, a wider confidence interval would suggest a lower certainty of evidence. . **Publication bias: no serious.**
9. (patients incorrectly classified as having positive fetal Rh D status)
10. **Risk of Bias: no serious.** Despite some gaps in reporting, most included studies were judged to be at low risk of bias. Concerns relating to selection bias (e.g. exclusion of multiple pregnancies or exclusion of sensitised women) or conduct of the index test (e.g. number of exons amplified and controls used) were small, and are not considered to have substantially altered the test results. Cord blood serology was the reference standard in all studies and was usually conducted independent of the index test. . **Inconsistency: no serious.** Almost all studies were consistent, and inconsistencies could be explained. Samples taken before 12 weeks of pregnancy would reduce confidence in the specificity of the test. Some studies did not report inconclusive results, which would favour the index test; however, this was not

considered to substantially reduce the confidence in the overall quality of the evidence.. **Indirectness: no serious.** The evidence was considered applicable to the Australian health care context with some caveats. Much of the evidence is from Northern European countries with a predominantly Caucasian majority. This was considered comparable to the Australian context in which the prevalence of the Rh D negative phenotype among donors is around 15%. The prevalence of Rh D negative babies born to Rh D negative women is estimated to be 38%, but the prevalence of specific RHD genotypes is not known. The meta-analyses by Zhu et al. (2014) and Geifman-Holtzman et al. (2006) were not included, because changes and improvements have occurred in how the test is conducted. It is expected that the screening test would, at a minimum, include primers for two exons (4, 5, 7 or 10), involve real-time quantitative polymerase chain reaction (RT-qPCR) and be conducted in duplicate. Diagnostic performance may by overestimated if only high-throughput studies are considered (as reported in Saramago et al. (2018)): therefore the inclusion of Mackie et al. (2017) and smaller studies was considered appropriate for the Australian context. Care should be taken when interpreting test results in women with multiple pregnancies, because this subgroup was excluded from the meta-analysis by Mackie et al. (2017) and other studies. . **Imprecision: no serious.** Many studies were included. Smaller CIs were observed in the large studies with central reference laboratories and those that used thresholds to maintain an acceptable level of sensitivity, and thus confidence in the evidence from those studies is high. In small, single-centre studies, a wider confidence interval would suggest a lower certainty of evidence. . **Publication bias: no serious.**

References

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86. Mackie FL, Hemming K., Allen S., Morris RK, Kilby MD. The accuracy of cell-free fetal DNA-based non-invasive prenatal testing in singleton pregnancies: a systematic review and bivariate meta-analysis. BJOG: An International Journal of Obstetrics and Gynaecology 2017;124(1):32-46 Journal

114. Saramago P., Yang H., Llewellyn A., Walker R., Harden M., Palmer S., et al. High-throughput non-invasive prenatal testing for fetal rhesus D status in RhD-negative women not known to be sensitised to the RhD antigen: A systematic review and economic evaluation. Health Technology Assessment 2018;22(13) Journal

145. Jorgensen M, Allerdice S. NIPT versus serological testing for fetal RHD status in pregnant RhD-women. 2021.

Strong recommendation , Low certainty evidence

R7: If fetal Rh D status is not available or is uncertain, the ERG **recommends** that antenatal Rh D immunoprophylaxis be offered to Rh D negative pregnant women with no preformed anti-D antibodies.

See R6 for Research evidence and References

Practical info

See Challenges - Consent and the choice to decline Rh D immunoglobulin

Evidence to decision

Benefits and harms

See Benefits and harms R6

Certainty of the Evidence

Low

See Certainty of the Evidence R6

Values and preferences

See Values and preferences R6

Resources and other considerations

See Resources and other considerations R6

Rationale

See Rationale R6

Strong recommendation , High certainty evidence

R8: The ERG currently **recommends** that postnatal Rh D immunoprophylaxis (Rh D immunoglobulin 625 IU) continue to be administered to all Rh D negative women with no preformed anti-D antibodies who have a baby who is predicted to be Rh D positive based on NIPT for fetal *RHD*, or cord blood or neonatal Rh D typing.*

The cord blood or neonatal testing should be performed regardless of the results of NIPT for fetal *RHD*, but need not delay administration of Rh D immunoprophylaxis when the fetus has been shown to be *RHD* positive by NIPT testing.

If the baby is Rh D positive, administer Rh D immunoglobulin even if the NIPT predicted an Rh D negative baby.

**If the newborn has a weak or variant Rh D type, consult a haematopathologist in regard to interpretation of results and management.*

See R6 for Research evidence and References

Practical info

See Dosing of Rh D immunoglobulin following fetomaternal haemorrhage quantitation

See Challenges - Consent and the choice to decline Rh D immunoglobulin

Evidence to decision

Benefits and harms

The 2003 guidelines *[1]* stated that *there is very strong evidence, from the late 1960s onwards, that the practice of administering Rh D immunoglobulin postpartum has dramatically reduced the incidence of immunisation and of HDN. Postpartum administration of Rh D immunoglobulin to all Rh D negative women with no preformed anti-D antibodies who deliver Rh D positive babies is standard practice in*

Australia and in most parts of the world, although the dose used varies between countries.

Certainty of the Evidence

High

The best evidence on the postpartum use of Rh D immunoglobulin comes from the Cochrane Database of Systematic Reviews, with the last substantive amendment made in February 1997 [57] Six randomised controlled trials in which postpartum Rh D immunoglobulin prophylaxis was compared with no treatment or placebo were considered eligible for analysis. The trials involved over 10,000 women, but trial quality varied.

On the basis of this evidence, the 2003 guidelines state that *Rh D immunoglobulin can be recommended for routine postpartum prophylaxis in Rh D negative women with no preformed antibodies following birth of an Rh D positive infant (level I evidence) [1].*

Values and preferences

See Values and preferences R6

Resources and other considerations

See Resources and other considerations R6

Rationale

See Rationale R6

Strong recommendation , High certainty evidence

R9: The ERG **recommends** the testing of maternal blood to determine fetal *RHD* genotype in all Rh D negative pregnant women to enable targeted antenatal Rh D immunoprophylaxis.*

**The ERG's recommendation on the use of NIPT for fetal RHD is not a policy statement on funding and supply arrangements for the national provisions of NIPT for blood group genotyping to determine the Rh D status of the fetus.*

As of February 2024, NIPT for fetal Rh D status is not widely available in Australia. Universal Rh D immunoprophylaxis should be maintained until NIPT is widely available.

Further details are provided on the NBA website.

See R6 for Research evidence and References

Practical info

The Royal College of Pathologists of Australia (RCPA) has developed Pathology The Facts which provides further information about pathology testing for consumers including an explanation of false negative and false positive results.

See Challenges - Consent and choice to decline Rd D immunoglobulin

Evidence to decision

Benefits and harms

See Benefits and harms R6

Certainty of the Evidence

High

See Certainty of the Evidence R6

Values and preferences

See Values and preferences R6

Resources and other considerations

See Resources and other considerations R6

Rationale

See Rationale R6

Strong recommendation , High certainty evidence

R10: The ERG **recommends** that test sensitivity be at least 99% in order to minimise the number of Rh D positive fetuses being missed by the test.

See R6 for Research evidence and References

Practical info

The RCPA has developed Pathology The Facts which provides further information about pathology testing for consumers including an explanation of false negative and false positive results.

Evidence to decision

Benefits and harms

See Benefits and harms R6

Certainty of the Evidence

High

See Certainty of the Evidence R6

Values and preferences

See Values and preferences R6

Resources and other considerations

See Resources and other considerations R6

Rationale

See Rationale R6

Strong recommendation , High certainty evidence

R11: The ERG recommends NIPT for fetal *RHD* from 11^{+0} weeks of pregnancy because of higher test accuracy than at earlier weeks.

See R6 for Research evidence and References

Practical info

The RCPA has developed Pathology The Facts which provides further information about pathology testing for consumers including an explanation of false negative and false positive results.

See Challenges - Consent and the choice to decline Rd D immunoglobulin

Evidence to decision

Benefits and harms

See Benefits and harms R6

Certainty of the Evidence

High

See Certainty of the Evidence R6

Values and preferences

See Values and preferences R6

Resources and other considerations

See Resources and other considerations R6

Rationale

See Rationale R6

6.4 Risk of failure of Rh D immunoprophylaxis due to high BMI

There is some concern that in Rh D negative pregnant or postpartum women with no preformed anti-D antibodies, a high BMI may increase the risk of failure of Rh D immunoglobulin administration.

Weak recommendation against , Very low certainty evidence

R12: The ERG **does not currently support** an increased dose of Rh D immunoglobulin or changes in laboratory testing on the basis of high BMI in Rh D negative pregnant women.

Evidence to decision

Benefits and harms

Small net benefit, or little difference between alternatives

All serious outcomes for Rh D alloimmunisation are uncommon in Australia. This is despite the fact that the proportion of women with a BMI of more than 30 is progressively increasing (such women now comprise almost one-third of all those giving birth in Australia).

Certainty of the Evidence

Very low

Increasing BMI has not been shown to have any effect on the incidence of Rh D alloimmunisation in Rh D negative women. Several studies suggest that increasing BMI may affect peak serum levels of anti-D antibodies; however, there is no clear evidence that increasing BMI affects the persistence of anti-D antibodies. There is no established relationship between lower post-administration serum levels of anti-D antibodies and Rh D alloimmunisation or poor clinical outcomes.

Values and preferences

No substantial variability expected

It is preferable to maintain a consistent dose of Rh D immunoglobulin for all women, rather than having a dose specific to women with a BMI of more than 30. Also, it is clear that there is no evidence of the need for a separate dose for such women.

Resources and other considerations

Important issues, or potential issues not investigated

There is insufficient evidence to support changes to the current recommendations.

Rationale

Question 4 – (Prognostic)

In Rh D negative pregnant or postpartum women with no preformed anti-D, does increasing BMI increase the risk of failure of anti-D administration?

The literature search for this question aimed to establish whether an increasing BMI, maternal weight or any other weight-related factors impact on the effectiveness of Rh D immunoglobulin administration.

Clinical question/ PICO

Population: Rh D negative pregnant women with increased BMI and no preformed anti-D
Intervention: Increased dose of RAADP
Comparator: Not applicable

Summary

There were two RCTs *[83][139]* and three nonrandomised studies *[35][75][138]* identified that provided some evidence relating maternal body weight to Rh D immunoglobulin administration.

Wikman et al. (2021) *[138]* retrospectively examined the proportion of Swedish women (out of 4280) with undetectable levels of prophylactic Rh D immunoglobulin at the time of delivery after RAADP (single dose of 1500 IU at 28-29 gestational weeks). The authors also prospectively administered a second dose (1500 IU) in 39 Rh D negative women carrying an Rh D positive fetus at 38 weeks gestation. The concentration of serum anti-D was then monitored weekly until 43 weeks gestation (including post-delivery).

MacKenzie et al. (2006) *[83]* was a prospective cohort study set in the UK, which evaluated serum levels of Rh D immunoglobulin with respect to BMI and body surface area (BSA). The study was assessed to have an overall serious risk of bias due to insufficient reporting of outcome data, and the cohort was too small (n = 45) to provide any useful information relating to the association between BMI and persistence of anti-D antibodies.

Woelfer et al. (2004) *[139]* was a cohort study conducted in Austria that evaluated the effect of increasing BMI on anti-D serum levels by constructing a multivariate linear regression model. The study was assessed to have a moderate risk of bias, but there was insufficient longer term data to provide useful information relating to an association between BMI and the incidence of Rh D alloimmunisation in a subsequent pregnancy.

Koelewijn et al. (2009) *[75]* was a case–control study set in the Netherlands that examined risk factors associated with Rh D alloimmunisation in Rh D negative women during their first pregnancy. The cases were 42 women who developed antibodies detected upon first trimester screening in their second pregnancy, who were identified from a nationwide study in the years 1999, 2000, 2002, 2003 and 2004. Controls were selected over a 10-month period between September 2002 and June 2003 among women who had registered a negative red cell antibody screening result in the first 12 weeks of pregnancy (includes Rh D positive and Rh D negative parae-1). RAADP (1000 IU, single dose at week 30) had been available in the Netherlands since 1 July 1998. The study was assessed to have an overall moderate risk of bias, with a key concern being confounding and women selection bias. The study authors acknowledged an over-representation of women from the primary care setting (midwives and general practitioners) in the control group (as compared with the obstetric setting) compared with cases. To compensate, weighted data was used in the analysis.

Bichler et al. (2003) *[35]* was a Phase II, open label, controlled trial conducted across seven gynaecological practices in Germany. The purpose of the study was to examine the pharmacokinetics of antenatal Rh D immunoglobulin when administered antenatally (intramuscular vs intravenous route). Serum Rh D immunoglobulin (1500 IU) was measured by flow cytometry, and the weight and height of each woman was provided. The study was assessed to have an overall critical risk of bias and was too problematic to provide any meaningful evidence.

Incidence of Rh D alloimmunisation (any timepoint)

One study *[75]* was identified that considered whether increasing BMI increased the risk of failure of Rh D immunoglobulin administration (measured by the incidence of Rh D alloimmunisation in a second pregnancy). The study examined various risk factors for Rh D alloimmunisation in Dutch primiparous women, with the univariate analysis of risk factors suggesting no significant association between BMI, mean body weight or increased body weight (>75 kg and >100 kg), and the incidence of Rh D alloimmunisation.

The mean BMI in the Rh D alloimmunised group was estimated to be 23.8 ± 4.5 compared with a

mean BMI of 24.0 ± 4.5 in the control group (mean difference [MD] –0.20; 95% CI –1.74, 1.34; p = 0.80). There was also no difference in mean body weight, being 67.6 ± 11.5 kg among the Rh D alloimmunised women and 69.6 ± 13.3 kg in the control group (MD –2.00; 95% CI –6.09, 2.09; p = 0.34). The authors also noted no association between Rh D alloimmunisation and maternal body weight greater than 75 kg, with 21.9% in the alloimmunised weighing more than 75 kg compared with 23.8% in the control group (p = 0.82). A similar observation was reported for women with maternal body weight greater than 100 kg (3.1% vs 3.3%, p = 0.71), although the number of cases may not have been sufficiently large to demonstrate an effect (there were fewer than two women in the alloimmunised group weighing > 100 kg).

This study may not have been sufficiently powered to detect a difference between populations due to the small number of cases (n = 42) and did not indicate when maternal body weight was measured. Also, the antenatal dose of Rh D immunoglobulin used in this study (1000 IU at 30 weeks gestation) differs from the current Australian regimen (625 IU at 28 and 34 weeks gestation).

Anti-D antibody levels (at any timepoint)

Four studies *[35][83][138][139]* suggested a correlation between higher maternal body weight and lower peak serum anti-D antibody levels; however, sample sizes were small and the evidence was of very low quality. Further research is needed to determine whether lower levels of measurable anti-D antibodies in obese women correlates to higher rates of Rh D alloimmunisation.

In the prospective cohort study conducted by Wikman et al. (2021) *[138]*, 7 out of 39 women (18%) did not have detectable levels of anti-D at screening (38 weeks gestation) and in 10 out of 39 women (26%) the anti-D levels were below the lower limit of quantification. After administration of the second dose of Rh D immunoglobulin (1500 IU), the mean increase in anti-D concentration (IU/mL) was 0.066 (SD 0.045) and showed a significant correlation with body mass index (p = 0.0118).

Woelfer et al. (2004) *[139]* assessed the influence of BMI on measurable anti-D antibody levels after delivery at one, two and three days, and at two weeks after administration. The study found that women with a BMI less than or equal to 27 kg/m^2 had significantly higher concentrations of serum anti-D antibodies (ng/mL) than women with a BMI higher than 27 kg/m^2. Using a general linear model, the study authors found each kg/m^2 BMI higher than 27 kg/m^2 reduced the serum concentration of anti-D antibodies by the calculated value (MD 4.2; 95% CI 6.4, 2.0; p < 0.002 at day one up to MD 8.4; 95% CI 15.8, 1.1; p = 0.03 at 2 weeks).

MacKenzie et al. (2006) *[83]* reported a significant inverse relationship between peak serum concentration of anti-D antibodies (ng/mL) and low BSA (R^2 = 0.299; p = 0.002) or low maternal body weight (R^2 = 0.171; p=0.006) when measured at seven days after the first dose (at 28 weeks of pregnancy). This did not significantly influence duration of persistence of anti-D antibodies at 12 weeks after the first dose when women with a maternal BSA of less than 1.80 m^2, 1.8–1.99 m^2 or greater than 2.00 m^2 were compared (p not reported).

The study by Bichler et al. (2003) *[35]* found that six women with a body weight less than 80 kg had a mean anti-D antibody level of 26.6 ng/mL, which was higher than the two women with a body weight greater than 80 kg (6.9 ng/mL and 10 ng/mL). Nevertheless, despite low peak serum levels of anti-D antibodies, the two women of higher body weight had quantifiable anti-D antibody levels up to the last scheduled blood sample (weeks 9 and 11, respectively).

Incidence of a positive Kleihauer test

The incidence of FMH after birth was analysed in one prospective cohort study (Wikman et al.

(2021)) *[138]*, which found the results to be negative in all patients tested (i.e. in 25 of 39 (64%) the test result was below the limit of detection being 1 mL of fetal blood in maternal circulation). Data were missing for 14 of 39 (36%) patients.

Adverse neonatal events

No studies were identified.

Maternal adverse events

No studies reported any maternal adverse events considered to be related to the study drug.

Outcome Timeframe	**Study results and measurements**	**Comparator**	**Intervention** Risk with increased dose of RAADP	**Certainty of the Evidence** (Quality of evidence)	**Summary**
Incidence of Rh D alloimmunsation any timepoint 9 Critical	Based on data from 188 participants in 1 studies. [1] (Observational (non-randomized))	No significant association between body mass index, mean body weight, weight >75 kg or weight >100 kg on the incidence of Rh alloimmunisation observed in one small case–control study.		Very low Due to very serious imprecision [2]	Increasing BMI does not appear to have any effect on the incidence of Rh D alloimmunisation in Rh D negative women, but the evidence is very uncertain.
Anti-D serum levels after administration of Rh D immunoglobulin [3] two doses, 28 and 34-38 weeks gestation 3 Not Important	Based on data from 45 participants in 2 studies. [4] (Observational (non-randomized))	One small study reported a correlation between peak anti-D serum levels and maternal body surface area (BSA) and weight measured at 7 days after the first dose but found no significant difference relating to persistence measured at 12 weeks after the first dose. One small study reported a significant correlation with BMI (p = 0.01) and detectable levels of serum anti-D after administration of a second dose (1500 IU) at 38 weeks gestation.		Very low Due to very serious risk of bias, Due to very serious imprecision [5]	Increasing BSA appears to have little or no effect on persistence of anti-D serum levels after administration of Rh D immunoglobulin (two doses, 28 and 34 weeks gestation) but the evidence is very uncertain.
Anti-D serum levels after administration of Rh D immunoglobulin single dose, 28 weeks gestation 3 Not Important	Based on data from 8 participants in 1 studies. [6] (Randomized controlled)	In a single arm of an RCT, women with body weight greater than 80 kg (n = 2) had lower peak serum levels than women who weighed less than 80 kg (n = 6); but anti-D immunoglobulin remained quantifiable in these women at last scheduled follow-up (week 9 and 11).		Very low Due to very serious risk of bias, Due to serious indirectness, Due to very serious imprecision [7]	Increased body weight appears to have little to no effect on persistence of anti-D serum levels after administration of Rh D immunoglobulin (single dose, 28 weeks gestation) but the evidence is very uncertain.
Anti-D serum levels after	Based on data from 26 participants in 1 studies.	Based on the general linear model over time, the study authors found		Very low Due to very serious	Increasing BMI may result in reduced anti-D serum concentration

Outcome Timeframe	**Study results and measurements**	**Comparator**	**Intervention** Risk with increased dose of RAADP	**Certainty of the Evidence** (Quality of evidence)	**Summary**
administration of Rh D immunoglobulin after delivery of Rh D positive baby 3 Not Important	[8] (Observational (non-randomized))	each kg/m2 BMI higher than 27 kg/m2 reduced the anti-D serum concentration by the calculated value.		imprecision [9]	after delivery of an Rh D positive baby but the evidence is very uncertain. The link between lower anti-D levels and incidence of Rh D alloimmunisation is unknown.
Incidence of a positive test for FMH		No studies reported this outcome			No studies were found that looked at incidence of a positive test for FMH in Rh D negative pregnant women with increased BMI after administration of Rh D immunoglobulin
Adverse neonatal events e.g. jaundice 6 Important		No studies reported this outcome			No studies were found that looked at adverse neonatal events in Rh D negative pregnant women with increased BMI after administration of Rh D immunoglobulin
Adverse maternal events [10] 6 Important	Based on data from 9 participants in 1 studies. [11] (Randomized controlled)	A total of seven adverse events reported among five women, none of which were considered related to study drug.		Very low Due to very serious risk of bias, Due to very serious imprecision [12]	

1. Systematic review [92]. No significant association between body mass index, mean body weight, weight >75 kg or weight >100 kg on the incidence of Rh alloimmunisation observed in a small case–control study.. **Supporting references:** [75],
2. **Risk of Bias: no serious.** One case-control study that appears to provide sound evidence for a non randomised study but cannot be considered comparable to a well-performed RCT. There was an over-representation of women from the primary versus obstetric setting (3:1) in the control group compared with cases, resulting in the use of weighted data in the analysis. This was not considered to seriously effect the overall direction of effect.. **Inconsistency: no serious.** Heterogeneity not assessed. Certainty of evidence not downgraded.. **Indirectness: no serious.** Evidence is directly generalisable to the target population and applicable to the Australian healthcare system with some caveats. The study was conducted in The Netherlands in Rh D negative women who received Rh D immunoglobulin 1000 IU at 30 weeks of pregnancy and within 48 hours of giving birth to an Rh D positive baby. This is different to the recommended dose in Australia of 625 IU at 28 and 34 weeks of pregnancy, and within 72 hours of giving birth to an Rh D positive baby.. **Imprecision: very serious.** The study is not statistically powered to inform decision making. A very small number of women with a high BMI were included.. **Publication bias:**

no serious.

3. One small study reported a correlation between peak anti-D serum levels and maternal body surface area and weight measured at 7 days after the first dose but found no significant difference relating to persistence measured at 12 weeks after the first dose. One small study reported a significant correlation with BMI (p = 0.01) and detectable levels of serum anti-D after administration of a second dose (1500 IU) at 38 weeks' gestation.
4. Systematic review [16]. One small study reported a correlation between peak anti-D serum levels and maternal body surface area and weight measured at 7 days after the first dose but found no significant difference relating to persistence measured at 12 weeks after the first dose. One small study reported a significant correlation with BMI (p = 0.01) and detectable levels of serum anti-D after administration of a second dose (1500 IU) at 38 weeks' gestation.. **Supporting references:** [83], [138],
5. **Risk of Bias: very serious.** Included studies have some important problems that seriously weaken the confidence in the results. Small cohort with some concerns with reporting bias and missing data.. **Inconsistency: no serious.** Heterogeneity not assessed. Certainty of evidence not downgraded.. **Indirectness: no serious.** Evidence is directly generalisable to the target population and applicable to the Australian healthcare system with some caveats. One study was conducted in the UK in Rh D negative pregnant women. Rh D immunoglobulin (500 IU) was administered at 28 and 34 weeks of pregnancy but the dose was lower than recommended in Australia (625 IU). One study was conducted in Sweden in Rh D negative pregnant women. Rh D immunoglobulin 1500 IU (which is higher dose than recommended in Australia) was administered at 28 and 38 weeks of pregnancy.. **Imprecision: very serious.** Small cohort with insufficient longer term data to provide meaningful information relating to BMI and incidence of Rh D alloimmunisation in a subsequent pregnancy.. **Publication bias: no serious.**
6. Primary study **Supporting references:** [35],
7. **Risk of Bias: very serious.** The study is too problematic to provide any useful evidence on the outcome of interest.. **Inconsistency: no serious.** Heterogeneity not assessed. Certainty of evidence not downgraded. . **Indirectness: serious.** Evidence is probably generalisable to the target population but difficult to judge whether it is sensible to apply it to the Australian health care system. The study was conducted in Germany in Rh D negative women. Rh D immunoglobulin (1500 IU) was administered at 28 weeks gestation, which is different to that recommended in Australia (Rh D immunoglobulin 625 IU at 28 and 34 weeks gestation). The correlation between body weight and BMI is poor.. **Imprecision: very serious.** Small cohort with insufficient longer term data to provide meaningful information relating to BMI and incidence of Rh D alloimmunisation in a subsequent pregnancy.. **Publication bias: no serious.**
8. Primary study Based on the general linear model over time, the study authors found each kg/m2 BMI higher than 27 kg/m2 reduced the Rh D immunoglobulin serum concentration by the calculated value.. **Supporting references:** [139],
9. **Risk of Bias: no serious.** One observational study that appears to provide sound evidence for a nonrandomised study but cannot be considered comparable to a well-performed RCT.. **Inconsistency: no serious.** Heterogeneity not assessed. Certainty of evidence not downgraded. **Indirectness: no serious.** Evidence is directly generalisable to the target population and is applicable to the Australian healthcare system with some caveats. The study was conducted in Austria in Rh D negative women who had delivered an Rh D positive baby. Rh D immunoglobulin was administered within 72 hours of birth, but at a dose higher than that recommended in Australia (1500 IU vs 625 IU). . **Imprecision: very serious.** Small cohort with insufficient long term data to provide meaningful information relating to BMI and incidence of Rh D alloimmunisation in a subsequent pregnancy.. **Publication bias: no serious.**
10. x
11. Primary study **Supporting references:** [35],
12. **Risk of Bias: very serious.** The study is too problematic to provide any useful evidence on the outcome of interest.. **Inconsistency: no serious.** Heterogeneity not assessed. Certainty of evidence not downgraded.. **Indirectness: no serious.** Evidence is directly generalisable to the target population and

applicable to the Australian healthcare system with some caveats. The study was conducted in The Netherlands in Rh D negative women who received Rh D immunoglobulin 1000 IU at 30 weeks of pregnancy and within 48 hours of giving birth to an Rh D positive baby. This is different to the recommended dose in Australia of Rh D immunoglobulin 625 IU at 28 and 34 weeks of pregnancy and within 72 hours of giving birth to an Rh D positive baby.. **Imprecision: very serious.** Small study unlikely to be sufficiently powered to detect a statistically significant difference.. **Publication bias: no serious.**

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75. Koelewijn JM, de Haas M., Vrijkotte TG, van der Schoot CE, Bonsel GJ. Risk factors for RhD immunisation despite antenatal and postnatal anti-D prophylaxis. BJOG: An International Journal of Obstetrics & Gynaecology 2009;116(10):1307-1314 Journal

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92. Jorgensen M, Allerdice S. Routine antenatal prophylaxis with RhD IgG for prevention of haemolytic disease of the fetus and newborn in Rh D negative pregnant women. RevMan 5.4 2021.

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Expert opinion point

EOP7: Rh D immunoglobulin must be given by deep intramuscular injection. For women with a BMI of more than 30, particular consideration should be given to factors that may affect the adequacy of the injection (e.g. the site of administration and the length of the needle used).

6.5 Dosing of Rh D immunoglobulin following fetomaternal haemorrhage quantitation

The purpose of this section is to guide the dosing of Rh D immunoglobulin following quantitation of fetomaternal haemorrhage (FMH) volume. Rh D immunoglobulin products that are currently available on the National Product list, and funded and supplied under the National Blood Agreement, are listed in Supply

considerations.

For Rh D negative pregnant women, a maternal blood sample should be collected for quantitation of FMH following sensitising events after 20 weeks of pregnancy and after giving birth; the routine dose of Rh D immunoglobulin of 625 international units (IU) should be administered. This dose is sufficient to cover FMH of up to 6 mL Rh D positive fetal red cells (equivalent to about 12 mL of fetal whole blood), which will account for 99% of FMH.

For FMH volumes greater than 6 mL fetal red cells, an additional dose of Rh D immunoglobulin is required, and should be calculated at 100 IU per mL of fetal red cells in excess of 6 mL covered by the standard initial 625 IU dose. The required dose should be rounded up to the nearest full vial or vials.

Doses that require intramuscular injection of a volume of Rh D immunoglobulin of more than 5 mL should be divided and administered in separate intramuscular injections. Intravenous Rh D immunoglobulin may be used for the management of large FMH where administration of intramuscular Rh D immunoglobulin is either contraindicated or not practical.

For very large FMH volumes that would require more than two intramuscular injections, use of intravenous product Rhophylac 1500 IU is recommended, at a dose of 100 IU/1 mL fetal red cells in excess of the 6 mL that is covered by the standard initial 625 IU dose.

After the initial 625 IU standard dose for sensitising events and following birth, the table below guides the additional Rh D immunoglobulin dosing for large FMH ≥ 6 mL.

FMH volume (fetal red cells)	Total dose of Rh D Ig required	Initial dose of Rh D Ig (625 IU) administered by IM injection for sensitising event or birth - covers FMH of up to 6 mL fetal red cells	Additional vials of Rh D Ig (625 IU) to be administered by IM injection	Additional vials of Rhophylac (1500 IU) to be administered IV
< 6 mL	600 IU	1	0	-
≥ 6 to < 12 mL	1200 IU	1	1	-
≥ 12 to < 18 mL	1800 IU	1	2*	-
		1	-	1*
≥ 18 to < 21 mL	2100 IU	1	-	1
≥ 21 to < 36 mL	3600 IU	1	-	2
≥ 36 mL	FMH volume in mL fetal red cells multiplied by 100 IU	1	-	Total Rh D Ig dose required (less 600 IU if already given initial dose) divided by 1500 IU and rounded up to nearest full number of vials
Specialist advice is recommended for any large FMH quantities and especially volumes ≥ 36 mL fetal red cells				

FMH, fetomaternal haemorrhage; IM, intramuscular; IU, international units; IV, intravenous

*2 vials of 625 IU can be administered as a single injection or as separate injections, however in either case to avoid discomfort associated with a larger volume IM injection or 2 additional injections, it may be more practical to offer IV Rhophylac 1500 IU instead.

7. Cost considerations

In 1999, the NHMRC published Guidelines for the use of Rh D immunoglobulin in obstetrics *[95]*, with the aim of balancing best practice in the use of Rh D immunoglobulin with limited supply. The guidelines were based on a review of the literature and a cost-effectiveness analysis of six alternative strategies for the prevention of Rh D alloimmunisation in Australia.

Although the review process supported universal prophylaxis with Rh D immunoglobulin to Rh D negative women at 28 and 34 weeks of pregnancy, supply constraints meant that the NHMRC Working Party was unable to recommend universal prophylaxis at that time. This situation highlighted the need to consider options to increase the supply of Rh D immunoglobulin, to enable implementation of a universal antenatal prophylaxis program for all Rh D negative pregnant women.

In 2001, the Working Party was reconvened to review and update the guidelines, given developments in the availability of Rh D immunoglobulin since the publication of the 1999 guidelines. A literature search was commissioned to update the evidence base for the guidelines, and the cost-effectiveness data were reviewed.

A revised guideline was published in 2003 *[1]* – it made various recommendations for the staged implementation of a full antenatal prophylaxis program, based on the results of the updated literature review and assessment of progress towards self-sufficiency in Rh D immunoglobulin. The intention of the 2003 guideline was to progress towards full antenatal prophylaxis; thus, the updated cost analysis focused on the effect of the price of Rh D immunoglobulin and on the cost-effectiveness of its antenatal and postnatal use. The aim of the analysis was to investigate whether full antenatal prophylaxis remained cost-effective at different costs of Rh D immunoglobulin (imported and domestic supply), taking into account current evidence.

The results of the updated cost-effectiveness analysis suggested that both a postpartum program, and a postpartum plus antenatal prophylaxis program, remained well within the usual bounds of cost-effectiveness, given the prices per vial of Rh D immunoglobulin at that time. The Working Party concluded that antenatal prophylaxis appeared to be a cost-effective addition to a postpartum program, even at a relatively high price of Rh D immunoglobulin of A$115 per vial.

In developing the research questions for this guideline, the ERG did not explicitly include search strategies to identify evidence related to cost-effectiveness or resource implications of practice. However, where the literature searches conducted for the four clinical questions found information on cost-effectiveness or economic evaluations, this information was reviewed. Also analysed were cost-effectiveness studies for RAADP and NIPT that had been published since the release of the 2003 guideline *[10][50][59][97][98][99][108][113][114][122]*.

The following issues were identified when reviewing the studies:

- age of the studies
- only one of the studies was in the Australian context
- cost assumptions and inclusion of specific costs need to be validated for the Australian setting
- costs have an impact on a decentralised and centralised supply chain, including costs of testing and the donor programs
- differences in cost-effectiveness of a one-dose or two-dose RAADP regimen were a result of the differences in price of the products and administration costs.

The previous cost assessments completed for the Australian context were based on data from 1996 *[131]*; therefore, we recommend that a new independent assessment be conducted to assess the cost-effectiveness of the following strategies for the prevention of Rh D alloimmunisation. The assessment should cover:

- universal RAADP using one or two doses
- immunoprophylaxis using fetal Rh D status, determined by NIPT for fetal *RHD* or cord serology

- targeted antenatal Rh D immunoprophylaxis
  - with or without postnatal cord serology
  - centralised compared with decentralised testing
  - timing of testing
- universal sensitising or long-term event immunoprophylaxis in the first 12 weeks of pregnancy for threatened miscarriage compared with targeted immunoprophylaxis.

To inform the economic models, there is a need for additional evidence regarding uptake, women's preferences, errors and adverse events relating to administration of Rh D immunoglobulin, and episodes of Rh D sensitisation despite immunoprophylaxis. Also, it may be relevant to include the economic model disutility due to loss of fetus or long-term sequelae of HDFN (both of which were not included in the previous assessment), in which case, additional information on these outcomes may be required.

The availability of a more contemporary cost-effectiveness analysis is particularly important because of the limited supply of Rh D immunoglobulin available relative to the number of women and babies who may benefit from its use. A systematic approach to comparing costs and benefits in a variety of scenarios could help to inform decisions about the allocation of a scarce resource.

8. Supply considerations

Products currently available under the national blood arrangements

In Australia, Rh D immunoglobulin products are supplied and funded through arrangements managed by the NBA under the *National Blood Authority Act 2003* and National Blood Agreement.

Product	Presentation	Volume	Administration
Rh(D) Immunoglobulin-VF	Single vial 250 IU	up to 2 mL	Slow deep intramuscular injection
Rh(D) Immunoglobulin-VF	Single vial 625 IU	up to 2 mL	Slow deep intramuscular injection
Rhophylac (imported)	Single use prefilled 2 mL syringe 1500 IU	2 mL	Intravenous or intramuscular injection Note: Available only where access to an intravenous preparation is required

Rh (D) Immunoglobulin-VF and Rhophylac are manufactured by CSL Behring and are distributed to approved health providers by Australian Red Cross Lifeblood (Lifeblood).

- Rh (D) Immunoglobulin-VF for intramuscular administration is manufactured from plasma collected in Australia. This product is supplied through the national prophylaxis program. Australia is self-sufficient in the supply of Rh (D) Immunoglobulin-VF for RAADP.
- Rhophylac is available only for exceptional circumstances where intravenous administration is required, for use in large FMH where administration of intramuscular Rh D immunoglobulin is contraindicated or not practical, or in the case of inadvertent or emergency transfusion of Rh D positive blood to an Rh D negative woman of childbearing potential. The NBA manages the importation of Rhophylac.

For detailed product information, see the CSL Behring website. A full list of products available under the national blood arrangements is provided on the NBA website.

Supply trends

The number of vials of Rh D immunoglobulin issued to health providers in Australia has remained steady since 2006–07, as highlighted in Figure 7.1.

Product issued under the national blood arrangements can be provided to health providers in Australia, including public and private hospital pharmacies, public and private pathology laboratories, medical providers, and medical centres or clinics. The number of vials administered is not known because details of clinical use, inventory levels and wastage are not recorded nationally. Also, where products are used, it is unclear whether they have been used appropriately, in accordance with the clinical practice guidelines. Monitoring the use of Rh D immunoglobulin provides organisational level guidance on documenting, monitoring and auditing Rh D immunoglobulin use.

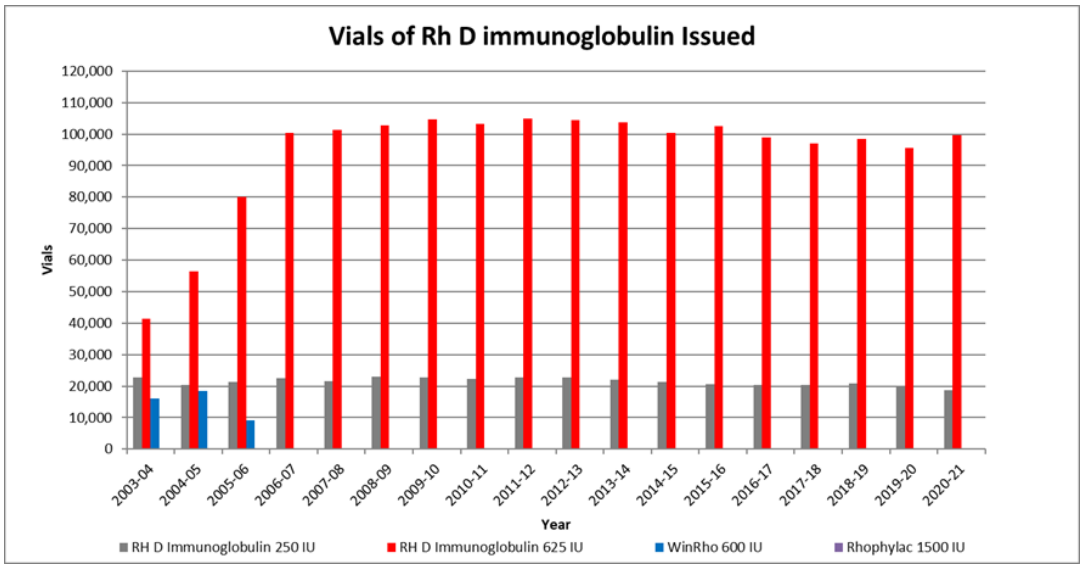

Figure 8.1 Vials of Rh D immunoglobulin issued since 2003–04

Note: Issues of Rhophylac are too small to appear on the graph.

The demand for products over recent years does not correlate with the change in births over the same period, as shown in Figure 8.2.

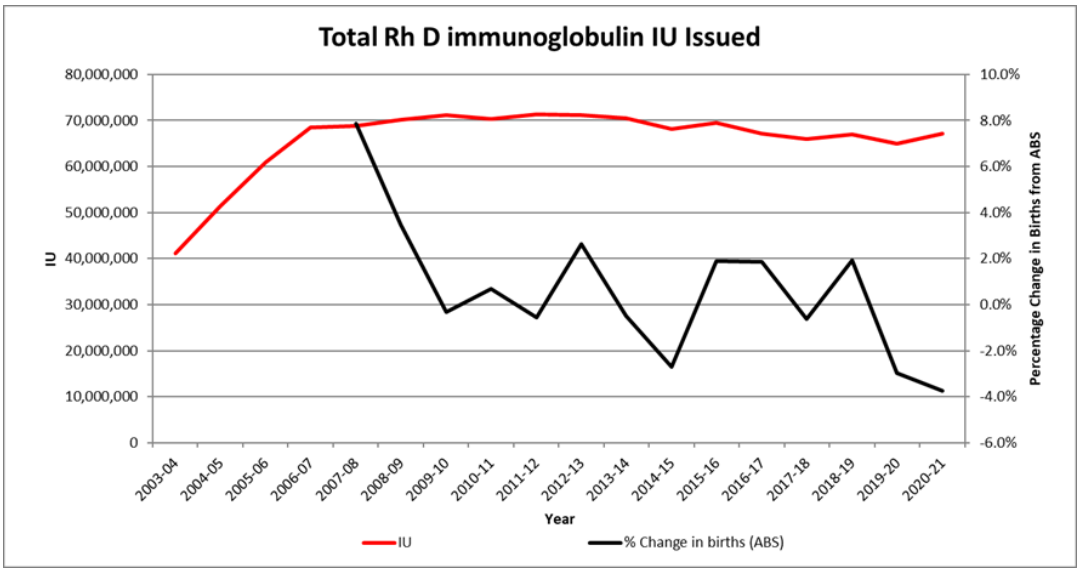

Figure 8.2 International units (IUs) of Rh D immunoglobulin issued since 2003–04

9. Safety of Rh D immunoglobulin

The effect of circulating prophylactically administered Rh D immunoglobulin in the fetal circulation

The literature search for the 2003 guideline found one study that evaluated signs of haemolysis in babies of Rh D negative mothers who underwent prophylaxis with one or two doses of Rh D immunoglobulin during pregnancy *[80]*. No statistically significant differences were found for any of the haematological variables between the babies of mothers who received one or two doses of Rh D immunoglobulin, or between the Rh D negative babies and the controls. Thus, the literature search of 2003 failed to find any new evidence for concern about fetal effects of prophylactic Rh D immunoglobulin (either one or two doses).

A search of the literature from 2001 to June 2019 found one study that matched babies born at 28–34 weeks of pregnancy after routine maternal Rh D immunoprophylaxis with controls *[79]*. That study found higher bilirubin at birth and peak bilirubin in the first three days, but no difference in haematocrits at birth or day three, or in haematocrit nadir or number of transfusions. The low number of participants (n = 94) and the exclusion of babies for ABO incompatibility between mother and baby, which is an uncommon cause of a positive direct antiglobulin test (DAT), or significant haemolysis or jaundice in babies born in this gestation range, reduce the certainty of the authors' conclusion that antenatal Rh D immunoprophylaxis does not cause clinically significant haemolysis in Rh D positive babies subsequently born preterm. A single case report of a 36-week gestation baby (born after maternal administration of 300 µg Rhogam at 28 weeks of pregnancy) identified marked jaundice (treated with phototherapy) and mild anaemia *[42]*. Detailed laboratory studies supported a diagnosis of Rh D immunoglobulin-associated haemolysis in the newborn. Nevertheless, most cases of significant HDFN in babies whose mothers have received antenatal immunoprophylaxis appear to be attributable to maternal alloimmunisation before or despite antenatal Rh D immunoglobulin, rather than to the immunoprophylaxis itself *[37]*.

There appear to have been no studies into the consequences of potential fetal exposure to high amounts of Rh D immunoglobulin after management of sensitising events.

Importantly, the investigation and management of Rh D positive, DAT-positive babies of Rh D negative mothers who have early or severe jaundice or anaemia should be similar, regardless of the suspected source of the antibody. Since clinically significant Rh D immunoglobulin-associated haemolysis in the newborn appears to be rare, the possibility of maternal alloimmunisation despite immunoprophylaxis should be investigated.

The risk of transmission of infectious organisms by administering Rh D immunoglobulin

Rh D immunoglobulin is derived from pooled donor plasma; therefore, it carries the potential of transmission of viral or other infectious organisms. To reduce the risk of such transmission, extra steps are taken when manufacturing Rh D immunoglobulin *[20]*. For example, strict controls are applied to the selection of blood donors and donations, and the product is specially treated to remove and kill certain viruses; these special treatments are considered effective against both enveloped viruses (e.g. human immunodeficiency virus (HIV), hepatitis B virus and hepatitis C virus) and non-enveloped viruses (e.g. hepatitis A virus and human parvovirus B19).

Despite these measures, it is not possible to totally eliminate the risk of infectivity from viruses and other agents; however, the systematic review did not identify any studies reporting adverse maternal events attributed to Rh D immunoglobulin administration.

Other risks and benefits

A few case reports of maternal hypersensitivity reactions *[110]* highlight the importance of administering Rh D immunoglobulin in locations where such reactions can be managed by appropriately trained providers.

Rh D immunoprophylaxis may have an added benefit of reducing risk of non-D alloimmunisation (e.g. alloimmunisation to other Rh antigens, or to Kell, Duffy or Kidd antigens) *[142]*.

10. Challenges

Consent and the choice to decline Rh D immunoglobulin

The Australian Commission on Safety and Quality in Health Care define informed consent as "a person's decision, given voluntarily, to agree to a healthcare treatment, procedure or other intervention that is made:

- Following the provision of accurate and relevant information about the healthcare intervention and alternative options available; and
- With adequate knowledge and understanding of the benefits and material risks of the proposed intervention relevant to the person who would be having the treatment, procedure or other intervention" *[146]*.

The National Safety and Quality Health Service (NSQHS) Standards *[21]* require health service organisations to partner with patients for their own care, and to ensure that patients and carers are informed about the risks and benefits of using blood and blood products, and all available treatment options. For private sector organisations where informed consent may be obtained in a process separate from the health service organisation, it is not intended that visiting medical officer practices are monitored. Rather, the health service organisation takes a risk management approach, and confirms with women on admission, or at the start of an episode of care, that they understand why they are there and what treatment they will receive.

As explained in the NSW Health *Guideline: Maternity Rh (D) immunoglobulin (anti D) [100]* women should be advised that Rh D immunoglobulin is a blood product, and should be given a clear explanation of the potential risks and benefits of receiving Rh D immunoglobulin. Written information should also be provided; for example, *You and your baby; important information for Rh (D) negative women [15]*.

Written consent may be obtained before administration of Rh D immunoglobulin immunoprophylaxis, by completing the appropriate records and documents. The discussion and the provision of written information should be documented in the medical record.

The ERG also recommends obtaining written or verbal informed consent for NIPT for fetal *RHD*. The information given to women should include:

- Who is tested?
- Why the testing is done?
- The only DNA test done will be for the gene that codes for the Rh D positive blood type in the fetus (modify if NIPT for fetal *RHD* is done in combination with NIPT for aneuploidy or other reasons).

NIPT for fetal *RHD* has no link to forensic identification testing.

Rh D negative mothers who decline NIPT for aneuploidy or other fetal diagnostic reasons should be offered NIPT for fetal *RHD*, and the differences in the purpose of testing should be explained.

Donors

To ensure that the Australian demand for Rh D immunoglobulin can be met from domestic supply, Lifeblood collects high-titre anti-D plasma from a group of about 120 donors to produce Rh D immunoglobulin. The volume of plasma collected varies considerably month to month because of the small donor pool.

Challenges in maintaining this donor program include:

- the progressive retirement of Rh D donors, primarily on the grounds of age
- declining levels of anti-D antibody in Rh D donors, which occurs over time
- a reduction in the number of potential donors with anti-D antibodies due to a fall in the number of women immunised during pregnancy, resulting from the success of the prophylaxis program

- ethical considerations associated with increasing the anti-D antibody levels in blood donors by primary immunisation and boosting, as this requires a small transfusion of incompatible blood
- the significant effect on input if a donor withdraws from the program.

The shelf-life of plasma is 12 months. The shelf-life of Rh D immunoglobulin is two years once it has been manufactured from plasma.

The following strategies will be pursued to maintain the production of Rh D immunoglobulin in a practical, sustainable and ethical way:

- the program of immunisation of new Rh D immunoglobulin donors by Lifeblood will be maintained - this involves actively recruiting new donors for Rh D primary immunisation, and boosting to increase the pool of donors contributing to the supply of plasma for the production of Rh D immunoglobulin
- CSL Behring and Lifeblood will continue to pursue ways of increasing anti-D plasma supply by increasing the yield of Rh D immunoglobulin from the anti-D plasma collected
- the NBA will pursue the development of an educational program for health professionals on the efficient use of Rh D immunoglobulin.

Care pathways

In Australia, there is a wide range of pregnancy care pathways, as outlined in the National Maternity Services Plan *[22]*. It is estimated that 92.7% of Australian women receive care through one of four models: private pregnancy care, combined pregnancy care, public hospital care and shared pregnancy care. The trend of population and workforce movements to larger centres over the past decade has seen a decline in the number of facilities able to provide full pregnancy care for women in rural and remote areas. Providing continuity of care across the entire pregnancy care continuum requires a collaborative and flexible approach from maternity services and the maternity workforce, supported by integration of services, including:

- effective consultation and referral pathways
- effective clinical networks
- collaborative interdisciplinary professional relationships
- sound information sharing and communication channels.

The provision of community-based pregnancy care in remote locations is also an important strategy for providing care to women in remote parts of Australia. This collaborative approach to pregnancy care is particularly important for those women and babies whose care requires linkages to specialist services.

The wide range of pregnancy care pathways in Australia is seen in the different categories of health providers supplied with Rh D immunoglobulin, shown in the following table. Details of who has prescribed and administered the products issued (e.g. midwives, nurses, obstetricians, medical officers or general practitioners) are not recorded at a state or national level.

Type	Category	Rh D immunoglobulin 250 IU	% Of total 250 IU	Rh D immunoglobulin 625 IU	% of total 625 IU	Rophylac 1500 IU
Private	Community pharmacy	8	0	–	0	–
	Hospital	1 769	9	2 782	3	–
	Hospital pharmacy	49	0	667	1	–

	Pathology laboratory	4 832	24	26 613	27	17
	Medical providers	5 621	27	8 828	9	–
	Other	6	0	6	0	–
Public	Hospital	1 322	6	8 847	9	16
	Hospital pharmacy	838	4	4 423	5	–
	Pathology laboratory	6 034	29	44 833	46	45
	Other	11	0	37	0	–
Total		**20 490**	**100**	**97 036**	**100**	**78**

Ig: immunoglobulin; IU: international units

Measurement of product usage against clinical guidance

Gordon et al. (2017) *[59]* estimated the number of women in 2017 requiring Rh D immunoglobulin for universal prophylaxis under the 2003 Guideline *[1]*.

Using the recommendations on dosing for the events, it is estimated that 122 839 vials of Rh D immunoglobulin 625 IU could have been issued. However, the actual number of vials issued in 2017–18 was 97 036 (as per Table 9.1), suggesting an uptake of 79% against the 2003 guideline. The following table summarises the results.

Event	Number of women	Dosing of 625 IU	Expected number of Rh D immunoglobulin 625 IU vials required
Antenatal	41 693	2 doses (28 and 34 weeks of pregnancy)	83 386
Postpartum	28 344	1 dose	28 344
Additional Rh D Ig for sensitising events and HDFN	11 109	1 dose	11 109
Total	**81 146**		**122 839**

HDFN: haemolytic disease of the fetus and newborn; IU: international units

Based on Gordon et al. (2017) *[59]*, with the estimate for the number of women requiring treatment for antenatal events adjusted to 95% for the uptake.

11. Monitoring the use of Rh D immunoglobulin

Documenting the use of Rh D immunoglobulin

As identified in the NSQHS Standards *[21]*, accurately recording and reviewing a woman's blood and blood product transfusion history, including any previous reactions and specific indications for use, in the woman's health care record is essential to enable easy and accurate review of records.

Identifying any red cell antibodies, transfusion reactions or individual requirements specific to the woman will improve transfusion safety by reducing the risk of an adverse transfusion reaction. In addition, recording detailed information about transfusion is important, to allow for an audit of the woman's health care record for quality improvement processes and for traceability of all blood products (including Rh D immunoglobulin) from donors to recipients.

Documenting the indications for transfusion is essential to allow transfusions to be audited against guidelines as outlined in the NSQHS Standards *[21]*.

Adverse event reporting and monitoring

Monitoring adverse events and analysing patterns of adverse events allows areas of risk to be identified and facilitates opportunities for improvement. Health professionals must report adverse events that occur as a result of administration of blood and blood products. Actions 7.7 and 7.8 of the NSQHS Blood Management Standard *[21]* provides guidance on reporting adverse blood management events and strategies for improvement.

Health providers who administer Rh D immunoglobulin should have processes for reporting adverse events experienced by women to the hospital incident management system, pathology service provider, the product manufacturer, and the Therapeutic Goods Administration (TGA), in accordance with their requirements.

In Victoria, the Blood Matters Serious Transfusion Incident Reporting (STIR) *[23]* system has started to report Rh D immunoglobulin administration incidents, and although the number of incidents was small in 2016-17, the types of incident reported through STIR mirror those identified by the *2017 Annual serious hazards of transfusion (SHOT) report [144]*. To understand current practice, Blood Matters conducted an audit *[36]* to assess compliance with the 2003 guideline *[1]* which revealed a number of areas for improvement, including reporting adverse events related to Rh D immunoglobulin.

Audits

Audits of practice should be undertaken on a continuing basis, to monitor uptake of these guidelines. Where variance is identified in relation to uptake, these instances should be addressed through a quality improvement program.

Suggested audits for health service organisations are as follows:

- identify where products are infused or wasted
- identify cold chain breaches
- identify where there has been uptake or a lack of uptake of relevant guidelines
- ensure that where a discrepancy between NIPT for fetal *RHD* and cord testing is noted, a report is sent to the laboratory that performed the NIPT for fetal *RHD*
- ensure that:
  - the woman's records are clearly updated and reviewed
  - the woman's consents are documented and placed in her medical record
- outcomes from haemovigilance activities for women and their babies

Audits could be developed as an accreditation activity for the NSQHS Standards *[21]*.

12. Implementing, evaluating and maintaining the guideline

Communication and education

This guideline will be available within the public domains of the NBA and RANZCOG websites. The availability of the guideline will be communicated with all relevant clinical colleges and societies.

Review of these guidelines

This guideline will be reviewed every five years unless data or new clinical evidence relevant to clinical practice triggers the need for an earlier review. At that time, the NBA will convene a multidisciplinary group of clinical experts to undertake the review.

Feedback

To provide feedback and inform future reviews of this guideline, please send comments to:

Email:	guidelines@blood.gov.au
Mail:	Guidelines National Blood Authority Locked Bag 8430 Canberra ACT 2601

Any correspondence should be forwarded to the project manager for consideration in the next scheduled review.

13. Evidence gaps for potential research priorities

The review of evidence identified a number of areas where the evidence is uncertain or unknown. These areas, which are listed below, may present avenues for further research:

- What are the incidence and causes of Rh D alloimmunisation during pregnancy?
- What are the consequences (if any) of moving to a single-dose Rh D immunoglobulin regimen in terms of safety, efficacy, uptake and acceptability to women?
- What is the correlation between low serum passive anti-D antibody levels in the late third trimester and incidence of Rh D alloimmunisation?
- What is the volume of fetal cells in the maternal circulation after the following sensitising events in the first 12 weeks of pregnancy: abdominal trauma, molar pregnancy, ectopic pregnancy, spontaneous miscarriage, threatened miscarriage or medical termination of pregnancy (with or without curettage)?
- What is the volume of fetal cells in the maternal circulation that increases the risk of Rh D alloimmunisation?
- What is the accuracy of non-invasive prenatal tests for fetal *RHD* in Rh D negative women with multiple pregnancies?
- What is the acceptability of the non-invasive prenatal testing for fetal *RHD* among users?
- Are there alternatives to non-invasive prenatal testing for fetal *RHD* for postnatal cord serology?
- Are neonatal exchange transfusion and intrauterine transfusion the most appropriate measures for assessing the number of fetuses with severe haemolytic disease of the fetus and newborn (HDFN), given clinical practice and thresholds for implementation have changed?
- What is the prevalence of the *RHD* genotype as it relates to pregnant women or the current ethnic populations in Australia?
- What is the incidence of Rh D alloimmunisation as it relates to BMI in the Australian population (in particular, in women with a BMI of >30)?
- What are the outcomes of the more conservative approaches to sensitising event indications adopted by some other countries?

14. Governance

Governance framework

A multi-tiered governance framework was established by the NBA for the development of the guideline.

The framework is depicted in the Figure 14.1.

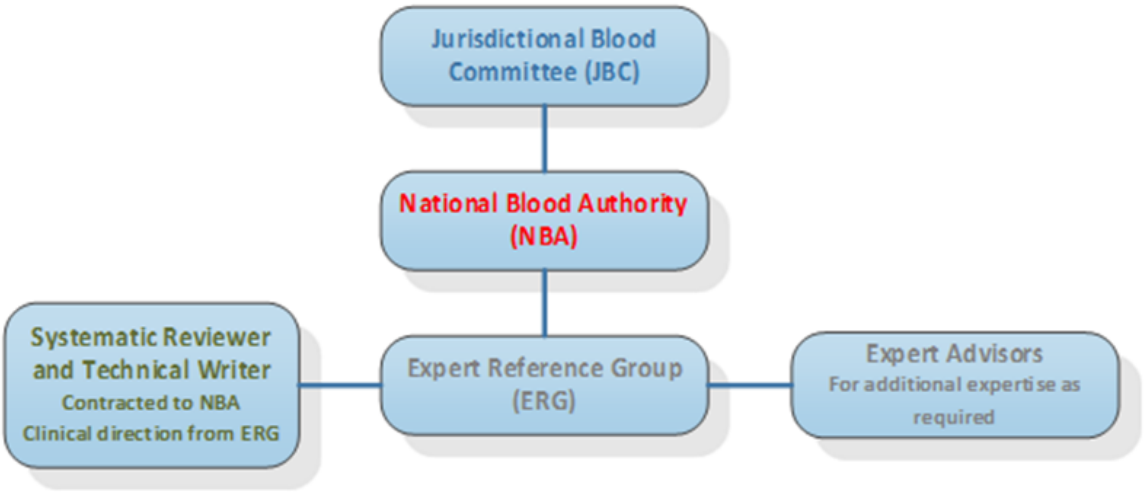

Figure 14.1: Governance arrangements

The Jurisdictional Blood Committee (JBC) is a committee of senior government officials with representation from the Australian Government, the six state governments and two territory governments. The JBC is responsible for all jurisdictional issues relating to the national blood supply, including planning, production, supply and budgeting. The JBC approved the process and expenditure to develop the guideline.

The NBA provided project management oversight and managed the procurement of all goods and services associated with the development of this guideline.

An evidence-based medicine expert was contracted by the NBA to assist the ERG with developing the scope of the research protocol to underpin the systematic review process.

A systematic review team and technical writer were contracted by the NBA to conduct systematic reviews of the scientific literature and provide technical writing services to produce the guideline and associated technical report in collaboration with the ERG.

A multidisciplinary ERG was established by the NBA to provide expert knowledge and input, with members representing a range of clinical colleges, societies and organisations. The ERG:

- identified and developed the research questions and research parameters (i.e. PICO criteria and search terms) for the systematic review, with support from an evidence-based medicine expert
- provided advice on the type of evidence review required to support the update
- reviewed the list of abstracts compiled by the systematic review team and advised which articles should be retained in the evidence base for data extraction and analyses
- provided advice and clinical interpretation to guide the systematic review team
- reviewed the findings from the systematic review, with support from the systematic reviewer
- provided advice on current clinical practices in specific areas of expertise
- drafted the clinical guidance, with support from a medical writer
- reviewed public consultation feedback and revised the guideline accordingly
- proposed tools and strategies to support implementation.

Membership

Expert Reference Group

Dr Marija Borosak	Royal College of Pathologists of Australasia
Dr James Daly	Australian and New Zealand Society of Blood Transfusion
Associate Professor Greg Duncombe	Royal Australian and New Zealand College of Obstetricians and Gynaecologists
Professor David Forbes	Jurisdictional Blood Committee
Professor Helen Liley	Royal Australasian College of Physicians
Dr Sharon Nowrojee	Jurisdictional Blood Committee
Professor Michael Peek	Royal Australian and New Zealand College of Obstetricians and Gynaecologists
Professor Michael Permezel	Royal Australian and New Zealand College of Obstetricians and Gynaecologists
Ms Kelley Stewart	Australian College of Midwives
Dr Amanda Thomson	Australian Red Cross Lifeblood
Dr Ken Wanguhu	Royal Australian College of General Practitioners
Ms Catherine Whitby	Consumer representative

Evidence-based medicine expert

Dr Sarah Norris	Project sponsor (research question development) Health Research Consulting
Dr Kristina Harvey	Project lead (research question development) Health Research Consulting
Dr Margaret Jorgensen	Project lead (research protocol, systematic review, evidence to decisions) Health Technology Analysts

Systematic review team

Ms Stephanie Allerdice	Systematic reviewer (evidence review) Health Technology Analysts
Mr Adrian Peacock	Systematic reviewer (literature search) Health Technology Analysts
Mr Kevin Phan	Systematic reviewer (literature search) Health Technology Analysts

Medical writing (guideline only) and technical editing

Dr Hilary Cadman	Cadman Editing Services

Project management and committee secretariat

Ms Donna Cassoni	Project manager National Blood Authority
Ms Sandra Cochrane	Project sponsor National Blood Authority
Ms Emma Johnson	Project officer National Blood Authority

15. Process report

Methodology

This guideline was developed by following the principles proposed by the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) Working Group *[116]*. It involved developing a set of research questions, systematically reviewing the scientific literature for evidence relating to those questions, and then developing and grading recommendations based on a structured assessment of the evidence. The methods used to apply this process are outlined in Methodology and are given in full in the accompanying technical reports *[16][17][18]*, which present in detail the methodology used to identify the evidence base (clinical questions addressed, documented systematic literature search, inclusion and exclusion criteria described), the characteristics and quality of the evidence base (data extraction and risk of bias forms), and detailed results presented by outcome (evidence summary tables and GRADE profiles).

The systematic review process was based on that described in the *Cochrane handbook for systematic reviews of interventions [123]*. Covidence, a web-based platform for producing systematic reviews was used to store data that are compatible with the Cochrane data collection tools. RevMan *[26]* was used for the main analyses and GRADEpro GDT software was used to record decisions and derive an overall GRADE (high, moderate, low, or very low) for the certainty of evidence for each outcome.

Consensus process

In circumstances where no or insufficient evidence was identified, clinical guidance was developed by the ERG through a consensus-based process.

The consensus process was used where:

- the systematic review found insufficient evidence to address the clinical question
- the ERG determined that additional clinical practice guidance (expert opinion) was required for the evidence-based recommendations
- the development of clinical commentary was required.

The consensus process followed is presented below.

Stage 1 – Introduction

The consensus process, participants' roles and responsibilities, ground rules and guiding principles are provided to members.

Stage 2 – Open discussion

The Chair opens the floor to a general discussion and suggestions for expert opinion or clinical commentary wording. The Chair provides an opportunity for concerns or issues to be raised.

Stage 3 – Resolve concerns

The Chair has the first option to resolve concerns by clarifying or changing the wording or seeing whether those with concerns will stand aside. Where concerns are not resolved and the time is short, the discussion will be carried over to a later meeting.

Stage 4 – First call for consensus

The Chair calls for consensus. If consensus is not reached, the ERG will consider the consensus process guiding principles and values before the Chair calls for consensus again.

Stage 5 – Second call for consensus

If consensus is not reached:

- the member stands aside, and the differing schools of thought are documented
- the member is not willing to withdraw the concern or stand aside, and the ERG declares itself blocked – the proposed clinical guidance is not accepted
- the member withdraws their concern and consensus is reached.

Conflict of interest

All members of the ERG were asked to declare any interests before starting work on the guidelines. Members were advised that the NBA regards a conflict of interest as referring to any situation where any professional, commercial, financial, personal or other interest or duty of the ERG member means that:

- the ERG member may not participate in the activity in a fair and impartial way; or
- the ERG member may have the opportunity to gain an improper benefit or advantage (for themselves or another person or organisation) as a result of participating in the activity.

ERG members were asked to take a broad and conservative view and were provided with a conflict of interest form to draw out the domains and topics that could provide a source of a conflict of interest and subsequently affect proceedings within the ERG. Members were asked to declare both pecuniary and non-pecuniary interests:

- **Pecuniary interests** are possible financial advantages or disadvantages of participating in a process associated with businesses or companies that are providers of products, viewpoints or information that could be relevant to the ERG.
- **Non-pecuniary interests** can include the notions of reputation, pursuing a particular favoured practice or supporting a particular viewpoint of a group with whom members are affiliated.

New declarations were required to be declared to the NBA and Chair before the start of each meeting as a standing agenda item on each day of a meeting. The NBA kept a register of all declared interests. If an interest was declared, and the Chair decided that it should be considered by the ERG, the ERG decided by consensus whether it affected the proceedings. If the interest was considered to be competing or in conflict, the Chair directly managed the participation of that member in relation to discussions and decisions pertaining to the declared interest.

The Chair considered all declarations and determined that none constituted a conflict of interest. The Chair's declarations were reviewed by the NBA project management team and were not considered a conflict of interest. None of the NBA and evidence review contractors had any declarations.

Public consultation

Public consultation was conducted for 7 weeks from 20 September 2019 to 8 November 2019, during which time the draft guideline was available on the NBA website. The NBA also sent formal notification to all organisations with a representative on the ERG, with a request that they disseminate the draft guideline within their networks.

Seventeen submissions were received. Some of those submissions included literature that had not been captured in the systematic review process due to it being published after the literature searches were conducted. The ERG met on 28 November 2019 to review the public consultation submissions and supporting documentation. Changes were made to the guideline to address comments and concerns raised in submissions, and to improve clarity. Where recommendations were revisited in light of new literature published, the ERG used an expert consensus process in reviewing and updating the clinical guidance.

Appraisal of the guideline

The *Appraisal of Guidelines for REsearch & Evaluation* (AGREE) II instrument was developed to address the issue of variability in guideline quality and assesses the methodological rigour and transparency in which a guideline is developed *[82]*. The post-public consultation version of the guideline was sent to two Australian reviewers,

independent to the guideline development process, who used the AGREE II tool to assess the quality and usability of the guideline against international quality standards.

Both reviewers recommended the guideline for use, with one reviewer giving a rating of six out of seven and the other reviewer giving a rating of seven out of seven for overall quality of the guideline. Seven is the highest possible quality rating.

16. Abbreviations and acronyms

anti-D	Rh D antibodies
BMI	body mass index
BSA	body surface area
cfDNA	cell-free DNA
CI	confidence interval
CVS	chorionic villus sampling
DAT	direct antiglobulin test
DNA	deoxyribonucleic acid
EOP	expert opinion point
ERG	Expert Reference Group
FMH	fetomaternal haemorrhage
FNR	false-negative rate
FPR	false-positive rate
GRADE	Grading of Recommendations, Assessment, Development and Evaluation
HDFN	haemolytic disease of the fetus and newborn
Ig	immunoglobulin
IM	intramuscular
IU	international units
IV	intravenous
JBC	Jurisdictional Blood Committee
MD	mean difference
NBA	National Blood Authority
NHMRC	National Health and Medical Research Council

NIPT	non-invasive prenatal testing
NSQHS	National Safety and Quality Health Service
NSW	New South Wales
PCR	polymerase chain reaction
PICO	population, intervention, comparator, outcome
R	recommendation
RAADP	routine antenatal anti-D prophylaxis
RANZCOG	Royal Australian and New Zealand College of Obstetricians and Gynaecologists
RBC	red blood cells
RCT	randomised controlled trial
RHD	refers to genotype
Rh D immunoglobulin	refers to the product
Rh D positive/negative	refers to blood type
RNA	ribonucleic acid
RT-PCR	real-time polymerase chain reaction
STIR	Serious Transfusion Incident Reporting (Victorian Blood Matters program)
UK	United Kingdom

17. Terminology

Terminology	Notes
250 IU, 625 IU, 1500 IU	Where the dose is presented in the guideline, it is given after the generic product name and in IU. Some other guidelines use micrograms (µg) as the unit of measurement – the conversion is as follows: 250 IU (50 µg), 625 IU (125 µg), 1500 IU (300 µg).
Antenatal, antepartum or prenatal	Each can be used depending on context. If referred to in the research questions, references or in content taken from published guidelines, the use is as stated in the original.
Anti-D antibodies	This term is used when referring to the circulating antibodies wherever possible. Some variation in terminology may be present in the summary of evidence tables to reflect the terminology used in the corresponding literature. Passive antibodies – Acquired from an external source such as administration of Rh D immunoglobulin. Preformed antibodies – Acquired when an Rh D negative woman is exposed to Rh D positive blood and develops antibodies to Rh D (known as sensitisation).
Baby or infant	The 2003 guideline *[1]* refers to *baby* and *infant*; this guideline uses the term baby throughout.
First trimester or first 12 weeks of pregnancy	If referred to in the research questions, references or in content from previous guidelines, the use is as stated in the original. In new recommendations, EOPs or commentaries, the term used is *first 12 weeks of pregnancy*, and refers to gestation up to 12^{+6} weeks and days.
Immunisation or alloimmunisation	*Immunisation* is used for donors and *alloimmunisation* for Rh D negative pregnant women.
Immunoprophylaxis or prophylaxis	If referred to in the research questions, references or content taken from published guidelines, the use is as stated in the original. In new recommendations, EOPs or commentaries, *immunoprophylaxis* is used.
Large fetomaternal haemorrhage (large FMH)	≥ 6 mL of fetal red cells (equivalent to 12 mL of whole blood)
Non-invasive prenatal testing (NIPT) for fetal *RHD*	Various terms are used to describe the test for determining the *RHD* genotype of a fetus, including non-invasive prenatal screening, non-invasive prenatal assessment, non-invasive prenatal testing (NIPT) and non-invasive fetal *RHD* genotype testing. The term NIPT for fetal *RHD* is used in this guideline.
Postnatal or postpartum	If referred to in the research questions, references or in content taken from published guidelines, the use is as stated in the original. In new recommendations, EOPs or commentaries, the term used is *postnatal*.
Primigravida/e or first pregnancy/ies	*First pregnancy/ies* is used in preference to *primigravida/e*; the latter is used only where it is referred to in a reference.
RHD	*RHD* is used to refer to the genotype.
Rh D immunoglobulin	The product *Rh D immunoglobulin* is discussed in generic terms (without brackets around the 'D'). Brackets around the 'D' are used only when referring specifically to the CSL Behring product.

Terminology	Notes
Rh D negative women or Rh D negative mothers	These are women who have the Rh D negative blood type. The term *Rh D negative women* is used in preference to *Rh D negative mothers*.
Rh D positive or Rh D negative	*Rh D positive* and *Rh D negative* are used in relation to blood type; the term *Rhesus* is used only where it is referred to in a reference.
Termination of pregnancy	Refers to either medical or surgical abortion. The RANZCOG Clinical Guideline for Abortion Care defines abortion as the removal of pregnancy tissue or the fetus and placenta from the uterus *[148]*.
Weeks gestation or weeks of pregnancy	If referred to in the research questions, references or content taken from published guidelines, the use is as stated in the original. In new recommendations, EOPs or commentaries, *weeks of pregnancy* is used.

EOP: expert opinion point; IU, international units;

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