Technical report

Volume 1 – Evidence review

Prepared for
National Blood Authority

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

Technical report prepared by
Health Technology Analysts Pty Ltd

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Technical report information

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Name	Contribution
Margaret Jorgensen	Project Manager responsible for leading the review. Design and development of the search methodology, search strategy, and research protocol Oversight of the technical report and guideline development process with contributions from other authors as described.
Stephanie Allerdice	Developed and tested the search strategy. Named reviewer with contributions to the selection of studies (screening), data extraction, appraisal of studies, and draft technical reports.
Santwona Baidya	Named reviewer with planned contributions to the selection of studies (screening), data extraction, appraisal of studies.
Adrian Peacock	Contributed to the selection of studies (screening), data extraction and appraisal of studies.
Kevin Phan	Contributed to the selection of studies (screening), data extraction and appraisal of studies.
Expert Reference Group	Provided expert clinical advice, especially in relation to research questions and eligibility criteria. Conducted critical review of the protocol.

Declarations of interest

All authors have no financial, personal or professional interests that could be construed to have influenced the conduct or results of this systematic review.

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History

The National Health and Medical Research Council (NHMRC) *1999 Guidelines on the Prophylactic Use of Rh D Immunoglobulin (Anti-D) in Obstetrics* were updated by the NBA in 2003, with the aim of updating the guidance on antenatal prophylaxis.

This technical report presents the main body of evidence found by a systematic literature review on the use of Rh D immunoglobulin in Rh D negative pregnant women. The review was guided by a Research Protocol that was developed by HTAnalysts (the systematic reviewers), in conjunction with the NBA and ERG. The Research Protocol provided a framework outlining the methodology that was to be used to update and/or replace evidence-based recommendations in the 2003 Anti-D Guidelines.

NHMRC approval has not been sought for this update, but all associated materials have been developed in a robust and transparent manner in accordance with relevant best practice standards.

Dates

The Research Protocol for the systematic review received approval from the Expert Reference Group (ERG) and National Blood Authority (NBA) on 18 July 2018.

Findings of the evidence review were presented to the ERG on 6 March 2019, 27 March 2019, and 3 April 2019 and draft recommendations were developed.

Three teleconferences were then held on the 6 May 2019, 8 May 2019, and 30 May 2019 to seek members' advice on how the evidence-based recommendations would impact current clinical practice and supply arrangements, to provide comments on the guideline's layout and structure, and to discuss any additional clinical guidance to support the evidence-based recommendations.

A further meeting was held 12 June 2019 to incorporate guidance from the 2003 Guideline that was not included in the systematic review and edits to the clinical guidance were reviewed via a teleconference held 8 July 2019. On 28 November 2019, a final meeting was held to consider and incorporate feedback received after public consultation related to the clinical guidance, after which access and implementation concerns were considered.

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Abbreviations

AGREE	Appraisal of Guidelines for Research and Evaluation
AHRQ	Agency for Healthcare Research and Quality
AMSTAR	A MeaSurement Tool to Assess systematic Reviews
ANZCTR	Australian and New Zealand clinical trial registry
BMI	Body mass index
BSA	Body surface area
CADTH	Canadian Agency for Drugs and Technologies in Health
CI	Confidence interval
CINAHL	The Cumulative Index to Nursing and Allied Health Literature
ERG	Expert Reference Group
FMH	Fetomaternal haemorrhage
FNR	False-negative rate
FPR	False-positive rate
GDT	Guideline development tool
GRADE	Grading of Recommendations Assessment, Development and Evaluation
GW	Gestational week
HDFN	Haemolytic disease of the fetus and newborn
HTA	Health technology assessment
IgG	Immunoglobulin G
IM	Intramuscular
ITP	Immune/idiopathic thrombocytopaenia
ITT	Intent-to-treat
IU	International units
IV	Intravenous
JCC	Joint Consultative Committee
MeSH	Medical Subject Headings
NBA	National Blood Authority
NICE	National Institute for Health and Care and Excellence
NIPT	Noninvasive prenatal testing
NHMRC	National Health and Medical Research Council
NR	not reported
PBM	Patient blood management
PCR	Polymerase chain reaction
P/I/C/O	Population/Intervention/Comparator/Outcome
PP	per protocol
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
RoB	Risk of bias
RR	Relative risk
SD	Standard deviation
SIGN	Scottish Intercollegiate Guidelines Network
SR	Systematic review
WHO ICTRP	World Health Organization International Clinical Trials Registry Platform

1 Background

The National Health and Medical Research Council's (NHMRC) *1999 Guidelines on the Prophylactic Use of Rh D Immunoglobulin (Anti-D) in Obstetrics* (NHMRC, 1999) were updated by the National Blood Authority (NBA) in 2003 (NBA, 2003), with the aim of updating the guidance on antenatal prophylaxis. The updated 2003 Anti-D Guidelines aimed to inform clinicians, other health professionals, and policy makers of new recommendations for the staged implementation of full antenatal prophylaxis in Australia. The 2003 Anti-D Guidelines also included policy intent as a national program to address the issue of sufficiency of supply of product and were intended to be reviewed within five years, according to the availability of Rh D immunoglobulin.

To ensure the Anti-D Guidelines continue to reflect current evidence and best clinical practice, the NBA undertook a scoping exercise in September 2016 to identify the extent of newly available evidence and areas of concerns (Health Research Consulting, 2017). Through a collaborative partnership with the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) and other relevant stakeholders, it was agreed that a new evidence-based guideline would be developed. Whilst a robust and transparent process will be followed, it was noted the guideline would not be submitted to NHMRC for approval.

Key areas of concern identified in the scoping report include the following:

1. Does the available evidence still support universal[1] routine antenatal prophylaxis?
2. Should universal routine antenatal prophylaxis be moved from a two-dose regimen to a one-dose regimen?[2]
3. Should the list of first trimester sensitising events be amended to include additional events?
4. To reduce unnecessary use of Rh D IgG, should noninvasive prenatal screening be used in the first trimester so that prophylaxis can be targeted?
5. Does higher body mass index (BMI) impact on the efficacy of Rh D immunoglobulin?[3]

In October 2017, a multidisciplinary Expert Reference Group (ERG) with expertise from a range of clinical settings met to discuss the scope of the updated guidelines and to provide advice on the existing and target evidence base. Four research questions (two with subquestions) and their associated PICO/PPO[4] criteria were developed and have been used inform the basis of this Research Protocol.

[1] i.e., all pregnant women who are Rh D negative with no preformed anti-D.

[2] In June 2010, the Rh(D) Joint Consultative Committee (JCC) considered the available evidence and the relative advantages and disadvantages, and strongly supported the move. A trial on this comparison has been conducted in Australia and the results were presented at the HAA Meeting in November 2016. Pennell, C., J. Cheng, B. P. Veselinovic, et al. (2017). Single dose Anti-D prophylaxis in pregnancy: is it time to change? *Journal of Paediatrics and Child Health* **53**(S2): 112-113.

[3] An Expert Panel was convened in May 2015 and the draft Consensus Statement indicates there is still uncertainty around this issue. see https://www.ranzcog.edu.au/RANZCOG_SITE/media/RANZCOG-MEDIA/Women%27s%20Health/Statement%20and%20guidelines/Clinical-Obstetrics/Consensus-Position-Statement-RhDIg-and-Women-with-High-BMI.pdf?ext=.pdf.

[4] Population. Intervention, Comparator, Outcome / Population, Predictor, Outcome

1.1 Description of condition and setting

Maternal Rh antibodies develop during pregnancy when an Rh negative woman carries an Rh positive fetus. Antibodies develop when fetal red blood cells (RBCs) enter the maternal circulation and antibodies are produced towards the fetal Rh antigen. Small fetomaternal haemorrhages at birth and silent transplacental haemorrhages in the antenatal period are believed to be the key source of fetal RBCs entering the maternal circulation (Bowman, 2003, Chilcott et al., 2003, McBain et al., 2015). 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, but haemolytic disease of the fetus and newborn (HDFN) can arise in an Rh positive fetus (usually in subsequent pregnancies).

HDFN occurs when maternal Rh 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 hyperbilirubinemia (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) (Bowman, 2003, McBain et al., 2015, Zwiers et al., 2018). In the absence of intervention, HDFN affects 1% of neonates, and is a significant cause of perinatal mortality, morbidity, and long-term disability (Bowman, 2003, Chilcott et al., 2003).

In Australia, about 17% of blood donors are Rh negative.[5] It 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 10% of live births are Rh D positive infants delivered to Rh D negative women (Chilcott et al., 2003); however, this number may be higher in the Australian setting (Hyland et al., 2013).

1.2 Description of intervention and how it might work

Rh D immunoglobulin G (IgG) antibodies are manufactured from pooled plasma of Rh negative blood donors who are stimulated to produce elevated levels of anti-D antibodies. The sterile solution (usually administered as an intramuscular [IM] injection[6]) is given to Rh D negative women with no preformed anti-D antibodies (during pregnancy or postpartum) and acts by preventing the mother from developing her own anti-D antibodies (though immune mediated immunosuppression).

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 (Bowman, 2003). 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 (Kinnock et al., 1970). 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 (Bowman, 2003).

[5] http://resources.transfusion.com.au/cdm/ref/collection/p16691coll1/id/233

[6] Intravenous Rh D IgG is available for instances of large fetomaternal haemorrhage, when IM is contraindicated or not practical.

Clinical trials demonstrated that Rh D immunoprophylaxis given immediately after birth decreases the risk about 10-fold to approximately 1% (Crowther et al., 2000), results supported by observational studies (Freda et al., 1975, Bowman, 1981). Adding antenatal immunoprophylaxis may reduce the risk to about 0.2% (McBain et al., 2015). As a result of programs of immunoprophylaxis, HDFN has gone from being a leading cause of fetal and neonatal illness and death (Fretts et al., 1992) 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 (Liley, 1997, Bowman, 2003), 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 burdens 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 immunoglobulin prophylaxis should be recommended unless it is certain that the anti-D is preformed (ANZSBT, 2020).

In Australia, prophylaxis with Rh D IgG is recommended for three groups of Rh D negative women with no preformed anti-D antibodies:

1. those subject to a sensitising event during pregnancy
2. as routine (*universal*) antenatal prophylaxis at 28 and 34 weeks' gestation (Level II evidence)
3. as *targeted* postnatal prophylaxis to those who have given birth to a Rh D positive baby (Level I evidence)

During pregnancy, Rh D IgG prophylaxis should be administered as soon as possible after the sensitising event, but always within 72 hours (Level I evidence). A dose of 250 international units (IU; 50 µg) should be offered to those in the first trimester (up to and including 12 weeks' gestation) (Level IV evidence) and a dose of 625 IU (125 µg) should be offered to those beyond the first trimester (Level IV evidence).

Routine antenatal anti-D prophylaxis (RAADP) can be administered at 28 weeks' gestation at a single dose (1500 IU) or two doses given at 28 and 32-34 weeks' gestation (dose range 500 to 625 IU) (Urbaniak, 1998). A summary of International Guidance is provided in **Appendix 1**.

Because Rh D IgG is a blood derivative, all women should be informed of its source and should give informed consent. Blood donors are carefully screened for transmissible infections, but there is always a minor risk of the transmission of blood-borne infections. A small risk of localised or generalised allergic reactions also exist.

A small proportion of HDFN is caused by antibodies to antigens other than D,[7] therefore Rh D IgG would not be effective in these cases.

[7] According to the Australian Red Cross, IgG antibodies against other Rh antigens (including c, e, C, E) and blood group antigens (including Fya and K) occur in about 0.5% of pregnancies. https://transfusion.com.au/disease_therapeutics/fetomaternal/HDN Accessed 22 May 2018.

2 Rationale and objectives

As noted in the scoping report, the rationale for conducting this review is related to updating the evidence and guidance involving universal antenatal prophylaxis in women who are Rh D negative (Health Research Consulting, 2017). This is because questions pertaining to appropriate use, avoiding unnecessary use, and reducing the burden on stimulated donors remain uncertain.

The four main clinical questions (and two subquestions) chosen for evidence review by the ERG are:

1. In Rh D negative pregnant women with no preformed anti-D, does universal routine antenatal prophylaxis with Rh D immunoglobulin (1 or 2 doses) prevent Rh D alloimmunisation?
    a. 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? (subquestion)
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/without a curette), does universal first trimester sensitising event prophylaxis with Rh D immunoglobulin prevent Rh D alloimmunisation?
3. In Rh D negative pregnant women with no preformed anti-D, does targeted routine antenatal or sensitising event prophylaxis to women with a Rh D positive fetus increase the incidence of Rh D alloimmunisation compared with universal routine antenatal or sensitising event prophylaxis?
    a. In Rh D negative pregnant women with no preformed anti-D, what is the diagnostic accuracy of noninvasive prenatal screening to identify fetal Rh D status? (subquestion)
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?

Question 1 is intended to update the evidence base regarding universal administration of routine antenatal prophylaxis at 28 and 34 weeks' gestation (intervention question). A subquestion will assess the evidence as to whether the two-dose strategy can be replaced with a single-dose strategy.

Question 2 is intended to examine whether universal administration of sensitising event prophylaxis in the first trimester should include the following additional events: abdominal trauma, molar pregnancy, threatened miscarriage and medical termination of pregnancy (intervention question).

Question 3 is intended to examine whether targeted administration can replace universal administration during pregnancy, thereby reducing the number of women who need to receive Rh D immunoglobulin (thereby reducing the amount of Rh D immunoglobulin that needs to be produced) (screening question). A subquestion will assess the diagnostic accuracy of the noninvasive prenatal screening test (diagnostic accuracy question).

Question 4 will examine whether an increasing BMI impacts on the effectiveness of Rh D immunoglobulin dosing (prognostic question).

Details of each research question are provided in **Appendix 2**.

The Research Protocol described the methodology intended to be used to (i) source the clinical evidence by performing a systematic literature search of the literature, (ii) selecting the best available evidence; (iii) critically appraising and presenting the evidence, and (iv) determining the quality of the evidence base for each question, using a structured assessment of the body of evidence in accordance GRADE[8] methodology.

This technical report presents, in detail, the evidence base for each research question by outcome and included the following information:

1. The methodology used to identify the evidence base (clinical questions addressed in the guidelines, documented systematic literature search, inclusion and exclusion criteria described).
2. The characteristics and quality of the evidence base (data extraction and risk of bias forms) (**Volume 2**).
3. Detailed results included in the evidence base, presented by outcome. That is, all evidence relating to a particular outcome will be presented together in an evidence summary tables and GRADE summary of findings tables.

[8] Grading of Recommendations Assessment, Development and Evaluation. Available at http://gdt.guidelinedevelopment.org/app/handbook/handbook.html

3 Methods

Methods used in this systematic review are based on that described in the *Cochrane Handbook for Systematic Reviews of Interventions* (The Cochrane Collaboration, 2011).

Covidence, a web-based platform for producing systematic reviews (www.covidence.org) was used to store data that are compatible with the Cochrane data collection tools. RevMan[9] was used for the main analyses and GRADEpro GDT software (www.gradepro.org) was used to record decisions and derive an overall GRADE (high, moderate, low, or very low) for the quality of evidence for each outcome.

To identify the evidence base for the four clinical questions detailed in **Appendix 1**, a systematic search of published medical literature was conducted and all potentially relevant studies were identified after applying prespecified inclusion and exclusion criteria as outlined below. Details of the systematic literature search are provided in **Volume 2** of the technical report.

No changes to the original protocol were made.

3.1 Criteria for selecting studies for this review

3.1.1 Types of participants

Questions 1, 2, and 3 included *pregnant* women who are Rh D negative and do not have pre-formed anti-D antibodies. The focus of these questions was *antenatal prophylaxis* (i.e. during pregnancy). Question 4 included women who are Rh D negative with no preformed anti-D antibodies receiving prophylaxis either *during pregnancy* or *postpartum* (after the birth of an Rh positive infant).

For questions 1 and 2, the focus was *universal* prophylaxis. That is, administration of Rh D IgG to *all* Rh D negative pregnant women who have no preformed anti-D antibodies.

For question 3, the focus was *targeted* prophylaxis. That is, administration of Rh D IgG to Rh D negative women with no preformed anti-D antibodies who are pregnant with an Rh D positive fetus identified via noninvasive prenatal screening.

For question 2, study participants were women who had experienced a *first trimester* sensitising event. This is an event that leads to the development of anti-D antibodies due to maternal-fetal blood exchange. The definition of first trimester varies across countries and was as defined by the literature.

Sensitising events specifically included were:

- abdominal trauma (blunt or penetrating injury) – e.g. after motor vehicle accident or fall
- molar pregnancy (nonviable fertilised egg fails to develop) – could be complete or partial
- ectopic pregnancy (the fertilised egg develops outside the uterus)
- spontaneous miscarriage (noninduced death of the embryo or fetus before 20 weeks' gestation)

[9] Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.

- threatened miscarriage (viable pregnancy associated with abnormal vaginal bleeding and abdominal pain)
- medical termination of pregnancy (with/without a curette) – i.e. removal of the lining and the contents of the uterus by suction with a small plastic tube or by medication with drug such as mifepristone.

Events such as surgical termination of pregnancy, amniocentesis, and chorionic villus sampling were to be excluded.

Restrictions: There were no limits to age, race or nationality; and studies examining use of Rh D immunoglobulin in women with immune/idiopathic thrombocytopaenia (ITP)[10] were excluded.

Geographical restrictions: Studies had to be set in countries with health systems broadly comparable to those in Australia[11], 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.

3.1.2 Types of interventions

Studies evaluating the effectiveness of Rh D IgG were eligible for inclusion. There was no restrictions on the product type, mode of administration, number of doses, or dosage.

In question 1, studies were stratified by those examining the effectiveness of (i) one or two doses, (ii) one dose, or (iii) two doses. Here, the comparator was placebo or no universal prophylaxis in the antenatal period. For the subquestion, studies comparing a one-dose regimen with a two-dose regimen were included.

For question 2, the comparator was placebo or no prophylaxis after a first trimester sensitising event.

For question 3, to provide *targeted* prophylaxis, identification of an Rh D positive fetus is required. The prenatal screening tests were to be noninvasive (i.e. a simple blood test that uses maternal blood to determine the baby's fetal *RHD* status), but there were no restrictions on the timing, product type, or testing methodology. For the main question, the comparator was *universal* prophylaxis with Rh D Ig G.

For the subquestion of question 3, there were two comparators: (i) postnatal cord blood testing (collection of fetal blood directly from the umbilical cord) and testing for fetal Rh D status using a direct antibody test and (ii) other noninvasive fetal Rh D diagnostic test. As with the main question, there were no restrictions on the timing, product type or testing methodology.

Question 4 is a prognostic question therefore no comparative interventions were assessed.

[10] An autoimmune disorder characterised by mucocutaneous bleeding and a markedly decreased blood platelet count.

[11] E.g. Australia, New Zealand, United Kingdom, Europe, Canada, the United States of America.

3.1.3 Types of outcome measures

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, and in subsequent pregnancies).

Other outcome measures included:

- the incidence of a positive test for fetomaternal haemorrhage (i.e. a test that detects fetal cells in the maternal blood such as the Kleihauer test[12] or flow cytometry). Additional data extracted related to timing (i.e. at potentially sensitising events and postpartum).
- Utilisation rates of Rh D immunoglobulin.
- Neonatal adverse events (e.g. jaundice) in current or subsequent pregnancies. Events attributed to Rh D immunoglobulin and the severity (mild, moderate, severe) of the adverse event were recorded.
- Maternal adverse events attributed to Rh D immunoprophylaxis (e.g. rash, headache, allergic response, infection). The severity (mild, moderate, severe) of the adverse event was noted.

For diagnostic accuracy, critical outcome measures include sensitivity, specificity, false positives, and false negatives. Important measures were positive likelihood ratio and negative likelihood ratio.

3.1.4 Types of studies

Characteristics of the ideal evidence base specific to each question is provided in Table 3-1.

The types and definition of study designs eligible for inclusion are based on guidance from the NHMRC levels of evidence[13]. The review considered both peer reviewed and 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 body of evidence was assessed before lower levels of evidence were considered (as depicted in Figure 3.1). Here, a systematic review of Level II studies was considered the highest level of evidence (Level I) for all question types. If the Level I evidence effectively addressed the specified outcomes of interest for each research question, assessment of Level II and III evidence was not be conducted. However, an update of the literature was conducted to identify any Level II studies published since the search date of the key Level I evidence.

If no relevant Level I evidence was identified for a specific research question, a literature search to identify Level II studies was conducted. If no studies are identified the process was to be repeated for lower level evidence (to the level specified in the PICO/PPO criteria). For critical and important outcomes not addressed in higher level evidence, a search of lower level evidence will be conducted for that outcome only.

[12] The ERG debated whether to include 'incidence of a positive Kleihauer test' as an outcome. Given its inclusion in the 2015 Cochrane review (http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD000020.pub3/full) the ERG agreed to include, but to rate the outcome as not important.

[13] https://www.nhmrc.gov.au/_files_nhmrc/file/guidelines/developers/nhmrc_levels_grades_evidence_120423.pdf

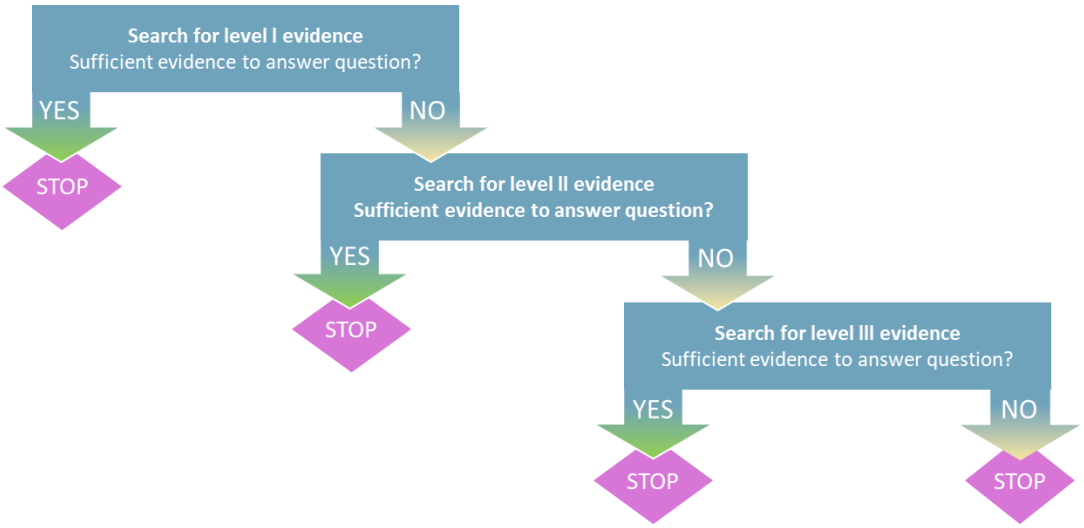
Table 3-1 Characteristics of the ideal evidence base specific to each question

Question	1	2	3	4
What type of question is this?	Interventional	Interventional	Screening Diagnostic (subquestion)	Prognostic
What type of evidence is appropriate?	SR of RCTs Individual RCT Non-RCT Observational study (with concurrent or nonconcurrent controls)	SR of RCTs Individual RCT Non-RCT Observational study (with concurrent or nonconcurrent controls)	*Screening* SR of RCTs Individual RCT Non-RCT Observational study (with concurrent or noncurrent controls) *Diagnostic* A study of test accuracy with: an independent, blinded comparison with a valid reference standard: (i) among consecutive persons with a defined clinical presentation or (ii) among nonconsecutive persons with a defined clinical presentation A comparison with reference standard that does not meet the criteria required for the above study types A diagnostic case-control study	SR of prospective cohort studies Prospective cohort study Single-arm RCT Retrospective cohort
What size of study is acceptable?	Any	Any	Any	Any
How should the impact of time be considered?	Reported as per included studies ERG to agree a clinically meaningful study duration and/or clinically sensible timepoints at which measurements should occur	Reported as per included studies ERG to agree a clinically meaningful study duration and/or clinically sensible timepoints at which measurements should occur	Reported as per included studies ERG to agree a clinically meaningful study duration and/or clinically sensible timepoints at which measurements should occur	Reported as per included studies ERG to agree a clinically meaningful study duration and/or clinically sensible timepoints at which measurements should occur
What publication time frame is appropriate?	6 months prior to search date of previous NBA guideline (June 2000) Study dates should be reported to ensure any changes in anti-D preparation or administration over time can be factored into the assessment of the evidence	Any Study dates should be reported to ensure any changes in anti-D preparation or administration over time can be factored into the assessment of the evidence	Any Study dates should be reported to ensure any changes in anti-D preparation or administration over time can be factored into the assessment of the evidence	Any Study dates should be reported to ensure any changes in anti-D preparation or administration over time can be factored into the assessment of the evidence

Source: Anti-D scoping report (Health Research Consulting, November 2017)

Abbreviations: ERG, Expert Reference Group; NBA, National Blood Authority; RCT, randomised controlled trial; SR, systematic review.

Figure 3.1 Schematic representation of literature review hierarchy



Further assessment down to Level IV was not conducted for any research question, irrespective of whether insufficient 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. Where there is insufficient or no Level I to III evidence available to answer a question, an 'expert opinion point' will be made.

3.2 Search methods for identification of studies

3.2.1 Search terms

The search strategy was developed in Ovid (for Embase and MEDLINE) based on key elements provided in the research questions (PICO/PPO criteria). The searches were not limited by outcome, but rather by population, intervention, and then study type (applied using the stepped approach outlined earlier). Search terms and results are provided in **Appendix A, Volume 2** of the technical report.

The search strategy was then adapted to suit CINAHL Plus, the Cochrane Library (database of systematic reviews, other reviews, clinical trials, technology assessments, economic evaluations)[14] and PubMed (limited to in-process citations and citations not indexed in MEDLINE).

Methodological filters for identifying different levels of evidence (Level I, Level II, and Level III) developed previously for the *Patient Blood Management Guidelines* were applied (based on NHMRC and SIGN[15]) and exclusions for publication types added. The search syntax from embase.com was converted to the Ovid platform.

In developing the search strategy, we appraised and adapted the search strategies suggested in the anti-D scoping report; with terms or concepts proven not suitable removed and other terms added.

[14] The Cochrane Library was searched via the EBM database provided in Ovid. The EBM covers the Cochrane Database of Systematic Reviews, Cochrane Clinical Answers, Cochrane Central Register of Controlled Trials, American College of Physicians Journal Club, Cochrane Methodology Register, Health Technology Assessments, The Database of Abstracts of Reviews of Effectiveness, and NHS Economic Evaluation Database

[15] Scottish Intercollegiate Guidelines Network

Systematic reviews identified in the scoping report and other health technology assessment reports were also reviewed to identify other potentially relevant concepts.

No date, language or geographic limitations were applied when conducting the search. Literature search start dates defined by the ERG for question one (2002) were applied once citations are imported in to the bibliographic management database.

3.2.2 Databases

In addition to the primary databases listed above (Embase, MedLine, CINHAL, the EBM, and PubMed), searches of additional secondary databases were conducted. This includes:

- OpenGrey and Clinical trial registers (ClinicalTrials.gov and WHO ICTRP[16])
- Health technology assessment and government websites that oversee maternal health (e.g., NICE[17], CADTH[18], and AHRQ[19])
- Guideline databases (Guidelines International Network, National Guidelines Clearing House)

3.2.3 Other sources

Hand-searching of reference lists of key relevant articles will be conducted to identify any additional studies not identified through searches of the primary databases. Relevant systematic reviews will be searched for additional studies, articles recommended by ERG members, and potentially relevant studies/systematic reviews identified in the scoping report were considered for inclusion if they satisfy eligibility criteria and were published within the specified search period of the systematic review.

3.3 Screening of studies

For each question, citations (title/abstracts) retrieved by the literature searches for each database were imported into EndNote and duplicates removed. Citations were then imported into Covidence (www.covidence.org), to streamline the screening and data extraction process. A prespecified, hierarchical approach was used to annotate reasons for exclusion. Citations that did not meet the inclusion criteria were excluded and the reason for exclusion noted.

One systematic reviewer (SA) independently screened citations relevant for full text review. A second reviewer (SB or AP) checked the screening process to ensure adherence to the *a priori* exclusion criteria and any differences were resolved by discussion with a third reviewer (MJ). Full text articles identified for possible inclusion in the evidence synthesis were retrieved and independently assessed for inclusion by two reviewers (SA, SB) disagreements were resolved by discussion with a third reviewer (MJ). Advice was sought from the ERG to confirm eligibility based on PICO/PPO criteria.

If a study did not contain enough information for a decision to be made about its eligibility, further information was sought from the study's authors.

[16] World Health Organization International Clinical Trials Registry Platform

[17] National Institute for Health and Care and Excellence

[18] Canadian Agency for Drugs and Technologies in Health

[19] Agency for Healthcare Research and Quality

Trial registration numbers, author names, and study titles, locations and dates were used to identify multiple reports arising from the same study.

A list of potentially relevant papers, and the existing clinical questions to which they apply, was then supplied to the NBA and ERG, with an understanding of the scope of new evidence to undergo full critical appraisal and data extraction reached before proceeding to the next Stage of the review.

The search strategy was not be limited by language; however, publications in languages other than English were only considered where a full text translation into English was available.

Literature search dates for question one commenced from 2002[20]. Questions 2, 3, and 4 were not date limited to ensure any changes in Rh D immunoglobulin preparation or administration over time can be factored into the assessment of the evidence.

To maintain the rigour of the systematic review process, studies published after the literature search date of the systematic review were not eligible for inclusion in the technical report. However, pivotal new evidence could be discussed in the guideline document and used to develop consensus-based 'expert opinion'.

3.4 Data collection, critical appraisal, and summary of the evidence

The methodological quality of included studies was critically appraised and appropriate data extracted into data extraction tables. The results were then analysed and summarised into appropriate categories or subquestions according to the key research question/s and evidence profiles developed guided by the GRADE framework, with relevance to the Australian context considered at this time.

Only data from systematic reviews (NHMRC Level I) of low risk of bias were to be extracted, with a verification of source data (from the primary studies) not to be completed. It was intended that a return to source documents would occur if the systematic review was assessed to be at moderate, high, or serious risk of bias.

For Question 1, it was determined that, although the identified systematic reviews (Chilcott et al., 2003, Pilgrim et al., 2009) were assessed to be at moderate risk of bias, the authors had provided sufficient (and additional information) regarding to the identified primary studies to warrant omission of this step. For Question 3, a return to the source documents was not done for a few reasons; either because sufficient (and additional) information had been provided (Saramago et al., 2018), there was insufficient information regarding the included citations (Zhu et al., 2014), or due to the high volume of primary studies identified (Geifman-Holtzman et al., 2006, Mackie et al., 2017), coupled with expert opinion that the available evidence was sufficient to inform clinical decision making.

3.4.1 Data extraction

For each included study, one reviewer extracted data using a pre-tested data extraction and coding form. Data extraction forms were then be checked by a second reviewer and disagreements resolved by discussion. Sample data extraction forms are provided in Appendix 3.

[20] The Research Protocol incorrectly noted the date limit as the year 2000.

The following characteristics of included studies were extracted:

- study design
- year conducted
- setting and location
- participant characteristics (including number of pregnancies, timing and nature of the sensitising event, or those needed to characterise risk group such as BMI or weight)
- intervention and comparator characteristics (including product, timing, mode, number of doses/dosage, and administration technique)
- outcomes measures (including measurement method, timing, or severity)
- results for critical and important outcomes
- funding sources and funder involvement in study

3.4.2 Critical appraisal

Critical appraisal methodology was applied to assess any study design, methodological or reporting bias', strengths and weaknesses. Here, the clarity and completeness or reporting, methods and processes, as well as the underlying assumptions and limitations was assessed. In general, the risk of bias associated with prespecified domains (such as selection of participants, outcome assessment bias, attrition bias) were assessed using the most appropriate risk of bias assessment tool according to study design as outlined in Appendix 4.

For each study, our judgement of risk of bias (e.g. low, moderate, high, critical, unclear) was reported for each domain, with a rationale for the judgement and supporting information also provided. Some domains were assessed separately for different outcome categories (blinding of outcome assessment, incomplete outcome data); and judgements reported by outcome for these domains. Overall risk of bias judgements are summarised in the characteristics of included studies table.

For GRADE assessments, the overall risk of bias for each outcome within a study was first assessed, and then the risk of bias assessments across studies for each outcome. These summary assessments of risk of bias were used in determining the overall quality of the body of evidence using GRADE, and the basis for each will be reported as footnotes to the summary of findings tables.

The risk of bias for each included study was assessed by one reviewer, with a second reviewer provided check and confirm assessments made. Disagreements will be resolved by discussion, with advice sought from a third reviewer if agreement cannot be reached.

3.4.2.1 Systematic reviews and meta-analyses

Systematic reviews and meta-analyses (NHMRC Level I) of Level II studies were assessed using the AMSTAR[21] quality assessment checklist (Shea et al., 2007). The AMSTAR tool consists of 11 research questions that are answered 'yes', 'no', 'can't answer', or 'not applicable'. A 'yes' answer denotes a positive result. For the purposes of this review, systematic reviews were assigned a descriptive risk of bias based on their AMSTAR score. Specifically, systematic reviews with an AMSTAR score of 9-11 were rated low risk of bias, systematic reviews with an AMSTAR score between 6 and 8 were rated

[21] A MeaSurement Tool to Assess systematic Reviews

as moderate risk of bias, and systematic reviews with an AMSTAR score of five or less were rated as high risk of bias.

Systematic reviews and meta-analysis of observational studies were assessed using the AMSTAR-2 quality assessment checklist (Shea et al., 2017). The AMSTAR-2 consists of 16 domain questions (classified as being critical flaws or weaknesses as shown in Table 3-2) that are answered as 'yes', 'no', 'partial yes'. A 'yes' answer denotes a positive result.

The overall risk of bias of the systematic literature review was determined based on the following criteria:

- *Low risk* (*no or one noncritical weakness)* – the systematic review provides an accurate and comprehensive summary of the results of the available studies that address the question of interest.
- *Moderate risk (more than one noncritical weakness)* – the systematic review has more than one weakness but no critical flaws. It may provide an accurate summary of the results of the available studies that were included in the review.
- *High risk (one critical flaw with or without noncritical weaknesses)* – the review has a critical flaw and may not provide an accurate and comprehensive summary of the available studies that address the question of interest.
- *Serious risk (more than one critical flaw with or without noncritical weaknesses)* – the review has more than one critical flaw and should not be relied on to provide an accurate and comprehensive summary of the available studies.

Table 3-2 Domain classification as critical weakness or critical flaw

Critical weakness	Critical flaw
Domain 1: Inclusion of PICO in research questions and inclusion criteria Domain 2: Registration of protocol before commencement of the review Domain 3: Discussion of selection of study designs for inclusion Domain 5: Duplicate study selection Domain 6: Duplicate data extraction Domain 8: Detailed description of included studies Domain 10: Review of sources of funding for included studies Domain 12: Discussion of impact of risk of bias of included studies on meta-analysis results Domain 14: Discussion of heterogeneity Domain 15: Assessment of presence and likely impact of publication bias	Domain 4: Adequacy of the literature search Domain 7: Justification for excluding individual studies Domain 9: Risk of bias from individual studies being included in the review Domain 11: Appropriateness of meta-analytical methods Domain 13: Consideration of risk of bias when interpreting the results of the review Domain 16: Reporting of potential sources of conflict of interest including any funding received

Source: Shea et al. 2017. BMJ 358: j4008 doi:10.1136 (https://doi.org/10.1136/bmj.j4008)

3.4.2.2 Randomised controlled trials

The risk of bias of included RCTs (Level II) was assessed using the Cochrane Collaboration's Risk of Bias tool (Higgins et al., 2011) This tool is made up six bias domains assessing seven sources of bias: selection bias (random sequence generation and allocation concealment), performance bias (blinding of researchers and subjects), detection bias (blinding of outcome assessment), attrition

bias (incomplete outcome data), reporting bias (selective reporting) and other bias. Each domain was assessed for bias and recorded as 'high', 'low', or 'unclear'.

Overall risk of bias was determined based on the following criteria:

- *overall low risk of bias* – low risk of bias for all key domains
- *overall unclear risk of bias* – low or unclear risk of bias for all key domains
- *overall high risk of bias* – high risk of bias for one or more key domains

3.4.2.3 Observational studies

Appraisal of observational studies was guided by the GRADE criteria for assessing risk of bias,[22] which focuses on bias for the following four domains: selection of participants, measurement of exposure/outcomes, confounding, and followup. Each domain was assessed for bias, which was recorded as 'low', 'moderate', 'serious', 'critical', or 'no information provided'.

The overall risk of bias judgement for a specific outcome used the following guide:

- *overall low risk of bias* – the study is comparable to a well-performed RCT and is judged to be a low risk of bias for ALL domains.
- *overall moderate risk of bias* – the study appears to provide sound evidence for a nonrandomised study but cannot be considered comparable to a well-performed randomised trial. The study is judged to be a low or moderate risk of bias for ALL domains.
- *overall serious risk of bias* – the study has some important problems and is judged to be at serious risk of bias in at least ONE domain, but not a critical risk of bias in any domain.
- *overall critical risk of bias* – the study is too problematic with regards to this domain to provide any useful evidence on the safety of the intervention. The study is judged to be at critical risk of bias in at least ONE domain.
- *no information* – there is no information on which to base a judgement about overall risk of bias. There is no clear indication that the study is at serious or critical risk of bias AND there is a lack of information in one or more key domains of bias.

3.4.2.4 Diagnostic accuracy studies

The risk of bias of the included diagnostic performance studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool (Whiting et al., 2011). This tool is made up of four domains: patient selection, index test, reference standard and patient flow. Each domain was assessed in terms of risk of bias, with the first three domains also considered in terms of applicability to the intended population for these Guidelines.

The overall risk of bias judgement for a specific outcome used the following guide:

- *overall low risk of bias* – the study is judged to be a low risk of bias (☺) for ALL four domains
- *overall unclear risk of bias* – there is no clear indication that the study is at high risk of bias AND there is a lack of information (?) in one or more key domains of bias
- *overall high risk of bias* – the study is judged to be at high risk of bias (⊗) in at least ONE domain

[22] Table 5.5 in GRADE handbook http://gdt.guidelinedevelopment.org/app/handbook/handbook.html#h.m9385o5z3li7

3.4.3 Data synthesis

Available effect estimates (95% confidence intervals, p-values) are presented, along with risk of bias assessments, and other intervention characteristics, in tables structured by comparison, outcome, and study design. For studies where the results are incompletely reported (e.g. no effect estimate is reported, but the direction of effect is reported along with a p-value), we report the available information. Forest plots are used to visually depict effect estimates, even when these effects are not meta-analysed.

Where possible, data synthesis of randomised trials was performed using RevMan 5.3. Within each comparison we combined effect estimates across studies for each outcome using a random effects model. Indirect treatment comparisons were not be conducted and effect estimates were not combined across outcome categories or from studies other than RCTs.

We did not plan to undertake any imputation for missing data.

Heterogeneity was assessed by visually inspecting the overlap of confidence intervals on the forest plots, formally test for heterogeneity using the Chi^2 test (using a significance level of $\alpha=0.1$), and quantified using the I^2 statistic (Higgins et al., 2002).

3.4.4 GRADE profiles and summary of findings

For each comparison and outcome, we assessed the quality of the evidence using the GRADE approach (Schünemann et al., 2013). This process provides a framework for determining the certainty of the evidence and is based on consideration of the following five 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. Publication bias would be suspected when the evidence is limited to a small number of small trials.

Summary of findings tables were prepared using the GRADEpro GDT software (www.gradepro.org), reporting estimates of treatment effects for each outcome as absolute and relative risks. In the absence of data, a narrative summary is provided.

For each domain, a judgement was made about whether there are 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. Here, scoring of the certainty of the evidence begins as 'high' for randomised trials (score=4) that was downgraded by –1 for each domain with serious concerns or –2 for very serious concerns. Observational studies being a 'low'. Footnotes are used to record judgements made about downgrading (or upgrading) of the evidence.

3.4.5 Draft recommendations

Results of the systematic review were presented to the ERG, with summary tables from the technical report used to inform translation of the evidence into recommendations for use in the clinical guidance chapter. A consensus process (see Appendix 5) was used to ensure the clinical guidance is consistent with the evidence presented.

Recommendations were structured based on the GRADE framework and guided by the questions provided in the GRADEpro GDT software (Hultcrantz et al., 2017).

Here, a weak (conditional) or strong (for or against an action) recommendation was made, based on four key concepts:

1. balance of benefits and risks
2. values and preferences
3. resource use
4. quality of evidence

As noted by GRADE

> *"In the context of a systematic review, the ratings of the quality of evidence reflect the extent of our confidence that the estimates of the effect are correct. In the context of making recommendations, the quality ratings reflect the extent of our confidence that the estimates of an effect are adequate to support a particular decision or recommendation."*

The certainty of the evidence was used to inform the strength of any evidence-based recommendations that were made, with higher certainty evidence starting as a strong recommendation for or against a particular action, and lower certainty resulting in a weak recommendation for or against a particular action. If, after weighing all factors, the ERG was certain that the desirable effects outweigh undesirable effects, a strong recommendation was made. Conversely, a weak recommendation was made when there was considerable uncertainty regarding desirable or undesirable effects. Where more careful consideration regarding an individual's circumstance, preference, or values were required, the recommendation was denoted as discretionary (action may be based on opinion) or qualified (issues that would lead to different decisions is offered).

In the absence of evidence, the panel were asked to develop a recommendation based on expert opinion; noting that in special circumstance they could either provide no recommendation (when balance of desirable/undesirable is too variable and risky) or recommend an intervention be used only in research (until more data is generated).

4 GRADE Summary of findings

4.1 Question 1 – *Routine* antenatal Rh D immunoprophylaxis

Summary of findings:

Universal RAADP (1 or 2 doses) compared to placebo or no universal RAADP in Rh D negative pregnant women with no preformed anti-D

Patient or population: Rh D negative pregnant women with no preformed anti-D

Setting: Obstetrics and maternity, primary

Intervention: universal antenatal Rh D immunoprophylaxis (1 or 2 doses)

Comparison: placebo or no universal antenatal Rh D immunoprophylaxis

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo or no universal RAADP	Risk with universal RAADP (1 or 2 doses)				
Incidence of Rh D alloimmunisation (any timepoint)	14 per 1,000	5 per 1,000 (1 to 22)	**RR 0.39** (0.09 to 1.63)	2297 (2 RCTs)	⨁⨁◯◯ LOW [a,b,c,d,e,f]	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 (any timepoint)	11 per 1,000	3 per 1,000 (2 to 6)	**RR 0.31** (0.18 to 0.54)	51 987 (8 observational studies)	⨁◯◯◯ VERY LOW [b,e,g,h,i]	
Incidence of Rh D alloimmunisation (in subsequent pregnancy)	8 per 1,000	3 per 1,000 (2 to 5)	**RR 0.43** (0.31 to 0.59)	31 826 (6 observational studies)	⨁⨁◯◯ LOW [b,e,g,h,j]	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 per 1,000	2 per 1,000 (0 to 8)	**RR 0.33** (0.08 to 1.37)	28 357 (4 observational studies) [k]	⨁◯◯◯ VERY LOW [a,b,c,e,f,g,h,i]	In Rh D negative 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 alloimmunisation (at birth of Rh positive newborn or within three days of delivery)	14 per 1,000	3 per 1,000 (1 to 6)	**RR 0.19** (0.08 to 0.45)	24 622 (8 observational studies) [l]	⨁◯◯◯ VERY LOW [a,b,c,e,g,h,i]	In Rh D negative women with no preformed anti-D, universal RAADP may reduce the incidence of Rh D alloimmunisation (at birth or within three days of delivery of a Rh D positive newborn) but we are very uncertain about the size of the effect.

Summary of findings:

Universal RAADP (1 or 2 doses) compared to placebo or no universal RAADP in Rh D negative pregnant women with no preformed anti-D

Patient or population: Rh D negative pregnant women with no preformed anti-D

Setting: Obstetrics and maternity, primary

Intervention: universal antenatal Rh D immunoprophylaxis (1 or 2 doses)

Comparison: placebo or no universal antenatal Rh D immunoprophylaxis

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo or no universal RAADP	Risk with universal RAADP (1 or 2 doses)				
Incidence of Rh D alloimmunisation (up to 12 months postnatal followup)	15 per 1,000	3 per 1,000 (2 to 4)	**RR 0.19** (0.13 to 0.29)	17 372 (8 observational studies) [m]	⨁⨁◯◯ LOW [a,b,c,e,g,h,j]	In Rh D negative 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 fetomaternal haemorrhage assessed with: Kleihauer test at 32 to 35 weeks' gestation	70 per 1,000	42 per 1,000 (29 to 62)	**RR 0.60** (0.41 to 0.88)	1884 (1 RCT)	⨁⨁⨁◯ MODERATE [a,b,e,n]	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 fetomaternal haemorrhage (assessed at 32-35 weeks' gestation).
Incidence of a positive test for fetomaternal haemorrhage assessed with: Kleihauer test at birth of Rh positive newborn	202 per 1,000	121 per 1,000 (93 to 159)	**RR 0.60** (0.46 to 0.79)	1189 (1 RCT)	⨁⨁⨁◯ MODERATE [a,b,c,e,n]	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 fetomaternal haemorrhage (assessed at birth of an Rh D positive newborn).
Adverse neonatal events: jaundice	4 per 1,000	1 per 1,000 (0 to 10)	**RR 0.26** (0.03 to 2.30)	1882 (1 RCT)	⨁⨁◯◯ LOW [a,b,c,e,f,n]	In Rh D negative women with no preformed anti-D, the effect of universal RAADP (1 or 2 doses) on neonatal jaundice is uncertain.
Adverse neonatal events: prevalence of severe HDFN (perinatal mortality, need for IUT and/or exchange transfusion)	2 per 1,000	1 per 1,000 (0 to 2)	**RR 0.51** (0.09 to 0.92)	21 221 (1 observational study)	⨁◯◯◯ VERY LOW [n,o,p]	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.

Summary of findings:

Universal RAADP (1 or 2 doses) compared to placebo or no universal RAADP in Rh D negative pregnant women with no preformed anti-D

Patient or population: Rh D negative pregnant women with no preformed anti-D

Setting: Obstetrics and maternity, primary

Intervention: universal antenatal Rh D immunoprophylaxis (1 or 2 doses)

Comparison: placebo or no universal antenatal Rh D immunoprophylaxis

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo or no universal RAADP	Risk with universal RAADP (1 or 2 doses)				
Adverse maternal events attributed to anti-D	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. [1,2]			-	-	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

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio

GRADE Working Group grades 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

Explanations

a. One or more randomised studies with plausible bias that raises serious doubts about the results.
b. Missing data and exclusion of some women may overestimate the clinical effectiveness of RAADP.
c. Includes one quasi-randomised trial with high risk of selection bias.
d. No significant heterogeneity, with variability in effect estimates assessed as moderate (I^2 statistic between 25% and 50%). Does not reduce confidence in results to inform decision-making.
e. 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.
f. Low event rate and/or wide confidence intervals that cross the line of no effect. Confidence in the results is weak.
g. One or more comparative observational studies with some important problems that seriously weaken the confidence in the results.
h. Studies include historical and/or geographic controls and it is not clear whether intervention and control groups are comparable at baseline.
i. Significant heterogeneity with substantial variability in effect estimates (I^2 statistic greater than 50%). Reduces confidence in the results to inform decision making.
j. No significant heterogeneity (I^2 statistic = 0%).
k. Includes one RCT and one quasi-RCT.
l. Includes one RCT, one quasi-RCT and six observational studies. One observational study does not contribute any data.
m. Includes one RCT, one quasi-RCT and six observational studies. Two observational studies do not contribute any data.
n. One study only. Heterogeneity not assessed.
o. 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.
p. Some concerns with reporting bias and missing data.

References

1. 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.
2. McBain, R. D., Crowther, C. A., Middleton, P.. Anti-D administration in pregnancy for preventing Rhesus alloimmunisation. Cochrane Database Syst Rev; Sep 3 2015.

4.1.1 Subquestion 1 – One-dose RAADP versus two-dose RAADP

Summary of findings:

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?

Patient or population: Rh D negative pregnant women with no preformed anti-D

Setting: Obstetrics and maternity, primary setting

Intervention: universal antenatal Rh D immunoprophylaxis (single dose)

Comparison: universal antenatal Rh D immunoprophylaxis (two-dose)

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)
	Risk with RAADP (one dose)	Risk with RAADP (two doses)			
Incidence of Rh D alloimmunisation	No evidence found			-	-
Incidence of a positive test for fetomaternal haemorrhage	No studies identified			-	-
Serum anti-D levels at birth	Complete data not available (abstract only). The proportion of women with undetectable anti-D at delivery was 45.2% vs 14.2% (OR 5.0; 95% CI NR; p<0.001), favouring the two-dose regimen			(1 RCT)	⨁◯◯◯ VERY LOW [a,b]
Adverse neonatal events	No studies identified		-	-	-
Adverse maternal events	No studies identified		-	-	-
	Risk with RAADP (one or two doses)	**Risk with no RAADP**			
Incidence of Rh D alloimmunisation (one dose, any timepoint)	12 per 1,000	4 per 1,000 (1 to 9)	**RR 0.31** (0.12 to 0.80)	36 555 (4 observational studies)	⨁◯◯◯ VERY LOW [c,d,e,f,g,h]
Incidence of Rh D alloimmunisation (two dose, any timepoint)	10 per 1,000	3 per 1,000 (2 to 5)	**RR 0.32** (0.20 to 0.51)	15 264 (6 observational studies) [i]	⨁◯◯◯ VERY LOW [c,d,e,f,h,j,k]
Incidence of Rh D alloimmunisation (one dose, estimated)	In a meta-regression model, Turner 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. [1] Using only studies relevant to the UK health system Pilgrim 2009 estimated the risk of sensitisation using a single dose to be 0.34% (0.28, 0.40). [2]			(10 observational studies)	⨁⨁◯◯ LOW [b,c,d,e,f,h,l]

Summary of findings:

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?

Patient or population: Rh D negative pregnant women with no preformed anti-D

Setting: Obstetrics and maternity, primary setting

Intervention: universal antenatal Rh D immunoprophylaxis (single dose)

Comparison: universal antenatal Rh D immunoprophylaxis (two-dose)

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)
	Risk with RAADP (one dose)	Risk with RAADP (two doses)			
Incidence of Rh D alloimmunisation (two dose, estimated)	In a meta-regression model, Turner 2012 estimated an OR of 0.31 (95%CI 0.09, 0.65) for two-doses of RAADP based on the relative effectiveness observed in published studies adjusted for bias and expert opinion. [1] Using only studies relevant to the UK health system, Pilgrim 2009 estimated the risk of sensitisation using two-doses to be 0.30% (95% CI 0.22, 0.38). [2]			(10 observational studies)	⨁⨁◯◯ LOW b,c,d,e,f,h,l

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio

GRADE Working Group grades 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

Explanations

a. Study is reported in a conference abstract and it is difficult to judge internal bias. Not all outcomes reported.
b. One study only. Heterogeneity not assessed.
c. One or more randomised studies with plausible bias that raises some doubts about the results
d. Missing data and exclusion of women may overestimate the clinical effectiveness of RAADP
e. One or more comparative observational studies with some important problems that seriously weaken the confidence in the results
f. Studies include historical and/or geographic controls and it is not clear whether intervention and control groups are comparable at baseline.
g. Significant heterogeneity with substantial variability in effect estimates (I2 statistic > 50%). Reduces confidence in the results to inform decision making.
h. 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.
i. Includes one RCT and one quasi-RCT
j. No heterogeneity (I^2 statistic = 0%). Does not reduce confidence in results to inform decision making.
k. Low event rate and/or wide confidence intervals that cross the line of no effect. Confidence in the results is weak.
l. Authors elicited expert opinion to estimate association between the relative and observed effectiveness for different dosing regimens.

References

1. Turner, R. M., Lloyd-Jones, M., Anumba, D. O. C., Smith, G. C. S., Spiegelhalter, D. J., Squires, H., Stevens, J. W., Sweeting, M. J., Urbaniak, S. J., Webster, R., Thompson, S. G.. 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.
2. 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.

4.2 Question 2 – Universal sensitising event prophylaxis in the first trimester

Summary of findings:

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/without a curette), does universal first trimester sensitising event prophylaxis with Rh D immunoglobulin prevent Rh D alloimmunisation?

Patient or population: Rh D negative women with no preformed anti-D with a first trimester sensitising event

Setting: Obstetrics and maternity, primary setting

Intervention: routine sensitising event prophylaxis

Comparison: placebo or no sensitising event prophylaxis

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo or no sensitising event prophylaxis	Risk with sensitising event prophylaxis				
Incidence of Rh D alloimmunisation (4-6 months after spontaneous miscarriage and/or therapeutic evacuation) assessed with: Enzyme-Coombs screening	0 per 1,000	0 per 1,000 (0 to 0)	not estimable	48 (1 RCT) [4]	⨁◯◯◯ VERY LOW a,b,c,d,e,f,g	The evidence is very uncertain about the effect of sensitising event prophylaxis 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 alloimmunisation (4-6 months after incomplete miscarriage or medical termination of pregnancy) assessed with: Indirect Coombs	56 per 1,000	19 per 1,000 (1 to 372)	**RR 0.34** (0.02 to 6.69)	57 (1 observational study) [1]	⨁◯◯◯ VERY LOW c,d,g,h,i,j,k	The evidence is very uncertain about the effect of sensitising event prophylaxis on the incidence of Rh D alloimmunisation 4-6 months after incomplete miscarriage or medical termination of pregnancy in Rh D negative women.
Incidence of Rh D alloimmunisation (at subsequent pregnancy after spontaneous miscarriage and/or therapeutic evacuation) assessed with: Enzyme-Coombs screening	0 per 1,000	0 per 1,000 (0 to 0)	not estimable	9 (1 RCT) [4]	⨁◯◯◯ VERY LOW a,b,c,d,e,f,g	The evidence is very uncertain about the effect of sensitising event prophylaxis on the incidence of Rh D alloimmunisation in a subsequent pregnancy after spontaneous miscarriage or therapeutic evacuation in Rh D negative women.

Summary of findings:

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/without a curette), does universal first trimester sensitising event prophylaxis with Rh D immunoglobulin prevent Rh D alloimmunisation?

Patient or population: Rh D negative women with no preformed anti-D with a first trimester sensitising event

Setting: Obstetrics and maternity, primary setting

Intervention: routine sensitising event prophylaxis

Comparison: placebo or no sensitising event prophylaxis

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo or no sensitising event prophylaxis	Risk with sensitising event prophylaxis				
Incidence of Rh D alloimmunisation (at subsequent pregnancy after inducted abortion) assessed with: Papain-treated cells or Indirect Coombs	14 per 1,000	10 per 1,000 (1 to 113)	**RR 0.76** (0.07 to 8.21)	241 (1 observational study) [5]	⨁◯◯◯ VERY LOW [c,g,h,i,k,l,m]	The evidence is very uncertain about the effect of sensitising event prophylaxis on the incidence of Rh D alloimmunisation in a subsequent pregnancy after induced abortion in Rh D negative pregnant women
Incidence of Rh D alloimmunisation (after abdominal trauma, molar pregnancy, ectopic pregnancy)	No comparative evidence found [2]			-	-	The effect of sensitising event prophylaxis 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 fetomaternal haemorrhage	No comparative evidence found [3]			-	-	The effect of sensitising event prophylaxis on the incidence of a positive test for fetomaternal haemorrhage after abdominal trauma, molar pregnancy, or ectopic pregnancy in Rh D negative women is unknown.
Adverse neonatal events (e.g. jaundice)	No comparative evidence found [3]			-	-	The effect of sensitising event prophylaxis 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 anti-D	No comparative evidence found [3]			-	-	The effect of sensitising event prophylaxis on the incidence of adverse maternal events after abdominal trauma, molar pregnancy, or ectopic pregnancy in Rh D negative women is unknown.

Summary of findings:

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/without a curette), does universal first trimester sensitising event prophylaxis with Rh D immunoglobulin prevent Rh D alloimmunisation?

Patient or population: Rh D negative women with no preformed anti-D with a first trimester sensitising event

Setting: Obstetrics and maternity, primary setting

Intervention: routine sensitising event prophylaxis

Comparison: placebo or no sensitising event prophylaxis

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo or no sensitising event prophylaxis	Risk with sensitising event prophylaxis				

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio

GRADE Working Group grades 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

Explanations

a. One randomised study with plausible bias that raises serious doubts about the results.
b. Method of randomisation not reported and unclear whether treatment allocation concealed. Some concerns with reporting bias and missing data.
c. Single study. Heterogeneity not assessed.
d. 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.
e. The study was conducted in the United States among RhD negative women with complete miscarriage (n=9) or incomplete miscarriage with curettage (n=48). An unknown proportion of women had miscarriage outside the first trimester (after 12 weeks' gestation) and the intervention was administered at a dose higher than recommended in Australia (1500 IU vs 625 IU).
f. Small study not sufficiently powered to detect a statistically significant difference.
g. Single study. Publication bias likely.
h. Comparative study with some important problems that seriously weakens the confidence in the results.
i. Method of treatment allocation or blinding not reported. Some concerns with reporting bias and missing data.
j. The study was conducted in the United States among Rh D negative women who had medical termination of pregnancy (n=33) or were treated for incomplete miscarriage (n=24). Thirteen (22.8%) women were treated outside the first trimester (>13 weeks' gestation) and the dose of Rhogam was not stated.
k. Low event rate and/or wide confidence intervals that cross the line of no effect. Confidence in the results is weak.
l. The evidence is probably applicable to the Australian population and healthcare context with some caveats.
m. 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).

References

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3. Karanth, L., Jaafar, S. H., Kanagasabai, S., Nair, N. S., Barua, A.. Anti-D administration after spontaneous miscarriage for preventing Rhesus alloimmunisation. Cochrane Database of Systematic Reviews; 2013.
4. Visscher, R. D.,& Visscher,H. C.. Do Rh-negative women with an early spontaneous abortion need Rh immune prophylaxis?. Am J Obstet Gynecol; 1972.
5. Simonovits, I.,Bajtai,G.,Kellner,R.,Kerenyl,M.,Rucz,L.,Szilvas,R.,& Takacs,S.. Immunization of RhO(D)-negative secundigravidae whose first pregnancy was terminated by induced abortion.. Haematologia (Budap); 1974.

4.3 Question 3 - *Targeted* routine antenatal or sensitising event prophylaxis

Summary of findings:

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

Patient or population: Rh D negative pregnant women with no preformed anti-D

Setting: Obstetrics and maternity, primary care

Intervention: targeted antenatal Rh D immunoprophylaxis (based on noninvasive prenatal screening)

Comparison: universal antenatal Rh D immunoprophylaxis

Outcomes	Impact	№ of participants (studies)	Certainty of the evidence (GRADE)
Incidence of Rh D alloimmunisation	No studies directly assessed effect of targeted routine antenatal or sensitising event prophylaxis on the incidence of RhD alloimmunisation. One study (Saramago 2018) conducted a simulation based on diagnostic accuracy of the test and expected management in women with positive and negative test results. The report estimated targeted RAADP increased the risk of Rh D alloimmunisation from 281 per 100 000 pregnancy women with universal RAADP to 284 (base case scenario) or 309 (worst case scenario) per 100 000 [1]	-	not reported
Utilisation of Rh D immunoglobulin	No comparative studies directly assessed the effect of targeted routine antenatal or sensitising event prophylaxis on utilisation of Rh D immunoglobulin. One study (Saramago 2018) conducted a simulation based on data from three noncomparative studies and estimated utilisation of Rh D immunoglobulin would decrease by approximately 33.1% to 36.9%. [1,2,3,4]	-	not reported
Incidence of a positive test for fetomaternal haemorrhage	No studies directly assessed effect of targeted routine antenatal or sensitising event prophylaxis on the incidence of a positive test for fetomaternal haemorrhage.	-	not reported
Adverse neonatal events	No studies were identified that reported any data on adverse neonatal events relating to NIPT or antenatal Rh D immunoglobulin administration.	-	not reported
Adverse maternal events attributed to Rh D immunoglobulin	No studies were identified that reported any data on adverse maternal events relating to NIPT or antenatal Rh D immunoglobulin administration.	-	not reported

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval

GRADE Working Group grades 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

References

1. Saramago, P., Yang, H., Llewellyn, A., Walker, R., Harden, M., Palmer, S., Griffin, S., Simmonds, M.. 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.
2. Grande M, Ordoñez E,Cirigliano V,Cid J,Grau E,Pericot A,et al.. Clinical application of midtrimester non-invasive fetal *RHD* genotyping and identification of *RHD* variants in a mixed-ethnic population.. Prenat Diagn; 2013.
3. 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.. Prenat Diagn; 2014.
4. 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; 2015.

Subquestion 3 – Diagnostic accuracy of noninvasive prenatal screening tests for fetal Rh D status

Summary of findings:

Should noninvasive prenatal screening tests be used to diagnose fetal Rh D status in Rh D negative pregnant women with no preformed anti-D (for routine or sensitising event prophylaxis)?

Patient or population: Rh D negative pregnant women with no preformed anti-D (for routine or sensitising event prophylaxis)

Setting: Obstetrics and maternity, primary setting

New test: noninvasive prenatal screening test for fetal Rh D status

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

Range of sensitivities: 0.93 to 1.00 | **Range of specificities**: 0.92 to 1.00

Test result	Number of results per 1,000 women tested (95% CI)			Number of participants (studies)	Certainty of the Evidence (GRADE)	Comments
	Prevalence 55% Assumed lower estimate	Prevalence 62% Likely estimate for Australia	Prevalence 75% Maximum reported prevalence in identified studies			
True positives	510 to 550	575 to 620	696 to 750	76 349 (48)	⨁⨁⨁⨁ **HIGH** [a,b,c,d,e]	Around 57.5% to 62.0% of Rh D negative women would receive Rh D IgG. [f]
False negatives	0 to 40	0 to 45	0 to 54			Around 0 to 4.5% of Rh D negative women with an Rh D positive fetus would not receive Rh D IgG. [g]
True negatives	412 to 450	348 to 380	229 to 250	76 349 (48)	⨁⨁⨁⨁ **HIGH** [a,b,c,d,e]	Around 34.8 to 38.0% of Rh D negative women would avoid unnecessary Rh D IgG. [h]
False positives	0 to 38	0 to 32	0 to 21			Around 0 to 3.2% of women would unnecessarily receive Rh D IgG. [i]
Inconclusive*	Where possible, inconclusive results were treated as test positive				-	Approximately 6.7% of results are estimated to be inconclusive (Saramago 2018).

CI: Confidence interval

GRADE Working Group grades 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

Explanations

a. 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, exclusion of sensitised women) or conduct of the index test (e.g., number of exons amplified, 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.

b. Almost all studies were consistent, and any inconsistencies could be explained. Samples taken before 12 weeks' gestation 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 have substantially reduced the confidence in the overall quality of evidence.

c. The evidence was considered applicable to the Australian healthcare 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 RhD

negative phenotype among donors is around 15%. The prevalence of RhD negative babies born to RhD negative women is estimated to be 38%, but the prevalence of specific *RHD* genotypes is not known. The meta-analyses by Zhu 2014 and Geifman-Holtzman 2006 were not included, because of changes and improvements in the how the test is conducted have occurred. It is expected that the screening test would, at a minimum, include primers for two exons (either 4, 5, 7, or 10), involve RT-qPCR, and be conducted in duplicate.

d. Diagnostic performance may by overestimated if only high-throughput studies are considered (as reported in Saramago 2018); therefore, the inclusion of Mackie (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 2017 and other studies.

e. Many studies were included. Smaller confidence intervals were observed in the large studies with central reference laboratories and those that used thresholds to maintain an acceptable level of sensitivity. Here, confidence in the evidence is high. In small, single centre studies, wider confidence interval would suggest a lower certainty of evidence.

f. 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 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.

g. 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% (Crowther et al., 1997). Of these events, the likelihood that sensitisation causes mild HDFN is 90% and that severe morbidity due to HDFN is 10%. Among those with severe morbidity, fetal death is estimated to occur in 5% (Gordon et al., 2017) .

h. These women would avoid two injections of Rh D immunoglobulin (current recommendation is two doses at 28 and 34 weeks' gestation). This assumes the sampling is derived from bloods already taken, and that they would also not receive postnatal Rh D immunoglobulin after cord serology.

i. This is much smaller than the current rate of 35% to 40%, which occurs with universal routine antenatal Rh D immunoprophylaxis. No adverse effects are anticipated to occur in these women.

Question 4 – Risk of failure of Rh D immunoprophylaxis administration due to increased BMI

Summary of findings:

Does increasing BMI increase the risk of failure of Rh D immunoprophylaxis in Rh D negative pregnant or postpartum women with no preformed anti-D antibodies?

Patient or population: Rh D negative women with increased BMI and no preformed anti-D antibodies

Setting: Obstetrics and maternity

Intervention: increased dose of Rh D immunoglobulin

Comparison: Not applicable

Outcomes	Anticipated absolute effects* (95% CI)		№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with	Risk with increased dose of RAADP			
Incidence of Rh D alloimmunisation (any timepoint)	No significant association between body mass index, mean body weight, weight >75 kg or weight >100 kg on the incidence of Rh alloimmunisation reported in a small case-control study.		42 cases 146 controls (1 observational study) [1]	⨁◯◯◯ VERY LOW [a,b,c,d]	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 IgG (2 doses, 28 and 34 weeks' gestation)	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.		45 (1 observational study) [3]	⨁◯◯◯ VERY LOW [b,e,f,g,h]	Increasing body surface area (BSA) appears to have little to no effect on persistence of anti-D serum levels after administration of Rh D IgG (two doses, 28 and 34 weeks' gestation) but the evidence is very uncertain.
Anti-D serum levels after administration of Rh D IgG (single dose, 28 weeks' gestation)	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 IgG remained quantifiable in both women at last scheduled followup (week 9 and 11).		(1 RCT) [4]	⨁◯◯◯ VERY LOW [b,h,i,j]	Increased body weight appears to have little to no effect on persistence of anti-D serum levels after administration of Rh D IgG (single dose, 28 weeks' gestation) but the evidence is very uncertain.
Anti-D serum levels after delivery of an Rh D positive child	Based on the general linear model over time, the study authors found each kg/m^2 BMI higher than 27 kg/m^2 reduced the Rh D Ig G serum concentration by the calculated value.		26 (1 observational study) [2]	⨁◯◯◯ VERY LOW [b,h,k,l]	Increasing BMI may result in reduced anti-D serum concentration after delivery of an Rh D positive child 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 fetomaternal haemorrhage	No studies reported this outcome.		-	-	not reported
Adverse neonatal events (e.g., jaundice)	No studies reported this outcome.		-	-	not reported
Adverse maternal events	Seven adverse events reported among five women; none of which were considered related to study drug.		(1 RCT)	⨁◯◯◯ VERY LOW [b,c,i,m]	

Summary of findings:

Does increasing BMI increase the risk of failure of Rh D immunoprophylaxis in Rh D negative pregnant or postpartum women with no preformed anti-D antibodies?

Patient or population: Rh D negative women with increased BMI and no preformed anti-D antibodies

Setting: Obstetrics and maternity

Intervention: increased dose of Rh D immunoglobulin

Comparison: Not applicable

Outcomes	Anticipated absolute effects* (95% CI)		№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with	Risk with increased dose of RAADP			

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio

GRADE Working Group grades 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

Explanations

a. One case-control study that appears to provide sound evidence for a nonrandomised 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 affect the overall direction of effect.
b. Single study. Heterogeneity not assessed. Certainty of evidence not downgraded.
c. 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 1000 IU of Rh D immunoglobulin at 30 weeks' gestation and within 48 hours of giving birth to an Rh D positive child. This is different to the recommended dose in Australia of 625 IU at 28 and 34 weeks' gestation and within 72 hours of giving birth to an Rh D positive child.
d. The study is not statistically powered to inform decision-making. A very small number of women with a high BMI were included.
e. One study with some important problems that seriously weaken the confidence in the results.
f. Small cohort with some concerns with reporting bias and missing data.
g. Evidence is directly generalisable to the target population and applicable to the Australian healthcare system with some caveats. The study was conducted in the UK in Rh D negative pregnant women. Rh D immunoglobulin (500 IU) was administered at 28 and 34 weeks' gestation but the dose was lower than recommended in Australia (625 IU).
h. Small cohort with insufficient longer term data to provide meaningful information relating to BMI and incidence of Rh D alloimmunisation in a subsequent pregnancy.
i. The study is too problematic to provide any useful evidence on the outcome of interest.
j. Evidence is probably generalisable to the target population but it is difficult to judge whether it is sensible to apply it to the Australian healthcare 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 (625 IU at 28 and 34 weeks' gestation). The correlation between body weight and BMI is poor, with the BMI of subject 12 being 26.79 and the BMI of subject 9 being 32.29.
k. One observational study that appears to provide sound evidence for a nonrandomised study but cannot be considered comparable to a well-performed RCT.
l. 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 child. Rh D immunoglobulin was administered with 72 hours of birth, but at a dose higher than that recommended in Australia (1500 IU vs 625 IU).
m. Small study unlikely to be sufficiently powered to detect a statistically significant difference.

References

1. Koelewijn, J. M., de Haas, M., Vrijkotte, T. G., van der Schoot, C. E., Bonsel, G. J.. Risk factors for RhD immunisation despite antenatal and postnatal anti-D prophylaxis. BJOG: An International Journal of Obstetrics & Gynaecology; 2009.
2. Woelfer, B., Schuchter, K., Janisiw, M., Hafner, E., Philipp, K., Panzer, S.. Postdelivery levels of anti-D IgG prophylaxis in D-- mothers depend on maternal body weight. Transfusion; 2004.
3. MacKenzie, I. Z., Roseman, F., Findlay, J., Thompson, K., Jackson, E., Scott, J., Reed, M.. The kinetics of routine antenatal prophylactic intramuscular injections of polyclonal anti-D immunoglobulin. BJOG: An International Journal of Obstetrics & Gynaecology; 2006.
4. Bichler J, Schondorfer G. Pabst G. Andresen I.. Pharmacokinetics of anti-D IgG in pregnant RhD-negative women. 2003.

5 Findings of the systematic review

5.1 Results of the literature search

The medical literature was searched on 19–20 July 2018 to identify relevant studies and systematic reviews published from database inception to the literature search date. Searches were conducted of the databases and sources described in Section 3.2.2. Manual searches of the reference lists of relevant articles were performed and we endeavoured to find unpublished or grey literature via the expert group.

Search terms are as described in **Appendix A, Volume 2** of the technical report, with methodological filters applied to identify specific study types. Studies were excluded based on hierarchical, prespecified exclusion criteria, with all citations returned by the literature searches reviewed based on information in the publication title and, where available, the abstract. Relevant publications were retrieved and reviewed in full text before a final decision was made on their inclusion or exclusion for the review. The expert group was consulted in cases where further judgement was required.

The results of the literature search and the application of the study selection criteria is provided in **Appendix B, Volume 2** of the technical report. Studies that could not be retrieved or that met the inclusion criteria but contained insufficient or inadequate data for inclusion are listed in **Appendix C, Volume 2** of the technical report.

A PRISMA flow summarising the screening results is provided in Figure 5.1 (all questions) and Figure 5.2 (subquestion 3, diagnostic accuracy).

Figure 5.1. Literature screening results. Questions 1 to 4.

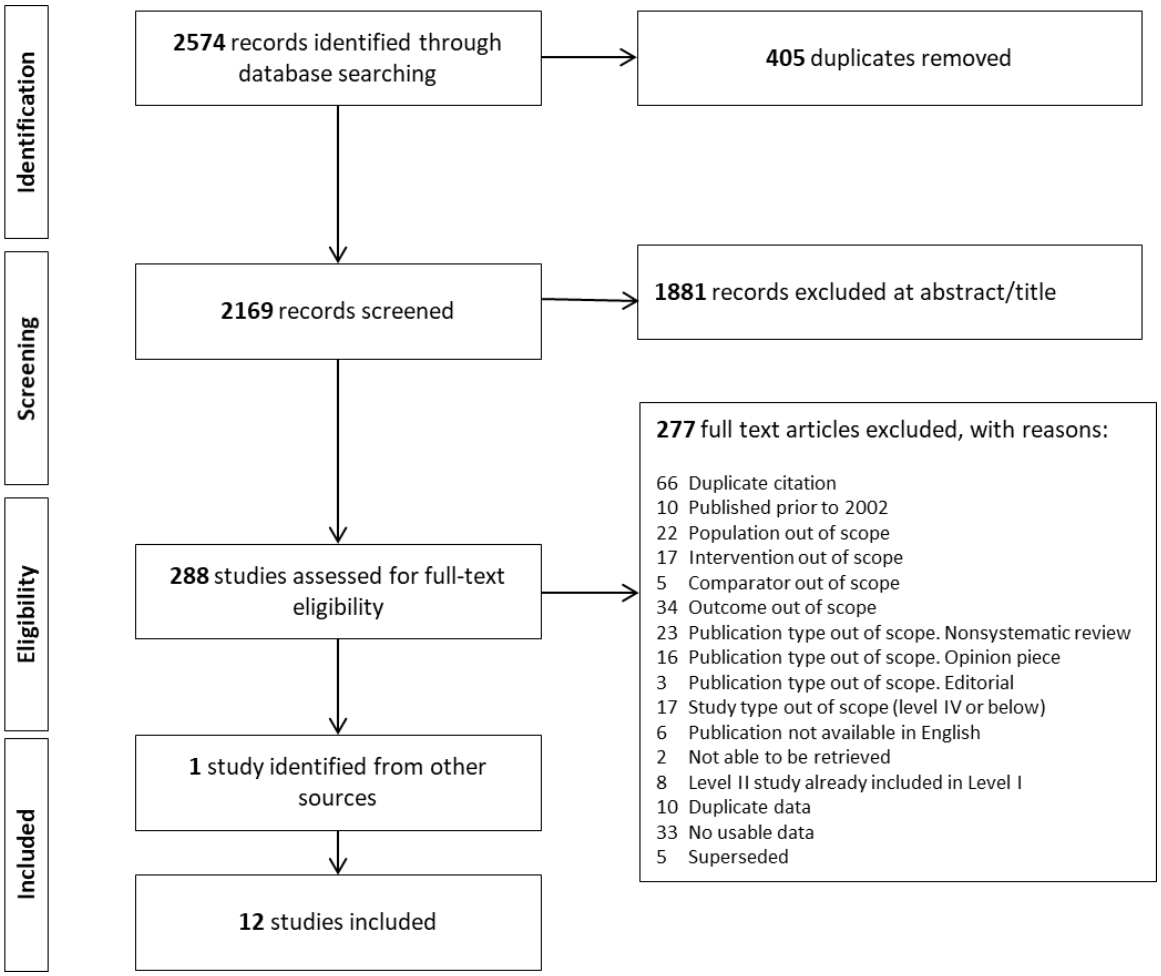
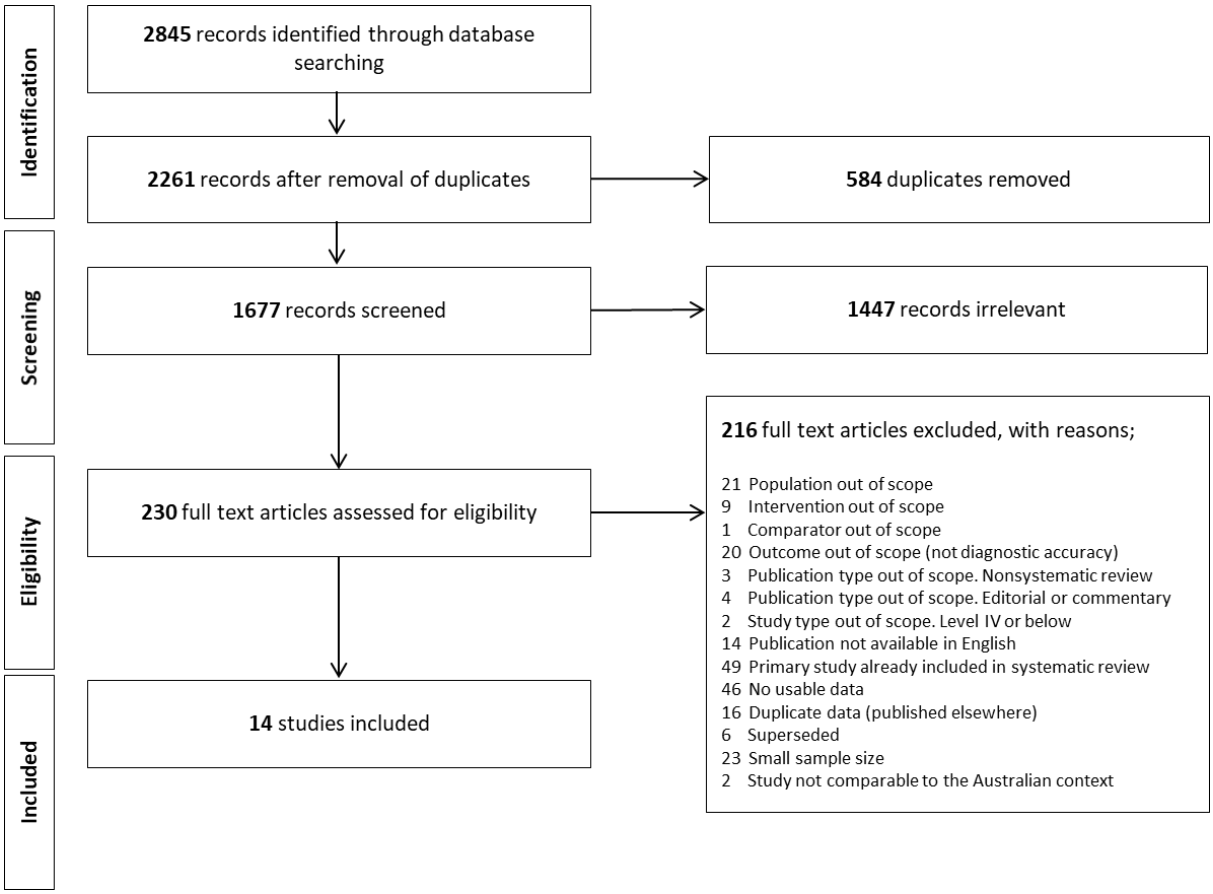


Figure 5.2. Literature screening results. Questions 3.



5.2 Question 1 - *Routine* antenatal Rh D immunoprophylaxis

Question 1 – (Intervention)

In Rh D negative pregnant women with no preformed anti-D, does universal *routine* antenatal prophylaxis with Rh D immunoglobulin (1 or 2 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?

5.2.1 Background

Question 1 intended to update the evidence base regarding universal administration of routine antenatal anti-D prophylaxis (RAADP) at 28 and 34 weeks' gestation in Rh D negative women. RAADP is aimed at all pregnant women who are Rh D negative with no preformed anti-D antibodies. A subquestion to assess whether the two-dose strategy can be replaced with a single-dose strategy was also included.

5.2.2 Methods

For this question study participants were *pregnant* women who are Rh D negative and do not have pre-formed anti-D antibodies. The focus was *universal antenatal prophylaxis*, that is, administration of Rh D Immunoglobulin to *all* Rh D negative pregnant women during pregnancy who have no preformed anti-D antibodies. There were no restrictions on whether women were primigravidae (pregnant for the first time), primiparae (giving birth for the first time at 24 weeks' gestation or more), or multigravidae[23] (pregnant for at least the second time).

Two comparisons were assessed in this review:

1. RAADP (1 or 2 doses) versus placebo or no RAADP, and
2. RAADP (1 dose) versus RAADP (2 doses).

Because this is an intervention question, the levels of evidence are as follows (see Section 3.1.4):

- Level I – a systematic review of two or more Level II studies
- Level II – an RCT
- Level III–1 – a pseudo-RCT
- Level III–2 – a comparative study with concurrent controls (including nonrandomised, experimental trials, cohort studies, case-control studies and interrupted time series with a control group)
- Level III–3 – a comparative study without concurrent controls (including historical control studies, two or more single-arm studies, and interrupted time series without a parallel control group)
- Level IV – case series with either post-test, or pre-test and post-test outcomes.

[23] It is recognised that, because women may not always reveal details of previous pregnancies, information on parity is likely to be the more reliable.

Level I, Level II, or Level III evidence was considered appropriate for inclusion. Studies using other designs (i.e. Level IV) were excluded because it is not possible to attribute observed changes in outcomes to the intervention with reasonable confidence. For the purposes of this review, a systematic review of Level III–2 or Level III–3 evidence was classified as Level III evidence.

For this question, the literature search was limited to studies published after 2002, as the previous guidelines (NBA, 2003) had searched and identified relevant studies published prior to 2002. Screening was conducted according to the hierarchy of evidence described in Section 3.3. Studies of lower level evidence were only screened for primary outcomes insufficiently addressed in higher level studies.

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

5.2.3 Summary of evidence

The systematic review and hand-searching process identified four systematic reviews (Chilcott et al., 2003, Pilgrim et al., 2009, Turner et al., 2012, McBain et al., 2015) that evaluated the effectiveness of RAADP in Rh D negative women. The reviews identified two Level II studies (Huchet et al., 1987, Lee et al., 1995) and nine Level III studies (Bowman et al., 1978, Bowman et al., 1978, Tovey et al., 1983, Hermann et al., 1984, Bowman et al., 1987, Trolle, 1989, Mayne et al., 1997, Parsons et al., 1998, MacKenzie et al., 1999) meeting their search criteria. A matrix illustrating the overlap of studies included in each review is provided in Table 5-1**.**

Assuming relevant primary studies had been identified in the included systematic reviews, the screening of the Level II and Level III citations was limited to those published after the literature search date of Pilgrim 2009. No additional Level II studies were found, and one Level III study (Koelewijn et al., 2008) was identified.

Table 5-1 Overlap table showing primary studies included in the Level I studies: RAADP (1 or 2 doses) versus placebo or no RAADP

	Review ID			
Study ID	**McBain 2015**	**Turner 2012**	**Pilgrim 2009**	**Chilcott 2003**
Level II				
Lee 1995	✓	✓		✓
Huchet 1987 (quasi)	✓	✓	✓	✓
Level III				
MacKenzie 2004 [a]			✓	
MacKenzie 1999		✓	✓	✓
Parsons 1998		✓		✓
Mayne 1997		✓	✓	✓
Trolle 1989		✓	✓	✓
Bowman 1987		✓	✓	✓
Hermann 1984		✓		✓
Tovey 1983		✓	✓	✓
Bowman and Pollock 1978		✓	✓	✓
Bowman 1978		✓	✓	✓

a. MacKenzie 2004 did not match our PICO criteria as it compared administration routes (IV versus IM)

5.2.3.1 Level I

One systematic review of RCTs (McBain 2015) was identified that compared the effectiveness of RAADP administration with no therapy.

McBain 2015 was a Cochrane review that included Rh D negative women without anti-D antibodies at 28 weeks' gestation. The review searched the Cochrane Pregnancy and Childbirth Group's Trials Register, and the search was conducted from database inception 31 May 2015. The intervention was Rh D immunoglobulin at 28 weeks' gestation or more, regardless of timing, dose and route of administration, compared to no treatment or comparisons of different treatments.

Two studies were identified; a quasi-randomised trial (Huchet 1987) and one RCT (Lee 1995). The characteristics of these studies are outlined in Section 5.2.3.2.

Table 5-2 Characteristics and quality of Level I evidence: RAADP (1 or 2 doses) versus placebo or no RAADP

Review ID	Study type *Risk of bias*	Population	Intervention	Comparator	Outcomes
McBain 2015	SR and meta-analysis of Level II studies *Low*	Pregnant women who are Rh D negative and have no anti-D antibodies at 28 weeks' gestation N = 4510 (2 studies)	RAADP at 28 weeks' gestation n = 2195	No treatment, placebo or other dosing regimes n = 2228	Rh alloimmunisation Positive test for fetomaternal haemorrhage Adverse neonatal events Adverse maternal events

RAADP, routine antenatal anti-D prophylaxis;

5.2.3.2 Level II

The two RCTs identified by McBain 2015 evaluated the effectiveness of a two-dose Rh D immunoprophylaxis regimen administered at 28 and 34 weeks' gestation. Huchet 1987 was a multicentre trial conducted between January 1983 and June 1984. The study, set in Paris, France, administered two intramuscular (IM) doses of 500IU Rh D IgG. Lee 1995 was a multicentre trial set in the UK that administered two IM doses of 250 IU Rh D IgG. In both trials, the controls received no treatment. Women who gave birth to an Rh positive baby (regardless of intervention group) were administered postpartum Rh D IgG. Huchet 1987 reported 500 IU was administered postpartum, while Lee 1995 reported that both groups 'were considered for anti-D Ig in the normal way at delivery'.

Both RCTs were considered by McBain 2015 to be affected by unclear to high risk of bias. Huchet 1987 was quasi-randomised (treatment allocation based on year of birth), and therefore at high risk of selection bias; Lee 1995 did not outline the method of randomisation despite noting use of sealed envelopes, thus selection bias was judged to be unclear. Neither trial used a placebo, therefore women were aware of the assigned intervention and details regarding blinding of outcome assessors to treatment allocation were not provided. Both trials also had high rates of attrition meaning outcome data were incomplete.

Table 5-3 Characteristics and quality of Level II evidence: RAADP (one or two doses) versus placebo or no RAADP

Study ID	Study type *Risk of bias*	Population	Intervention	Comparator	Outcomes
Identified by McBain 2015, Pilgrim 2009 and Chilcott 2003					
Lee 1995	RCT, MC *High*	Pregnant women who are Rh D negative primigravidae prior to 28 weeks N = 2541	Rh D IgG 2 x 250 IU IM at 28 and 34 weeks' gestation n = 1268[a]	No treatment n = 1273[b]	Rh D alloimmunisation (at birth, 6 months following delivery)
Huchet 1987	quasi-RCT, MC *High*	Pregnant women who are Rh D negative and primipara (not all of whom were primigravidae) N = 1969[c]	Rh D IgG 2 x 500 IU IM at 28 and 34 weeks' gestation n = 927[d]	No treatment n = 955[e]	Rh D alloimmunisation (during pregnancy, 2–12 months following delivery) Positive Kleihauer test (during pregnancy, at delivery, postpartum)

IgG, immunoglobulin G; IM, intramuscular; MC, multicentre; RAADP, routine antenatal anti-D prophylaxis; RCT, randomised controlled trial; IU, international units

a. McBain 2015 reported that 532 women gave birth to an Rh+ infant (of whom, 52 did not receive two doses of Rh D IgG). Chilcott 2003 reported 513 women gave birth to an Rh+ infant.

b. McBain 2015 reported that 649 women gave birth to an Rh+ infant (21 infants of unknown blood group). One woman was found to be alloimmunised, therefore 648 were included in the analysis.

c. A total of 1969 women began the study, 1882 were monitored until they went into labour (i.e. 87 women were lost to followup)

d. A total of 472 women were followed up postpartum (127 lost to followup). McBain 2015 reported 99 women gave birth to an Rh+ infant.

e. McBain 2015 reported 590 gave birth to an Rh+ infant. Two women were excluded due to fetal-maternal haemorrhage, 955 monitored until labour. 468 women were followed up postpartum (122 lost to followup)

5.2.3.3 Level III

Three systematic reviews of Level III studies (Chilcott 2003, Pilgrim 2009, Turner 2012) and one Level III–3 study (Koelewijn 2008) were identified that examined the effectiveness of RAADP compared to no treatment in pregnant women who are Rh D negative. The characteristics of these studies and the relevant outcomes assessed are summarised in Table 5-4.

Table 5-4 Characteristics and quality of Level III evidence identified in this review: RAADP (one or two doses) versus placebo or no RAADP

Study ID	Study type *Risk of bias*	Population [a]	Intervention	Comparator	Outcomes
Level I (III)					
Chilcott 2003	SR and meta-analysis of RCTs and observational studies *Moderate*	Pregnant women who are Rh D negative N = 41 441 11 studies	RAADP at any dose n = 29 288 11 studies	No treatment or placebo n = 12 153 10 studies	Rh D alloimmunisation Adverse neonatal events
Pilgrim 2009	SR and meta-analysis of RCTs and observational studies *Moderate*	Pregnant women who are Rh D negative N = 30 768 8 studies	RAADP (either two doses of at least 500 IU at 28 and 34 weeks' gestation or one dose of 1500 IU at 28 weeks' gestation) followed by another dose within 72 hours of birth if required n = 19 719	No treatment, placebo or RAADP using different dosing regimes n = 11 049	Rh D alloimmunisation Adverse neonatal events
Turner 2012	Meta-analysis of studies included in Chilcott 2003 and Pilgrim 2009 *Moderate*	Pregnant women who are Rh D negative N = NR 10 studies	RAADP at any dose n = NR	No treatment or placebo n = NR	Rh D alloimmunisation
Level III					
Koelewijn 2008	Retrospective Coh, MC *Moderate*	Pregnant women who are Rh D negative and primiparae-1 N = 21 221	Rh D IgG 1000 IU at 30 weeks' gestation n = 12 576	No treatment and postpartum Rh D IgG n = 8645	Rh D alloimmunisation Adverse neonatal events

Coh, cohort; IgG, immunoglobulin G; IU, international units; NR, not reported; RCT, randomised controlled trial; SR, systematic review
a. As reported by the original authors.

Chilcott 2003 was a published health technology assessment (HTA) report conducted for the UK National Health Service (NHS) with the aim of determining clinical effectiveness of RAADP regardless of timing, dose and route of administration. The search date is unclear, but databases were searched from inception to 2000. A search of the last four months of PubMed was undertaken on 30 November 2000, and a MEDLINE search was updated in September 2001. Chilcott 2003 identified 11 relevant studies (Bowman 1978, Bowman and Pollack 1978, Bowman 1987, Hermann 1984, Huchet 1987, Lee 1995, Parsons 1998, Mayne 1997, MacKenzie 1999, Tovey 1983 and the associated publications Thornton 1989 and Trolle 1989) conducted in various locations including Canada, France, Denmark, and the UK. All 11 studies met the inclusion criteria of this review, the details of which are summarised in Table 5-5.

Pilgrim 2009 was an update of the Chilcott 2003 HTA report, with databases searched from inception to between May and August 2007; however, the inclusion criteria were modified. Pilgrim 2009 included studies that used prescribed doses of Rh D IgG (either two doses of at least 500 IU at 28 and 34 weeks' gestation or one dose of 1500 IU at 28 weeks' gestation) and provided postnatal Rh D IgG within 72 hours following the birth of an Rh D positive infant. Three studies (Hermann 1984, Lee 1995, Parsons 1998) identified by Chilcott 2003 were therefore excluded by Pilgrim 2009 but, as they aligned with our PICO criteria, both systematic reviews were included.

A potential updated search was identified in this review in the form of a structured abstract (Chilcott 2016), however, no relevant information was able to be retrieved, and therefore Pilgrim 2009 was used as the most recent HTA assessment.

Pilgrim 2009 identified 12 citations relating to eight studies of clinical effectiveness, with four additional studies (MacKenzie 2004, MacKenzie 1998, Bowman 1980, Bowman 1982) identified by the updated search. MacKenzie 2004 did not meet the PICO of this review as it compared administration routes (IV and IM) and did not include a placebo or no treatment arm. MacKenzie 1998 was an additional publication relating to MacKenzie 1999, and Bowman 1980 and Bowman 1982 were additional publications relating to Bowman and Pollack 1987.

Turner 2012 was a meta-analysis of studies identified by Chilcott 2003 and Pilgrim 2009 that examined different licensed doses and adjusted for difference in study design and quality. No additional literature search was carried out. The purpose of the study was to assess the relevance of the studies to the target setting of interest, which reflects the objectives of the NICE appraisal. Four independent researchers assessed internal biases, and the studies were weighted based on the assessed bias. A meta-regression analysis was performed to inform about the relative effectiveness of the three licensed doses.

Koelewijn 2008 was published after the literature search date of the Pilgrim 2009 review and examined the effectiveness of the Dutch national routine antenatal anti-D immunisation prevention program, which comprised of a single dose of 1000 IU of Rh D immunoglobulin at 30 weeks' gestation. Women who were Rh D negative pregnant parae-1 in The Netherlands in 1999, 2002 and 2004 were studied, before and after the routine antenatal anti-D program was implemented.

Table 5-5 Characteristics and quality of Level III evidence identified by Pilgrim 2009 & Chilcott 2003: RAADP (1 or 2 doses) versus placebo or no RAADP

Study ID	Study type *Risk of bias* [a]	Population [b]	Intervention	Comparator	Outcomes
Bowman 1978	Prospective Coh, historic and geographic controls *High*	Pregnant women who are Rh D negative and primigravidae	Rh_o(D) IgG 2 x 1500 IU IM at 28 and 34 weeks' gestation n = 1357[c,d] Dec 1968 – Aug 1976 Winnipeg, Canada	No treatment n = 2768[d] Mar 1967–Dec 1974 Manitoba, Canada	Incidence of Rh D alloimmunisation (during pregnancy, within three days of delivery, at 6–9 months following delivery, in a subsequent pregnancy)
Bowman and Pollock 1978	Prospective Coh with historic controls *Moderate*	Pregnant women who are Rh D negative, primigravidae and unsensitised multigravidae	Rh_o(D) IgG 1 x 1500 IU IM at 28 weeks' gestation n = 1804[d] Mar 1976–June 1977 Manitoba, Canada	No treatment n = 3533[d] Mar 1967–Dec 1974 Manitoba, Canada	Incidence of Rh D alloimmunisation (during pregnancy, within three days of delivery, at 6–9 months following delivery)

Study ID	Study type *Risk of bias* [a]	Population [b]	Intervention	Comparator	Outcomes
Bowman 1987	Retrospective Coh, historical controls *High*	Pregnant women who are Rh D negative and primigravidae and unsensitised multigravidae	WinRho 1 x 1500 IU IM or IV at 28 weeks' gestation n = 9303[d] June 1977–Feb 1986 Manitoba, Canada	No treatment n = 3533[d] Mar 1967–Dec 1974 Manitoba, Canada	Incidence of Rh D alloimmunisation
Herman 1984	Prospective Coh, historical controls *High*	Pregnant women who are Rh D negative and primigravidae and unsensitised multigravidae	Rhesonativ 1 x 1250 IU at 32–34 weeks' gestation, im n = 568[e] NR Växjö, Sweeden	No treatment n = 645[e] 1968–1977 Växjö, Sweeden	Incidence of Rh D alloimmunisation
Mayne 1997	Retrospective before and after study *Moderate*	Pregnant women who are Rh D negative and primiparae	Rh D Ig G 2 x 500 IU at 28 and 34 weeks' gestation, NR n = 1425[d] 1993–1995 Southern Derbyshire, UK	No treatment n = 1426[d] 1988–1990 Southern Derbyshire, UK	Incidence of Rh D alloimmunisation (subsequent pregnancy)
MacKenzie 1999	Community intervention trial (controlled before and after study) *Low*	Pregnant women who are Rh D negative and primiparae	Rh D Ig G 2 x 500 IU at 28 and 34 weeks' gestation n = 3320[d] NR 1990–1996 Oxfordshire UK	No treatment n = 3146[d] 1990–1996 Northamptonshire, UK	Incidence of Rh D alloimmunisation (subsequent pregnancy)
Parsons 1998	Retrospective survey, geographical controls	NR	Rh D Ig G 1 x unknown dose at 28 weeks' gestation n = 9684[e] NR 1988–1995 Nova Scotia	No treatment n = NR NR 1998–1995 Scotland	Incidence of Rh D alloimmunisation
Trolle 1989	Prospective Coh, historical controls *High*	Pregnant women who are Rh D negative and primigravidae and unsensitised multigravidae	Rh D Ig G 1 x 1500 IU at 28 weeks' gestation n = 346[d] NR 1980–1985	No treatment n = 354[d] NR NR	Incidence of Rh D alloimmunisation
Tovey 1983	Prospective Coh with historical controls *Moderate*	Pregnant women who are Rh D negative and primigravidae	Rh D Ig G 2 x 500 IU at 28 and 34 weeks' gestation n = 1238[d] 1980–1981 Yorkshire	No treatment n = 2000[d] 1978–1979 Yorkshire	Incidence of Rh D alloimmunisation (at delivery, at 9–12 months following delivery, subsequent pregnancy[f])

Coh, cohort; IgG, immunoglobulin G; IU, international units; NR, not reported; RCT, randomised controlled trial; SR, systematic review

a. Study quality was assessed by Pilgrim 2009 primarily on the basis of two key factors: the comparability of the intervention and control groups, and the use of intention-to-treat analysis. The study quality was transcribed as follows: poor = high risk of bias; fair = moderate risk of bias, good = low risk of bias.

b. As reported by the original authors.

c. 153 women received one dose only at 34 weeks' gestation.

d. Number of Rh D negative women who delivered an Rh D+ infant, as reported by Pilgrim 2009. This overstates the effect of universal prophylaxis.

e. Number of Rh D negative women who delivered an Rh D+ infant, as reported by Chilcott 2003. This overstates the effect of universal prophylaxis.

f. As reported by Thornton 1989.

5.2.4 Results

A GRADE evidence profile summarising the evidence for universal *routine* antenatal prophylaxis with Rh D immunoglobulin (1 or 2 doses) in Rh D negative pregnant women with no preformed anti-D antibodies is provided in Appendix 6.

5.2.4.1 Incidence of Rh D alloimmunisation

Four systematic reviews (Chilcott et al., 2003, Pilgrim et al., 2009, Turner et al., 2012, McBain et al., 2015) were included that informed on the effectiveness of routine antenatal prophylaxis with Rh D IgG (one or two doses) on the incidence of Rh D alloimmunisation in Rh D negative pregnant women.

The primary studies included in the identified systematic reviews each varied with regards to the total dose of Rh D IgG administered (ranging from 500 IU up to 3000 IU) and the timing of outcome measurement, therefore several analyses were conducted to assess the implications for effectiveness.

One or two doses, any timepoint

A summary of the evidence for the incidence of Rh D alloimmunisation at any timepoint (one or two doses) is presented in Table 5-6. The evidence stratified by number of doses (any timepoint) is presented in Table 5-7.

The meta-analyses of the two available RCTs (Lee 1995, Huchet 1987) demonstrated a trend towards favouring antenatal administration of Rh D immunoprophylaxis, with lower incidence of Rh D alloimmunisation at any timepoint 6/1112 (0.5%) versus 16/1185 (1.4%) (RR 0.39, 95%CI 0.09) however, the effect was not significant (p = 0.20) and moderate heterogeneity was noted (I^2 = 40%); *GRADE: low quality evidence*. McBain 2015 reported there was no conclusive evidence that use of Rh D immunoglobulin during pregnancy was beneficial to the mother or baby. It was noted that the study by Lee 1995 used a lower dose than is currently used in the Australian context (250 IU at 28 and 34 weeks' gestation).

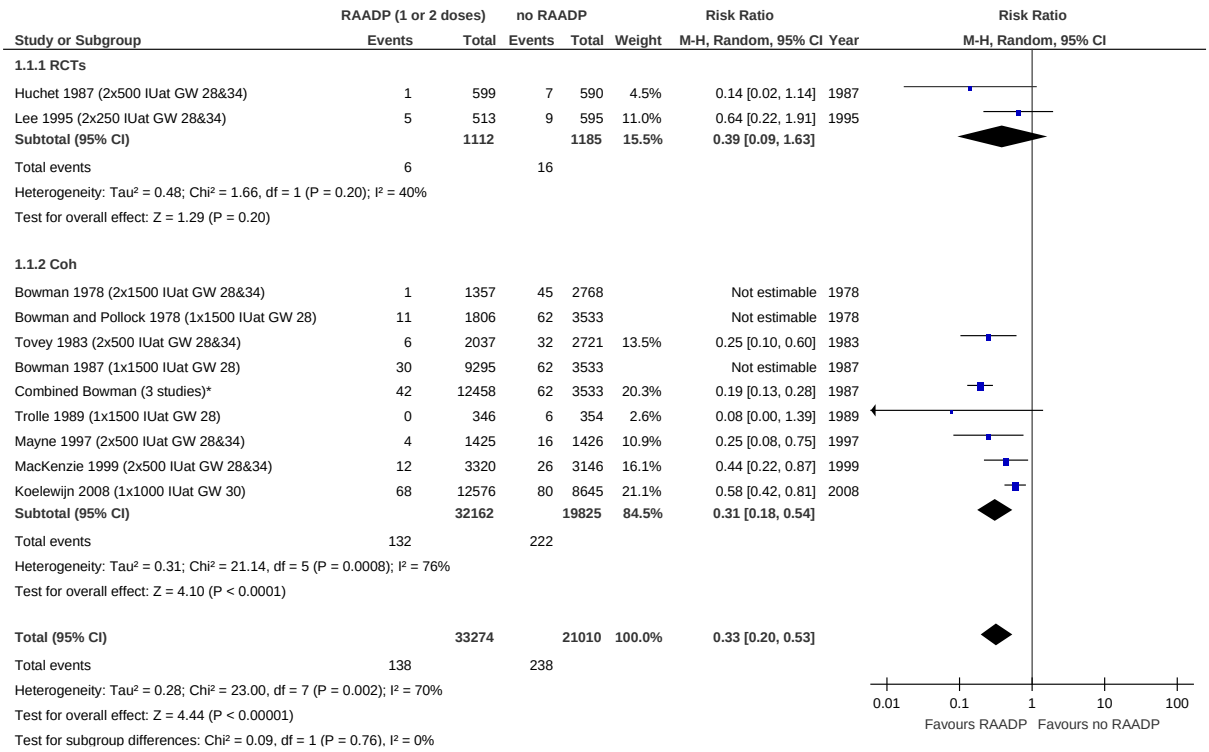
The meta-analyses reported by Turner 2012, Pilgrim 2009, and Chilcott 2003 each showed an effect favouring RAADP, regardless of dose or timing of outcome measurement when compared with no RAADP (odds ratio ranging from 0.22 to 0.31). Turner 2012 estimated the odds of Rh D alloimmunisation (during pregnancy, at birth, or in subsequent pregnancy) to be 0.31 (95%CI 0.17, 0.56), after adjusting for internal biases related to study design (e.g., patient selection, performance, attrition, outcome measurement) and external biases related to Rh D immunoprophylaxis (as rated by four assessors).

A meta-analysis of the eight Level III studies identified in this review (see Figure 5.3) revealed a significant effect favouring RAADP (any dose, any timepoint) compared with no RAADP for the incidence of Rh D alloimmunisation (RR 0.31; 95%CI 0.18, 0.54; p < 0.00001); however, heterogeneity was substantial (I^2 = 76%); *GRADE: very low quality evidence*. Data for the control group for one study (Parsons 1998) was not available therefore could not be included in this meta-analysis. The overall effect (including the RCTs) was also significant (RR 0.33; 95%CI 0.20, 0.53; p < 0.00001), with significant heterogeneity between studies noted (I^2 = 70%).

Many of the included studies were had problems with study design, with concerns raised regarding the comparability of treatment groups and missing data that may overestimate the degree of

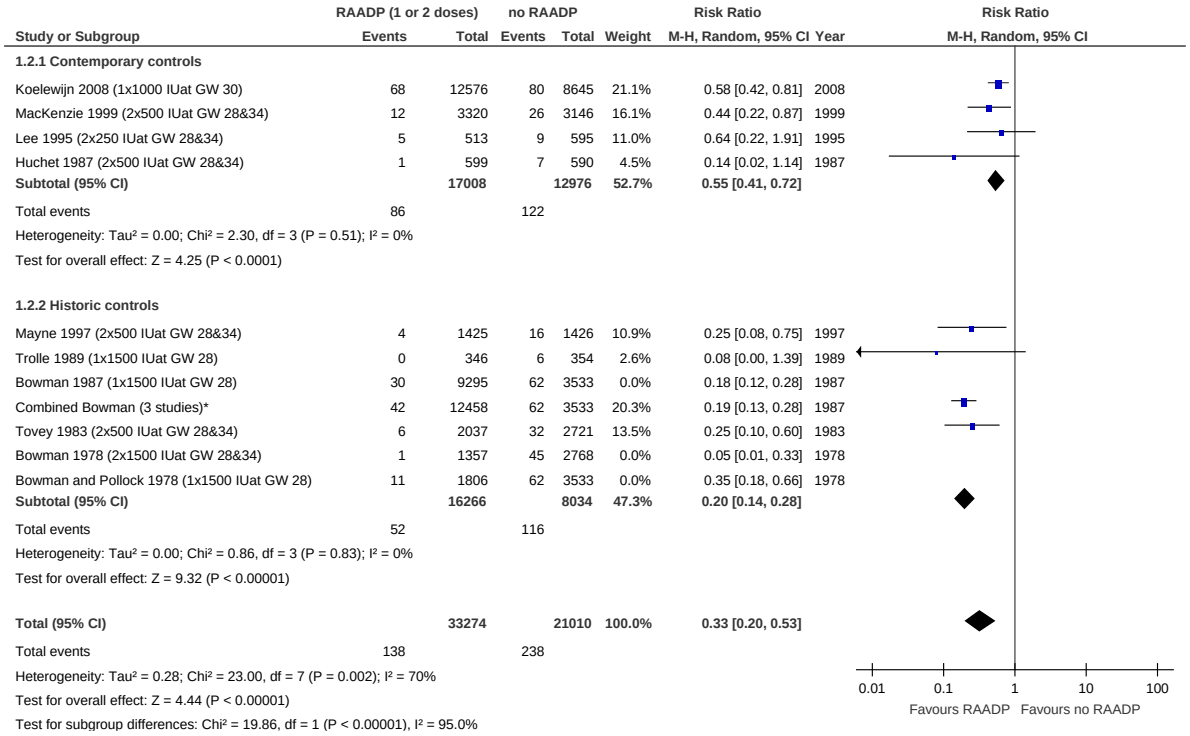
protection provided by RAADP (see Figure 5.4); therefore, interpretation of results should be made with caution.

Figure 5.3 Forest plot of comparison: RAADP (1 or 2 doses) vs no RAADP, outcome: Incidence of Rh D alloimmunisation, any timepoint.



* To avoid double counting of the controls in the studies reported by Bowman, 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.

Figure 5.4 Forest plot of comparison: RAADP (1 or 2 doses) vs no RAADP, outcome: Incidence of Rh D alloimmunisation, any timepoint (by control group).



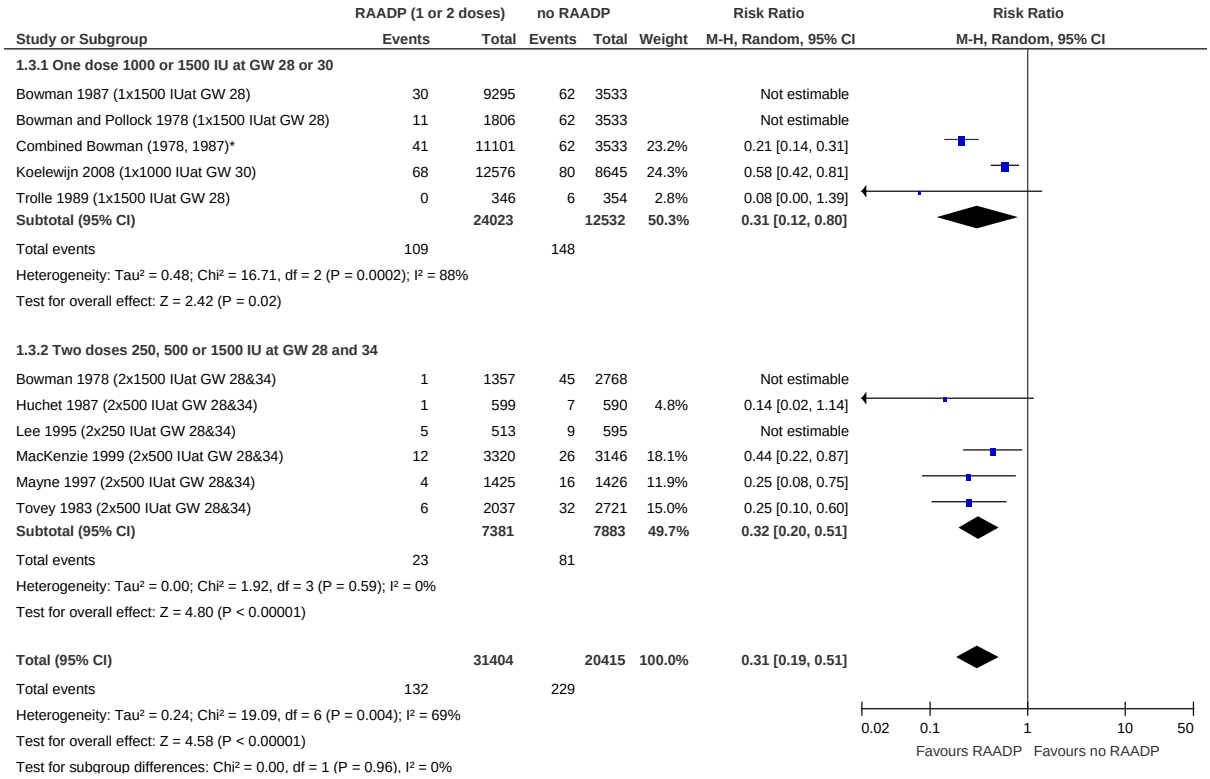
* To avoid double counting of the controls in the studies reported by Bowman, data for the intervention group were combined. It is not clear if some of the women included in the intervention group were reported in one, two or all three studies.

Both Turner 2012 and Pilgrim 2009 also assessed whether the different dosing regimens influenced the effectiveness of Rh D IgG, but found no evidence to suggest that one or two doses was superior. Turner 2012 noted that the subgroup analyses were not conclusive therefore the authors elicited expert opinion on the relative effectiveness of all RAADP treatment regimens based on a meta-regression model. The authors estimated the odds of Rh D alloimmunisation (any timepoint) to be 0.42 (95%CI 0.17, 0.73) with a single dose (1500 IU) at 28 weeks' gestation and 0.31 (95%CI 0.09, 0.65) with two doses (500 IU) at gestational week 28 and 34. This is different to the unadjusted point estimates reported by Pilgrim 2009, with pooled data from three studies that used a single dose (1500 IU) at 28 weeks' gestation showing an odds ratio of 0.20 (95%CI 0.13, 0.29), and pooled data from four studies that used two doses (500 IU) at 28 and 34 weeks' gestation showing an odd ratio of 0.33 (95%CI 0.20, 0.55).

Pooled data from the studies identified for this review (see Figure 5.5) reveal a significant effect favouring RAADP (any timepoint) compared with no RAADP for the incidence of Rh D alloimmunisation using a single dose (RR 0.31; 95%CI 0.12, 0.80; $p = 0.02$) and when using a two-dose regimen (RR 0.32; 95%CI 0.20, 0.51; $p < 0.00001$). No significant subgroup differences were observed ($Chi^2 = 0.00$, $p = 0.96$), $I^2 = 0\%$).

When pooled data were assessed based on the total administered dose, an effect favouring a higher dose was observed (see Figure 5.6). However, given the heterogeneity and quality of the included studies and variability of the interventions, controls, and outcomes reported, caution should be taken when interpreting these results.

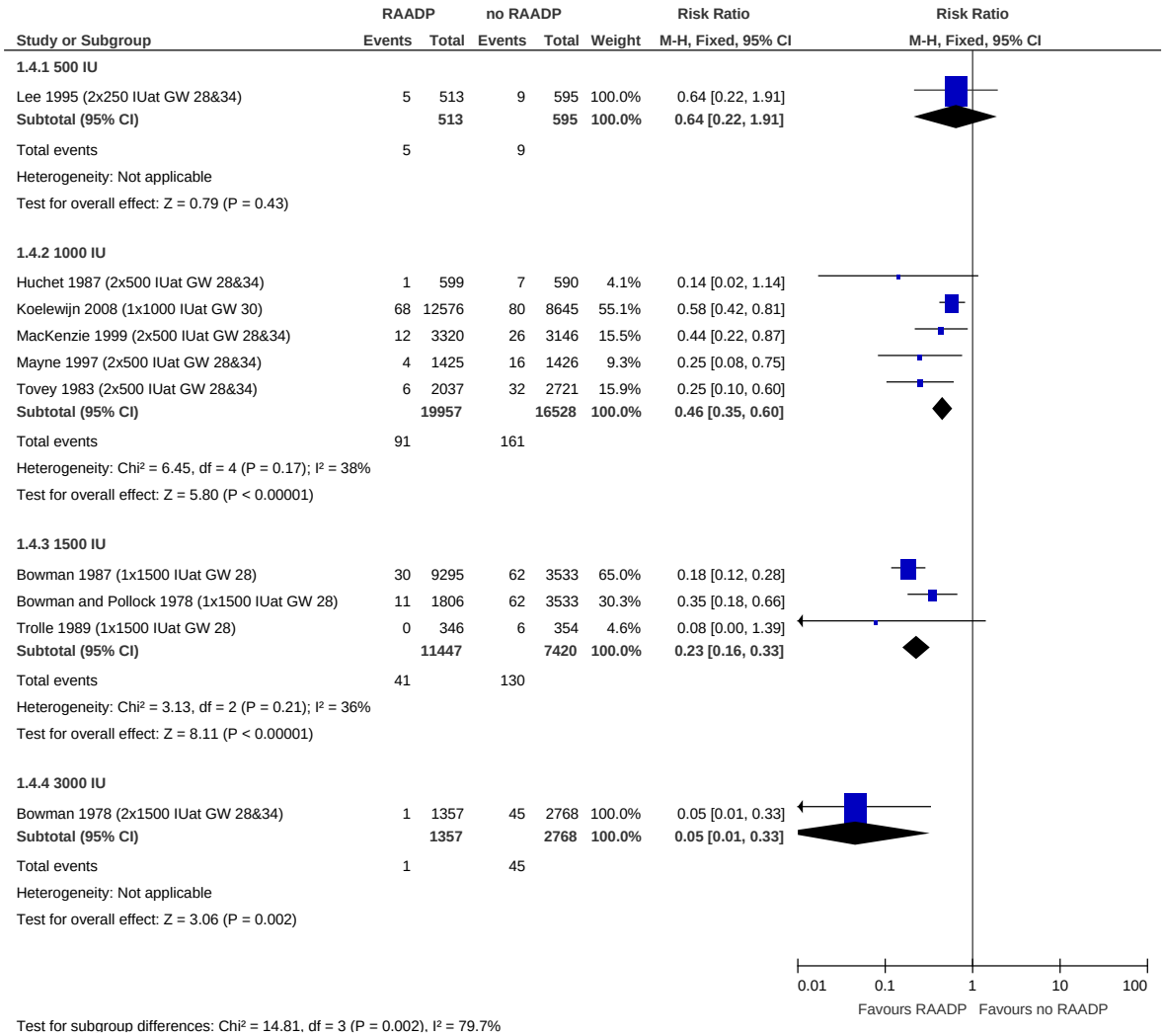
Figure 5.5 Forest plot of comparison: RAADP (1 or 2 doses) vs no RAADP, outcome: Incidence of Rh D alloimmunisation, any time point (one or two doses).



* 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.

Bowman 1978 (2x1500 IU at GW 28&34) and Lee 1995 (2x250IU at GW 28&34) were not included in the analysis for two doses regimens. This is because the total administered dose of RhD immunoglobulin in both studies is outside that recommended in Australia (300 IU and 500 IU, respectively). Inclusion of these studies results in a RR of 0.29 (95% CI 0.16, 0.53; $p < 0.00001$).

Figure 5.6 Forest plot of comparison: RAADP (1 or 2 doses) vs no RAADP, outcome: Incidence of Rh D alloimmunisation, any timepoint (by total dose).



Test for subgroup differences: Chi² = 14.81, df = 3 (P = 0.002), I² = 79.7%

Table 5-6 Results for *routine* antenatal prophylaxis with Rh D immunoglobulin (1 or 2 doses) versus placebo or no *routine* antenatal prophylaxis: Rh D negative women with no anti-D antibodies – Incidence of Rh D alloimmunisation (any timepoint)

Study ID Level of evidence [a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						RAADP **n/N (%)** ***% (95%CI)***	**No RAADP** **n/N (%)** ***% (95%CI)***	**Risk estimate (95% CI)**	***Statistical significance*** ***p*-value** **Heterogeneity [b]**
RAADP (1 or 2 doses) vs no RAADP									
Turner 2012 Level III *Moderate*	N = NR (10 studies) (MacKenzie 1999, Mayne 1997, Lee 1995, Trolle 1989, Huchet 1987, Bowman 1987, Hermann 1984, Tovey 1983, Bowman 1978, Bowman & Pollock 1978)	Non-sensitised Rh D negative pregnant women* *includes studies selected for primigravidae women only	Obstetric and maternity (Canada, Denmark, UK, Sweden, France)	RAADP (1 or 2 doses) vs routine care and no RAADP	Incidence of Rh D alloimmunisation (bias-adjusted) (during pregnancy, at birth, or in subsequent pregnancy)	NR	NR	OR 0.31 (0.17, 0.56) The authors adjusted each study for internal and external biases as rated by four assessors after consideration of a bias checklist. Internal biases related to study design (e.g., subject selection, performance, attrition, outcome) and external biases related to Rh D immunoprophylaxis. - naive conventional random effects: (OR 0.25; 95% CI 0.18, 0.36; I^2 = 19%) - adjustment for internal biases only (OR 0.28; 95% CI 0.15, 0.53; I^2 = 0%)	*Favours RAADP* *p* = NR No significant heterogeneity I^2 = 0% (*p* = NR)
Turner 2012 Level III *Moderate*	N = NR (8 studies) (MacKenzie 1999, Mayne 1997, Lee 1995, Trolle 1989, Huchet 1987, Hermann 1984, Tovey 1983, Bowman 1978)	Non-sensitised Rh D negative pregnant women* *includes studies selected for primigravidae women only	Obstetric and maternity (Canada, Denmark, UK, Sweden, France)	RAADP (1 or 2 doses) vs routine care and no RAADP	Incidence of Rh D alloimmunisation (during pregnancy, at birth, or in subsequent pregnancy)	NR	NR	OR 0.31 (0.16, 0.61) Excludes two studies (Bowman 1987 and Bowman & Pollock 1978) that share a control group with Bowman 1978.	*Favours RAADP* *p* = NR No significant heterogeneity I^2 = 0% (*p* = NR)
Pilgrim 2009 Level III *Moderate*	N = 31 961 (8 studies)	Rh D negative pregnant women	Obstetric and maternity (Canada, Denmark, France, US and UK)	2 x at least 500 IU at GW 28 and 34 or 1 x at least 1500 IU at GW 28 vs no RAADP	Incidence of Rh D alloimmunisation (authors' figures)	54/20191 (0.27)	149/11770 (1.27)	M-H Random[c] OR 0.22 (0.13, 0.36) RR 0.22 (0.14, 0.37)	*Favours RAADP* *p* < 0.00001 Moderate heterogeneity I^2 = 41% (*p* = 0.13)

Study ID Level of evidence [a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						RAADP n/N (%) *% (95%CI)*	**No RAADP n/N (%) *% (95%CI)***	**Risk estimate (95% CI)**	***Statistical significance p*-value Heterogeneity [b]**
	(MacKenzie 1999, Mayne 1997, Trolle 1989, Bowman 1987, Huchet 1987, Tovey 1983, Bowman & Pollock 1978, Bowman 1978)			* all received further dose at or within 72 hrs of delivery of a positive infant	(during pregnancy, at birth, or in subsequent pregnancy)	Intervention arms of three studies (Bowman 1978, Bowman & Pollock 1978, and Bowman 1987) combined to avoid triple counting of the control group.[d]			
Pilgrim 2009 Level III *Moderate*	N = 31 955 (8 studies) (MacKenzie 1999, Mayne 1997, Trolle 1989, Bowman 1987, Huchet 1987, Tovey 1983, Bowman & Pollock 1978, Bowman 1978)	Rh D negative pregnant women	Obstetric and maternity (Canada, Denmark, France, US and UK)	2 x at least 500 IU at GW 28 and 34 or 1 x at least 1500 IU at GW 28 vs no RAADP * all received further dose at or within 72 hrs of delivery of a positive infant	Incidence of Rh D alloimmunisation (authors' figures, including women excluded from published analysis for various reasons) (during pregnancy, at birth, or in subsequent pregnancy)	65/20185 (0.32)	149/11770 (1.27)	M-H Fixed[c] OR 0.23 (0.17, 0.32) RR 0.24 (0.18, 0.32)	*Favours RAADP* $p < 0.00001$ No significant heterogeneity $I^2 = 1\%$ ($p = 0.41$)
						Intervention arms of three studies (Bowman 1978, Bowman & Pollock 1978, and Bowman 1987) combined to avoid triple counting of the control group.[d]			
Pilgrim 2009 Level III *Moderate*	N = 30 598 (7 studies) (MacKenzie 1999, Mayne 1997, Trolle 1989, Bowman 1987, Huchet 1987, Tovey 1983, Bowman & Pollock 1978)	Rh D negative pregnant women	Obstetric and maternity (Canada, Denmark, France, US and UK)	2 x at least 500 IU at GW 28 and 34 or 1 x at least 1500 IU at GW 28 vs no RAADP * all received further dose at or within 72 hrs of delivery of a positive infant	Incidence of Rh D alloimmunisation (authors' figures) (during pregnancy, at birth, or in subsequent pregnancy)	53/18834 (0.28)	149/11770 (1.27)	M-H Random [c] OR 0.23 (0.14, 0.36) RR 0.23 (0.15, 0.36)	*Favours RAADP* $p < 0.00001$ Moderate heterogeneity $I^2 = 31\%$ ($p = 0.20$)
						Excludes Bowman 1978. Intervention arms of two studies (Bowman & Pollock 1978 and Bowman 1987) combined to avoid double counting controls. [d]			
Pilgrim 2009 Level III *Moderate*	N = 30 598 (7 studies)	Rh D negative pregnant women	Obstetric and maternity (Canada, Denmark, France, US and UK)	2 x at least 500 IU at GW 28 and 34 or 1 x at least 1500 IU at GW 28 vs no RAADP	Overall incidence of Rh D alloimmunisation	64/18828 (0.34)	149/11770 (1.27)	M-H Fixed [c] OR 0.24 (0.18, 0.33) RR 0.25 (0.18, 0.33)	*Favours RAADP* $p < 0.00001$ No significant heterogeneity $I^2 = 0\%$ ($p = 0.52$)

Study ID Level of evidence [a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						RAADP n/N (%) *% (95%CI)*	**No RAADP n/N (%) *% (95%CI)***	**Risk estimate (95% CI)**	***Statistical significance p*-value Heterogeneity [b]**
	(MacKenzie 1999, Mayne 1997, Trolle 1989, Bowman 1987, Huchet 1987, Tovey 1983, Bowman & Pollock 1978)			* all received further dose at or within 72 hrs of delivery of a positive infant	(authors' figures, including women excluded from published analysis for various reasons) (during pregnancy, at birth, or in subsequent pregnancy)	Excludes Bowman 1978. Intervention arms of two studies (Bowman & Pollock 1978 and Bowman 1987) combined to avoid double counting controls. [d]			
Chilcott 2003 Level III *Moderate*	N = 41 441 (11 studies) (MacKenzie 1999, Parsons 1998, Mayne 1997, Lee 1995, Trolle 1989, Huchet 1987, Hermann 1984, Tovey 1983, Bowman 1987, Bowman & Pollock 1978, Bowman 1978)	Rh D negative pregnant women	Obstetric and maternity (Canada, Denmark, France, Sweden and UK)	RAADP (any dose) vs no RAADP	Overall incidence of Rh D alloimmunisation (during pregnancy, at birth, or in subsequent pregnancy)	147/29288 (0.50)	167/12153 (1.37) (10 studies)	NR	NR
						Not clear how authors calculated number of women who received or were eligible for RAADP, or number of women in comparator group. Data were not available for control group in one study (Parsons 1998).			
Chilcott 2003 Level III *Moderate*	N = 34 282 (10 studies) (MacKenzie 1999, Mayne 1997, Lee 1995, Trolle 1989, Huchet 1987, Hermann 1984, Tovey 1983, Bowman 1987, Bowman & Pollock 1978, Bowman 1978)	Rh D negative pregnant women	Obstetric and maternity (Canada, Denmark, France, Sweden and UK)	RAADP (any dose) vs no RAADP	Incidence of Rh D alloimmunisation (authors' figures) (during pregnancy, at birth, or in subsequent pregnancy)	61/21272 (0.29)	170/13010 (1.31)	M-H Random [c] OR 0.25 (0.15, 0.40) RR 0.25 (0.16, 0.40)	*Favours RAADP* $p < 0.00001$ Moderate heterogeneity $I^2 = 44\%$ ($p = 0.08$)
						Excludes Parsons 1998. Intervention arms of three studies combined (Bowman 1978, Bowman & Pollock 1978, and Bowman 1987) to avoid triple counting of control group.			

Study ID Level of evidence [a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						RAADP **n/N (%)** ***% (95%CI)***	**No RAADP** **n/N (%)** ***% (95%CI)***	**Risk estimate (95% CI)**	***Statistical significance*** ***p*-value** **Heterogeneity [b]**
Chilcott 2003 Level III *Moderate*	N = 34 276 (10 studies) (MacKenzie 1999, Mayne 1997, Lee 1995, Trolle 1989, Huchet 1987, Hermann 1984, Tovey 1983, Bowman 1987, Bowman & Pollock 1978, Bowman 1978)	Rh D negative pregnant women	Obstetric and maternity (Canada, Denmark, France, Sweden and UK)	RAADP (any dose) vs no RAADP	Overall incidence of Rh D alloimmunisation (authors' figures, including women excluded from published analysis for various reasons) (during pregnancy, at birth, or in subsequent pregnancy)	75/21266 (0.36)	170/13010 (1.31)	M-H Fixed [c] OR 0.26 (0.20, 0.35) RR 0.27 (0.20, 0.35)	*Favours RAADP* $p < 0.00001$ Moderate heterogeneity $I^2 = 27\%$ ($p = 0.22$)
						Excludes Parsons 1998. Intervention arms of three studies combined (Bowman 1978, Bowman & Pollock 1978, and Bowman 1987) to avoid double counting of control group.			
Koelewijn 2008 Level III-3 *Moderate*	N = NR	Non-sensitised Rh D negative pregnant women *primiparae	Obstetrics and maternity (Netherlands)	1 x 1000 IU at Week 30 vs no RAADP *all received further dose after delivery of a positive infant	Rh D alloimmunisation (detected at Week 12 and/or Week 30)	68/NR [e] *0.56%* *(0.43, 0.69)*	79.5/NR [e] *0.92%* *(0.71, 1.12)*	RR 0.61 (0.22, 1.01)	*No significant difference* p = NR

CI, confidence interval; hrs, hours; im, intramuscular; IU, international units; M-H, Mantzel-Hentzel; NHMRC, National Health and Medical Research Council; NR, not reported; OR, odds ratio; RCT, randomised controlled trial; RR, relative risk; RAADP, routine antenatal anti-D prophylaxis; SR, systematic review; UK, United Kingdom; US, United States; vs, versus

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The risk of bias of the included primary studies is based on the quality assessment reported by the systematic review authors.

b. Only applicable to Level I studies with formal meta-analysis. Heterogeneity defined as follows: (i) no significant heterogeneity if $P_{het} > 0.1$ and $I^2 < 25\%$; (ii) mild heterogeneity if $I^2 < 25\%$; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity $I^2 > 50\%$.

c. Calculated post-hoc using RevMan 5.3.

d. The control group of Bowman 1978, Bowman & Pollock 1978 and Bowman 1987 are believed to be the same women (Pilgrim 2009, p 42).

e. Receipt of postnatal and/or antenatal prophylaxis was unknown in four cases in the intervention group. In the prevalence calculation, two cases were considered as received because anti-D antibodies were detected at Week 12 (i.e. included in the 29), and two were estimated as 0.5 (i.e. 2*0.5=1, meaning 1 valid event not counted). In the control group, receipt of postnatal prophylaxis was unknown in three cases. In the prevalence calculation, two cases were considered as received because anti-D antibodies was detected at Week 12 (i.e. included in the 21.5), and one was estimated as 0.5 (i.e. 1*0.5=0.5, meaning 0.5 valid event not counted).

Table 5-7 Results for *routine* antenatal prophylaxis with Rh D immunoglobulin (by dose) versus placebo or no *routine* antenatal prophylaxis: Rh D negative women with no anti-D antibodies – Incidence of Rh D alloimmunisation (any timepoint)

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						RAADP n/N (%) *% (95%CI)*	No RAADP n/N (%) *% (95%CI)*	Risk estimate (95% CI)	*Statistical significance* *p*-value Heterogeneity[b]
RAADP (1 dose) vs no RAADP									
McBain 2015 Level I *Low*	No RCTs identified	Rh negative women without anti-D antibodies at 28 weeks' gestation	Obstetric and maternity	RAADP (1 dose) vs no RAADP	Incidence of Rh D alloimmunisation (during pregnancy, at birth, or in subsequent pregnancy)	No data	No data	No data	No data
Turner 2012 Level III *Moderate*	N = NR (10 studies) (MacKenzie 1999, Mayne 1997, Lee 1995, Trolle 1989, Huchet 1987, Bowman 1987, Hermann 1984, Tovey 1983, Bowman 1978, Bowman & Pollock 1978)	Non-sensitised Rh D negative pregnant women* *includes studies selected for primigravidae women only	Obstetric and maternity (Canada, Denmark, UK, Sweden, France)	1500 IU at GW 28 to 30 vs routine care and no RAADP	Incidence of Rh D alloimmunisation (during pregnancy, at birth, or in subsequent pregnancy) (estimated)	NR	NR	OR 0.42 (0.17, 0.73)	*Favours RAADP* *p* = NR Heterogeneity not reported
						Subgroup analysis of different dosing regimens were not conclusive, therefore the authors elicited expert opinion on the relative effectiveness of all RAADP treatment regimens and performed a meta-regression model that estimated the association between the relative and observed effectiveness for different treatment regimes.			
Pilgrim 2009 Level III *Moderate*	N = 15 334 (3 studies) (Trolle 1989, Bowman 1987, Bowman & Pollock 1978)	Rh D negative pregnant women* *unselected primigravidae and multigravidae	Obstetric and maternity (Canada, Denmark)	1 x 1500 IU at GW 28 vs no RAADP * all received further dose at or within 72 hrs of delivery of a positive infant	Overall incidence of Rh D alloimmunisation (during pregnancy, at birth, or in subsequent pregnancy) (authors' figures, including women excluded from published analysis for various reasons)	41/11447 (0.36) *0.34% (0.28, 0.40%)*	68/3887 (1.75) *1.60% (0.37, 2.83%)*	OR 0.20 (0.13, 0.29)[c]	*Favours RAADP* *p* = NR No significant heterogeneity I^2 = NR (*p* = 0.940)

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						RAADP **n/N (%)** ***% (95%CI)***	**No RAADP** **n/N (%)** ***% (95%CI)***	**Risk estimate (95% CI)**	***Statistical significance*** ***p*-value** **Heterogeneity[b]**
Koelewijn 2008 Level III-3 *Moderate*	N = NR	Non-sensitised Rh D negative pregnant women *primiparae	Obstetrics and maternity (Netherlands)	1 x 1000 IU at GW 30 versus no RAADP *all received further dose after delivery of a positive infant	Prevalence of Rh D alloimmunisation (detected at Week 12 and Week 30)	68/NR [d] *0.56% (0.43, 0.69)*	79.5/NR [d] *0.92% (0.71, 1.12)*	RR 0.61 (0.22, 1.01)	*No significant difference* *p* = NR
RAADP (2 doses) vs no RAADP									
McBain 2015 Level I *Low*	No RCTs identified	Rh negative women without anti-D antibodies at 28 weeks' gestation	Obstetric and maternity (any)	RAADP (2 doses) versus no RAADP	Incidence of Rh D alloimmunisation (during pregnancy, at birth, or in subsequent pregnancy)	No data	No data	No data	No data
Turner 2012 Level III *Moderate*	N = NR (10 studies) (MacKenzie 1999, Mayne 1997, Lee 1995, Trolle 1989, Huchet 1987, Bowman 1987, Hermann 1984, Tovey 1983, Bowman 1978, Bowman & Pollock 1978)	Non-sensitised Rh D negative pregnant women* *includes studies selected for primigravidae women only	Obstetric and maternity (Canada, Denmark, UK, Sweden, France)	500 IU at GW 28 and 34 vs routine care and no RAADP	Incidence of Rh D alloimmunisation (during pregnancy, at birth, or in subsequent pregnancy) (estimated)	NR	NR	OR 0.31 (0.09, 0.65) Subgroup analysis of different dosing regimens were not conclusive, therefore the authors elicited opinion on the relative effectiveness of all RAADP treatment regimens and performed a meta-regression model that estimated the association between the relative and observed effectiveness for different treatment regimes.	*Favours RAADP* *p* = NR Heterogeneity not reported
Turner 2012 Level III *Moderate*	N = NR (10 studies) (MacKenzie 1999, Mayne 1997, Lee 1995, Trolle 1989, Huchet 1987, Bowman 1987, Hermann 1984, Tovey 1983, Bowman 1978, Bowman & Pollock 1978)	Non-sensitised Rh D negative pregnant women* *includes studies selected for primigravidae women only	Obstetric and maternity (Canada, Denmark, UK, Sweden, France)	1250 IU at GW 28 and 34 vs routine care and no RAADP	Incidence of Rh D alloimmunisation (estimated)	NR	NR	OR 0.18 (0.03, 0.53) Subgroup analysis of different dosing regimens were not conclusive, therefore the authors elicited opinion on the relative effectiveness of all RAADP treatment regimens and performed a meta-regression model that estimated the association between the relative and observed effectiveness for different treatment regimes.	*Favours RAADP* *p* = NR Heterogeneity not reported

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						RAADP n/N (%) *% (95%CI)*	No RAADP n/N (%) *% (95%CI)*	Risk estimate (95% CI)	*Statistical significance* *p*-value Heterogeneity[b]
Pilgrim 2009 Level III *Moderate*	N = 13 470 (4 studies) (MacKenzie 1999, Mayne 1997, Huchet 1987, Tovey 1983)	Rh D negative pregnant women* *primigravidae only	Obstetric and maternity (France, UK)	2 x 500 IU at GW 28 and 34 vs no RAADP * all received further dose at or within 72 hrs of delivery of a positive infant	Overall incidence of Rh D alloimmunisation (during pregnancy, at birth, or in subsequent pregnancy) (authors' figures, including women excluded from published analysis for various reasons)	20/6444 (0.31) *0.30% (0.22, 0.38)*	65/7026 (0.93) *0.89% (0.21, 1.56)*	OR 0.33 (0.20, 0.55)	*Favours RAADP* *p* = NR No significant heterogeneity I^2 = NR (*p* = 0.812)
Pilgrim 2009 Level III *Moderate*	N = 9317 (2 studies) (MacKenzie 1999, Mayne 1997)	Rh D negative pregnant women* *primigravidae only	Obstetric and maternity (UK)	2 x 500 IU at GW 28 and 34 vs routine care and no RAADP * all received further dose at or within 72 hrs of delivery of a positive infant	Overall incidence of Rh D alloimmunisation (during pregnancy, at birth, or in subsequent pregnancy) (authors' figures, including women excluded from published analysis for various reasons)	16/4745 (0.34) *0.35% (0.29, 0.40*)	42/4572 (0.92) *0.95% (0.18, 1.71)*	OR 0.37 (0.21, 0.65)	*Favours RAADP* *p* = NR No significant heterogeneity I^2 = NR (*p* = 0.976)

CI, confidence interval; GW, gestational week; hrs, hours; IM, intramuscular; IU, international units; M-H, Mantzel-Hentzel; NHMRC, National Health and Medical Research Council; NR, not reported; OR, odds ratio; RCT, randomised controlled trial; RR, relative risk; RAADP, routine antenatal anti-D prophylaxis; SR, systematic review; UK, United Kingdom; US, United States; vs, versus

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The risk of bias of the included primary studies is based on the quality assessment reported by the systematic review authors.

b. Only applicable to Level I studies with formal meta-analysis. Heterogeneity defined as follows: (i) no significant heterogeneity if P_{het} >0.1 and I^2 <25%; (ii) mild heterogeneity if I^2 <25%; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity I^2 >50%.

c. This meta-analysis appears to have double counted the control groups (Bowman & Pollock 1978 and Bowman 1987).

d. Receipt of postnatal and/or antenatal prophylaxis was unknown in four cases in the intervention group. In the prevalence calculation, two cases were considered as received because anti-D antibodies were detected at Week 12 (i.e. included in the 29), and two were estimated as 0.5 (i.e. 2*0.5=1, meaning one valid event not counted). In the control group, receipt of postnatal prophylaxis was unknown in three cases. In the prevalence calculation, two cases were considered as received because anti-D antibodies were detected at Week 12 (i.e. included in the 21.5), and one was estimated as 0.5 (i.e. 1*0.5=0.5, meaning 0.5 valid event not counted).

One or two doses, timing of outcome measurement

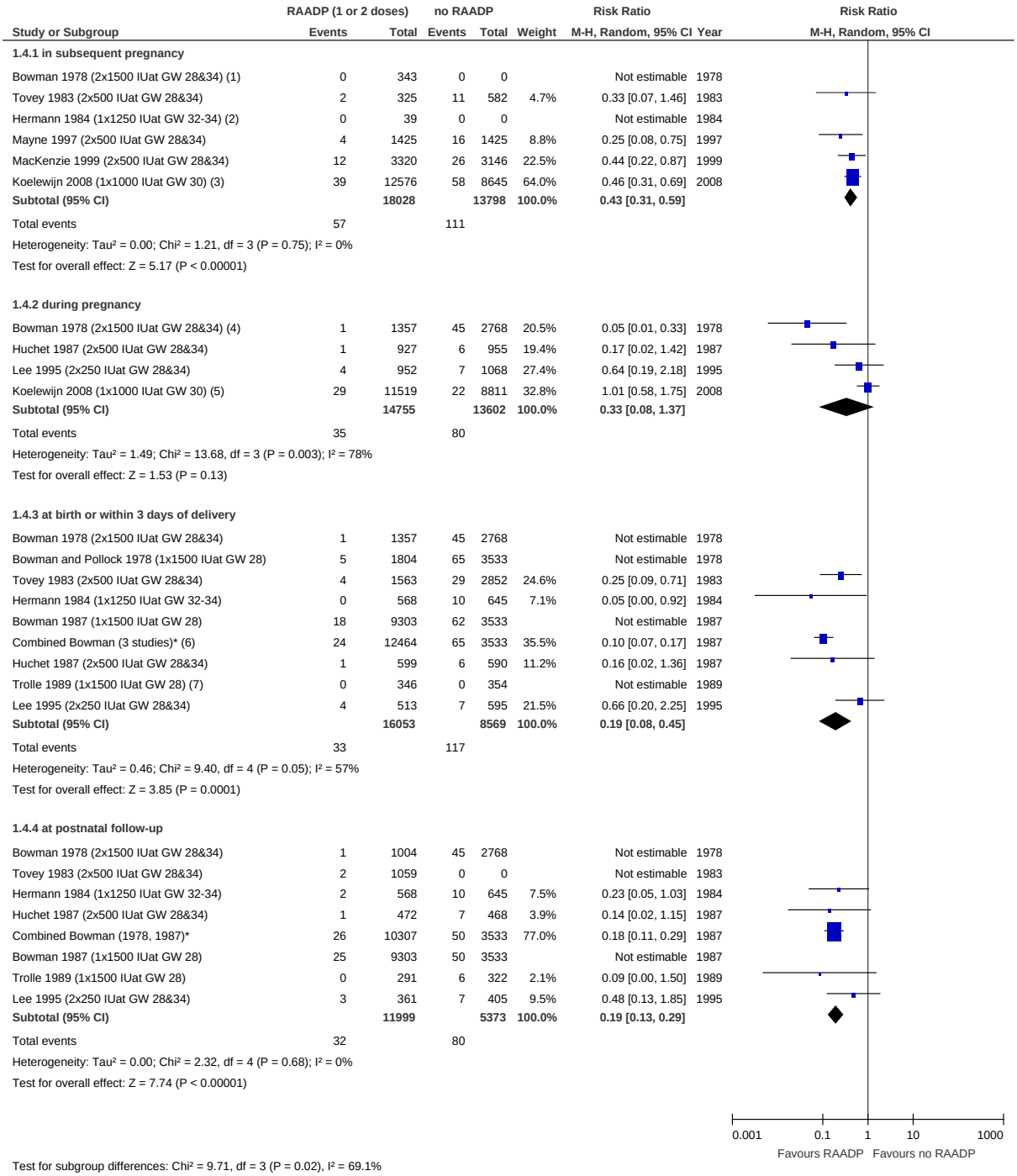
The included primary studies measured the incidence of Rh D alloimmunisation at varying timepoints including those detected in a subsequent pregnancy, during pregnancy, at birth or within 3 days after delivery, or at postnatal followup. A summary of results from identified studies stratified by timing of outcome measurement is presented in Table 5-8. A forest plot is presented in Figure 5.7.

Six studies reported incidence of Rh D alloimmunisation in a subsequent pregnancy (up to 12 week's gestation). Data for the control group were not available in two studies. Pooled data from these studies found a significant effect favouring RAADP (RR 0.43; 95%CI 0.31, 0.59; $p < 0.00001$; $I^2 = 0\%$); *GRADE: low quality evidence*.

Four studies reported the incidence of Rh D alloimmunisation detected during pregnancy. Pooled data from these studies showed a nonsignificant effect comparing RAADP with no RAADP (RR 0.33; 95%CI 0.08, 1.37; $p = 0.13$; $I^2 = 78\%$); *GRADE: very low quality evidence*. It is noticeable, however, that the risk reduction associated with RAADP decreased over time, with fewer women in the control group sensitised in the later studies. This is likely in response to changes in obstetric practice and better management in the antenatal period.

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; $p = 0.0001$; $I^2 = 57\%$); *GRADE: very low quality evidence* and in the seven studies that assessed the incidence of Rh D alloimmunisation at postnatal followup (RR 0.19; 95% CI 0.13, 0.29; $p < 0.00001$; $I^2 = 0\%$); *GRADE: low quality evidence*.

Figure 5.7 Forest plot of comparison: RAADP (1 or 2 doses) vs no RAADP, outcome: Incidence of Rh D alloimmunisation, any time point (timing of event).



Test for subgroup differences: Chi² = 9.71, df = 3 (P = 0.02), I² = 69.1%

Footnotes

(1) Bowman 1978. No data for comparator group.

(2) Herman 1984. No data for comparator group

(3) Koelewjin 2008. All women had previously delivered an D+ child after GW30. Rh D alloimmunisation detected at Week 12.

(4) Bowman 1978. Includes immunisations detected during pregnancy and within 3 days.

(5) Koelewjin 2008. All women had previously delivered an D+ child after GW30. Rh D alloimmunisation detected at Week 30

(6) Bowman 1978. Includes immunisations detected during pregnancy and within 3 days.

(7) Trolle 1989. No data reported for incidence at birth or within 3 days of delivery.

* To avoid double counting of the control group for the studies reported by Bowman, 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.

Table 5-8 Results for *routine* antenatal prophylaxis with Rh D immunoglobulin versus placebo or no *routine* antenatal prophylaxis: Rh D negative women with no anti-D antibodies – Incidence of Rh D alloimmunisation (timing of event)

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						RAADP n/N (%) *% (95%CI)*	No RAADP n/N (%) *% (95%CI)*	Risk estimate (95% CI)	*Statistical significance* *p*-value Heterogeneity[b]
Subsequent pregnancy									
McBain 2015 Level I *Low*	No RCTs identified	Rh negative women without anti-D antibodies at 28 weeks' gestation	Obstetric and maternity (Any)	RAADP (1 or 2 doses) vs no RAADP	Incidence of Rh D alloimmunisation (subsequent pregnancy)	No data	No data	No data	No data
Bowman 1978 Level III-3 *Serious*	N = 4125	Non-sensitised Rh D negative pregnant women	Obstetric and maternity (Canada)	2 x 1500 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (subsequent pregnancy)	0/343 (0.0) *0.0 (0.0, 0.0)*	No data	Not calculable	Not calculable
Tovey 1983 Level III-3 *Serious*	N = 3238	Non-sensitised Rh D negative pregnant women *primigravidae	Obstetric and maternity (UK)	2 x 500 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (subsequent pregnancy)	2/325 (0.6) *0.6 (–0.2, 1.5)*	11/582 (1.9) *1.9 (0.8, 3.0)*	OR 0.16 (0.04, 0.67)[c]	NR
Hermann 1984 Level III-3 *Serious*	N = 1033	Non-sensitised Rh D negative pregnant women	Obstetric and maternity (Sweden)	1 x 1250 IU at GW 28 vs no RAADP	Incidence of Rh D alloimmunisation (subsequent pregnancy)	0/39 (0.00) *0.0 (0.0, 0.0)*	No data	Not calculable	Not calculable
Mayne 1997 Level III-3 *Serious*	N = 2850	Non-sensitised Rh D negative pregnant women *primigravidae	Obstetric and maternity (UK)	2 x 500 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (subsequent pregnancy)	4/1425 (0.3) *0.3 (0.0, 0.6)*	16/1425 (1.1) *1.1 (0.6, 1.7)*	OR 0.25 (0.08, 0.74)[c]	NR

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						RAADP n/N (%) ***% (95%CI)***	**No RAADP n/N (%)** ***% (95%CI)***	**Risk estimate (95% CI)**	***Statistical significance p*-value** **Heterogeneity[b]**
MacKenzie 1999 Level III-2 *Serious*	N = 6466	Non-sensitised Rh D negative pregnant women *primigravidae	Obstetric and maternity (UK)	2 x 500 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (subsequent pregnancy)	12/3320 (0.4) *0.4 (0.2, 06)*	26/3146 (0.8) *0.8 (0.5, 1.1)*	OR 0.44 (0.22, 0.86) [c]	NR
Koelewijn 2008 Level III-3 *Moderate*	N = 21 221	Non-sensitised Rh D negative pregnant women *primiparae	Obstetrics and maternity (Netherlands)	1 x 1000 IU at GW 30 vs no RAADP *all received further dose after delivery of a positive infant	Rh D alloimmunisation (detected at GW 12)	39/12576 *0.31% (0.21, 0.41)*	58/8645 *0.67% (0.50, 0.84)*	RR 0.46 (0.09, 0.84) [d]	*Favours RAADP* *p* = NR
					Risk of Rh D alloimmunisation in first trimester* *assumes 95% RAADP coverage during the study period	0.33% (NR)	0.63% (NR)	RR 0.52 (0.10, 0.95)	*Favours RAADP* *p* = NR
During pregnancy or within 3 days after delivery									
McBain 2015 Level I *Low*	N = 3902 (2 trials) (Lee 1995, Huchet 1987)	Rh negative women without anti-D antibodies at 28 weeks' gestation	Obstetric and maternity (UK, France)	250 IU or 500 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (during pregnancy)	5/1879 (0.27)	13/2023 (0.64)	RR 0.42 (0.15, 1.17)	*No significant difference* *p* = 0.096 No significant heterogeneity I^2 = 13% (*p* = 0.28)
Bowman 1978 Level III-3 *Serious*	N = 4125	Non-sensitised Rh D negative pregnant women	Obstetric and maternity (Canada)	2 x 1500 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (during pregnancy or within 3 days of delivery)	1/1357 (0.1) *0.1 (–0.1, 0.3)*	45/2768 (1.6) *1.6 (1.2, 2.1)*	OR 0.02 (0.001, 0.33) [c]	NR

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						RAADP **n/N (%)** ***% (95%CI)***	**No RAADP** **n/N (%)** ***% (95%CI)***	**Risk estimate (95% CI)**	***Statistical significance*** ***p*-value** **Heterogeneity[b]**
Koelewijn 2008 Level III-3 *Moderate*	N = 20 330	Non-sensitised Rh D negative pregnant women *primiparae	Obstetrics and maternity (Netherlands)	1 x 1000 IU at GW 30 vs no RAADP *all received further dose after delivery of a positive infant	Rh D alloimmunisation (detected at GW 30)	29/11519 *0.25% (0.16, 0.34)*	21.5/8811 *0.24% (0.14, 0.35)*	RR 1.03 (0.00, 2.18) [d]	*No significant difference* *p* = NR
At birth or within 3 days after delivery									
McBain 2015 Level I *Low*	N = 2297 (2 trials) (Lee 1995, Huchet 1987)	Rh negative women without anti-D antibodies at 28 weeks' gestation	Obstetric and maternity (UK, France)	250 IU or 500 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (at birth of Rh positive infant)	5/1112 (0.45)	13/1185 (1.10)	RR 0.42 (0.15, 1.17)	*No significant difference* *p* = 0.096 No significant heterogeneity I^2 = 22% (*p* = 0.26)
Bowman 1978 Level III-3 *Serious*	N = 4125	Non-sensitised Rh D negative pregnant women	Obstetric and maternity (Canada)	2 x 1500 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (during pregnancy or within 3 days of delivery)	1/1357 (0.1) *0.1 (–0.1, 0.3)*	45/2768 (1.6) *1.6 (1.2, 2.1)*	OR 0.02 (0.001, 0.33)[c]	NR
Bowman & Pollock 1978 Level III-3 *Serious*	N = 5337	Non-sensitised Rh D negative pregnant women	Obstetric and maternity (Canada)	1 x 1500 IU at GW 28 vs no RAADP	Incidence of Rh D alloimmunisation (at delivery)	5/1804 (0.3) *0.3 (0.0, 0.5)*	62/3533 (1.8) *1.8 (1.3, 2.2)*	OR 0.34 (0.18, 0.65)[c]	NR
Tovey 1983 Level III-3 *Serious*	N = 4145	Non-sensitised Rh D negative pregnant women *primigravidae	Obstetric and maternity (Canada)	2 x 500 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (within 3 days of delivery)	4/1563 (0.3) *0.3 (0.0, 0.6)*	29/2582 (1.1) *1.1 (0.7, 1.5)*	NR	NR
Hermann 1984 Level III-3 *Serious*	N = 1213	Non-sensitised Rh D negative pregnant women	Obstetric and maternity (Sweden)	1 x 1250 IU at GW 32–34 vs no RAADP	Incidence of Rh D alloimmunisation (within 3 days of delivery)	0/568 (0.0) *0.0 (0.0, 0.0)*	10/645 (1.6) *1.6 (0.6, 2.5)*	NR	NR

Study ID **Level of evidence[a]** ***Risk of bias***	**Sample size (no. of trials) included in analysis**	**Population**	**Setting (Location)**	**Comparison**	**Outcome (timing)**	**Results**			
						RAADP **n/N (%)** ***% (95%CI)***	**No RAADP** **n/N (%)** ***% (95%CI)***	**Risk estimate (95% CI)**	***Statistical significance*** ***p*-value** **Heterogeneity[b]**
Bowman 1987 Level III-3 *Serious*	N = 12 836	Non-sensitised Rh D negative pregnant women	Obstetric and maternity (Canada)	1 x 1500 IU at GW 28 vs no RAADP	Incidence of Rh D alloimmunisation (within 3 days of delivery)	18/9303 (0.2) *0.2 (0.1, 0.3)*	62/3533 (1.8) *1.8 (1.3, 2.2)*	OR 0.18 (0.12, 0.65)[c]	NR
Huchet 1987 Level III-1 *High*	N = 1189	Non-sensitised Rh D negative pregnant women *primiparae	Obstetric and maternity (France)	2 x 500 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (within 3 days of delivery)	1/599 (0.2) *0.2 (0.0, 0.5)*	6/590 (1.0) *1.0 (0.2, 1.8)*	NR	NR
Trolle 1989 Level III-3 *Serious*	N = 700	Non-sensitised Rh D negative pregnant women	Obstetric and maternity (Denmark)	1 x 1500 IU at GW 28 vs no RAADP	Incidence of Rh D alloimmunisation (within 3 days of delivery)	No data/346	No data/354	not calculable	not calculable
Lee 1995 Level II *Unclear*	N = 1108	Non-sensitised Rh D negative pregnant women	Obstetric and maternity (UK)	2 x 250 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (within 3 days of delivery)	4/513 (0.8) *0.8 (0.0, 1.5)*	7/595 (1.2) *1.2 (0.3, 2.0)*	NR	NR
At postnatal followup									
McBain 2015 Level I *Low*	N = 2048 (2 trials) (Lee 1995, Huchet 1987)	Rh negative women without anti-D antibodies at 28 weeks' gestation	Obstetric and maternity (UK, France)	250IU or 500 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (at birth of Rh positive infant and at up to 12 months followup)	6/985 (0.61)	16/1063 (1.51)	RR 0.39 (0.10, 1.62)	*No significant difference* $p = 0.20$ Moderate heterogeneity $I^2 = 39\%$ ($p = 0.20$)
McBain 2015 Level II *Low*	N = 1696 (1 trial) (Huchet 1987)	Rh negative women without anti-D antibodies at 28 weeks' gestation *primigravidae only	Obstetric and maternity (France)	500 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (after birth of Rh positive infant and at 2–12 months followup)	0/362 (0)	4/360 (1.11)	RR 0.11 (0.01, 2.04)	*No significant difference* $p = 0.14$

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						RAADP n/N (%) *% (95%CI)*	No RAADP n/N (%) *% (95%CI)*	Risk estimate (95% CI)	*Statistical significance* *p*-value Heterogeneity[b]
Bowman 1978 Level III *Serious*	N = 4125	Non-sensitised Rh D negative pregnant women	Obstetric and maternity (Canada)	2 x 1500 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (at postnatal followup)[d]	1/1004 (0.1) *0.1 (0.0, 0.3)*	45/2768 (1.6) *1.6 (1.2, 2.1)*	NR	NR
Bowman & Pollock 1978 Level III *Serious*	N = 5337	Non-sensitised Rh D negative pregnant women	Obstetric and maternity (Canada)	1 x 1500 IU at GW 28 vs no RAADP	Incidence of Rh D alloimmunisation (at postnatal followup*) *not clear if all women were screened at postnatal followup	No data/807	50/3533 (1.4) *1.4 (1.0, 1.8)*	not calculable	not calculable
Tovey 1983 Level III-3 *Serious*	N = 3238	Non-sensitised Rh D negative pregnant women *primigravidae	Obstetric and maternity (Canada)	2 x 500 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (at postnatal followup)	2/1059 (0.2) *0.2 (–0.1, 0.5)*	No data	not calculable	not calculable
Hermann 1984 Level III-3 *Serious*	N = 1213	Non-sensitised Rh D negative pregnant women	Obstetric and maternity (Sweden)	1 x 1250 IU at GW 32 to 34 vs no RAADP	Incidence of Rh D alloimmunisation (at 8 months postnatal followup*) *not clear if all women were screened at postnatal followup	2/568 (0.4) *0.4 (0.0, 0.9)*	10/645 (1.6) *1.6 (0.6, 2.5)*	OR 0.24 [c] (0.05, 1.10)	*No significant difference* *p* = NR
Huchet 1987 Level III-1 *High*	N = 1696	Non-sensitised Rh D negative pregnant women *primiparae	Obstetric and maternity (France)	2 x 500 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (at 2–12 months postnatal followup)	1/472 (0.2) *0.2 (0.0, 0.6)*	7/468 (1.5) *1.5 (0.4, 2.6)*	OR 0.14 [c] (0.02, 1.14)	*No significant difference* *p* = NR

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						RAADP n/N (%) *% (95%CI)*	No RAADP n/N (%) *% (95%CI)*	Risk estimate (95% CI)	*Statistical significance* *p*-value Heterogeneity[b]
Bowman 1987 Level III-3 *Serious*	N = 12 836	Non-sensitised Rh D negative pregnant women	Obstetric and maternity (Canada)	1 x 1500 IU at GW 28 vs no RAADP	Incidence of Rh D alloimmunisation (at postnatal followup*) *not clear if all women were screened at postnatal followup	25/9303 (0.3) *0.3 (0.2, 0.4)*	50/3533 (1.4) *1.4 (1.0, 1.0)*	NR	NR
Trolle 1989 Level III-3 *Serious*	N = 700	Non-sensitised Rh D negative pregnant women	Obstetric and maternity (Denmark)	1 x 1500 IU at GW 28 vs no RAADP	Incidence of Rh D alloimmunisation (at postnatal followup*) *screened at 10 months or at next pregnancy	0/291 (0.0) *0.0 (0.0, 0.0)*	6/322 (1.9) *1.9 (0.4, 3.3)*	OR 0.08 [c] (0.005, 1.49)	NR
Lee 1995 Level II *Unclear*	N = 2541	Non-sensitised Rh D negative pregnant women *primigravidae	Obstetric and maternity (UK)	2 x 250 IU at GW 28 and 34 vs no RAADP	Incidence of Rh D alloimmunisation (at 6 months postnatal followup)	3/361 (0.8) *0.8 (0.0, 1.8)*	7/405 (1.7) *1.7 (0.5, 3.0)*	OR 0.56 [c] (0.14, 2.24)	*No significant difference* *p* = NR

CI, confidence interval; GW, gestational week; hrs, hours; IU, international units; M-H, Mantzel-Hentzel; NHMRC, National Health and Medical Research Council; NR, not reported; OR, odds ratio; RAADP, routine antenatal anti-D prophylaxis; RCT, randomised controlled trial; RR, relative risk; UK, United Kingdom; vs, versus

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The risk of bias of the included primary studies is based on the quality assessment reported by the systematic review authors.

b. Only applicable to Level I studies with formal meta-analysis. Heterogeneity defined as follows: (i) no significant heterogeneity if P_{het} >0.1 and I^2 <25%; (ii) mild heterogeneity if I^2 <25%; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity I^2 >50%.

c. OR reported in Turner (2012).

d. In the intervention group, receipt of postnatal and/or antenatal prophylaxis was unknown in seven cases (n = 2, Week 12; n = 5, Week 30). In the prevalence calculation, five cases were considered as received because anti-D antibodies were detected at Week 12 or the husband was typed as homozygous D positive, and two case were estimated as 0.5 (i.e. 2*0.5=1, meaning 1 valid event not counted). In the control group, receipt of postnatal prophylaxis was unknown in eight cases (n = 5, Week 12; n = 3, Week 30). In the prevalence calculation, seven cases were considered as received because anti-D antibodies were detected at Week 12 or the husband was typed as homozygous D positive, and one was estimated as 0.5 (i.e. 1*0.5=0.5, meaning 0.5 valid event not counted).

5.2.4.2 Incidence of a positive test for fetomaternal haemorrhage

One Level I study (McBain 2015) reported data from one Level II study (Huchet 1987) that reported on the incidence of a positive Kleihauer test. A summary of these results is presented in Table 5-9.

A positive Kleihauer result was reported by Huchet 1987 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); *GRADE: moderate quality evidence* and at birth of an Rh D positive infant (12.2% vs 20.2%; RR 0.60; 95% CI 0.46, 0.79; *p* = 0.00023) *GRADE: moderate quality evidence* 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).

Table 5-9 Results for *routine* antenatal prophylaxis with Rh D immunoglobulin versus placebo or no *routine* antenatal prophylaxis: Rh D negative women with no anti-D antibodies– Incidence of a positive Kleihauer test

Study ID Level of evidence [a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome	Results			
						RAADP n/N (%)	No RAADP n/N (%)	Risk estimate (95% CI)	*Statistical significance* *p*-value Heterogeneity [b]
RAADP (1 or 2 doses) vs no RAADP									
McBain 2015 Level II *Low*	N = 1884 (1 trial) (Huchet 1987)	Rh negative women without anti-D antibodies at 28 weeks' gestation	Obstetric and maternity (France)	500 IU at Week 28 and 34 vs no RAADP	Incidence of a positive Kleihauer test (32 to 35 weeks' gestation)	39/927 (4.21)	67/957 (7.00)	RR 0.60 (0.41, 0.88)	*Favours RAADP* *p* = 0.0094
McBain 2015 Level II *Low*	N = 1189 (1 trial) (Huchet 1987)	Rh negative women without anti-D antibodies at 28 weeks' gestation	Obstetric and maternity (France)	500 IU at Week 28 and 34 vs no RAADP	Incidence of a positive Kleihauer test (at birth of Rh positive infant)	73/599 (12.19)	119/590 (20.17)	RR 0.60 (0.46, 0.79)	*Favours RAADP* *p* = 0.00023
McBain 2015 Level II *Low*	N = 1189 (1 trial) (Huchet 1987)	Rh negative women without anti-D antibodies at 28 weeks' gestation	Obstetric and maternity (France)	500 IU at Week 28 and 34 vs no RAADP	Incidence of positive Kleihauer test (Kleihauer > 1/10,000, Rh positive infant)	31/599 (5.18)	32/590 (5.42)	RR 0.95 (0.59, 1.54)	*No significant difference* *p* = 0.85

CI, confidence interval; hrs, hours; IU, international units; M-H, Mantzel-Hentzel; NHMRC, National Health and Medical Research Council; NR, not reported; OR, odds ratio; RR, relative risk; RAADP, routine antenatal anti-D prophylaxis; UK, United Kingdom; US, United States

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The risk of bias of the included primary studies is based on the quality assessment reported by the systematic review authors.

b. Only applicable to Level I studies with formal meta-analysis. Heterogeneity defined as follows: (i) no significant heterogeneity if Phet >0.1 and I^2 <25%; (ii) mild heterogeneity if I^2 <25%; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity I^2 >50%.

5.2.4.3 Adverse neonatal events

One Level II study (Huchet 1987) and three Level III studies (Bowman 1987, Tovey 1983, Koelewjin 2008) provided limited data on adverse neonatal events relating to RAADP. A summary of results is provided in Table 5-10.

Huchet 1987 reported one case of neonatal jaundice among a neonate born to an Rh D negative women who had received RAADP compared with four cases among neonates born to women who did not receive RAADP (0.11% vs 0.42%; RR 0.26; 95% CI 0.03, 2.30; *p* = 0.22); *GRADE: low quality evidence*.

Both Tovey 1984 and Bowman 1987 reported several cases of treatment related to haemolytic disease of the fetus and newborn (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.

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]). 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). When they excluded cases in which the history of postnatal and antenatal prophylaxis was unknown, an effect favouring RAADP was observed (RR 0.51, 95% CI 0.9, 0.92; *p* = NR); *GRADE: very low quality evidence*. No HDFN perinatal mortality was reported in either group.

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).

Table 5-10 Results for *routine* antenatal prophylaxis with Rh D immunoglobulin versus placebo or no *routine* antenatal prophylaxis: Rh D negative women with no anti-D antibodies – Adverse neonatal events

Study ID Level of evidence [a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting Location	Comparison	Outcome	Results			
						RAADP n/N (%)	No RAADP n/N (%)	Risk estimate (95% CI)	*Statistical significance* *p*-value Heterogeneity [b]
McBain 2015 Level I *Low*	N = 1882 (1 trial) (Huchet 1987)	Rh negative women without anti-D antibodies at 28 weeks' gestation	Obstetric and maternity (France)	500 IU at GW 28 and 34 vs no RAADP	Neonatal morbidity (jaundice)	1/927 (0.11)	4/955 (0.42)	RR 0.26 (0.03, 2.30)	*No significant difference* *p* = 0.22
	No studies identified				Neonatal morbidity (other)	No data	No data	No data	No data
Pilgrim 2009 Level III *Moderate*	N = NR (1 study) (Tovey 1983)	Rh D negative pregnant women	Obstetric and maternity (UK)	2 x 500 IU at GW 28 and 34 vs no RAADP * all received further dose at or within 72 hrs of delivery of a positive infant	Treatment related to HDFN in first pregnancy	NR	2/18 (11)	NR	NR
	N = NR (1 study) (Bowman 1987)			1 x 1500 IU at GW 28 vs no RAADP * all received further dose at or within 72 hrs of delivery of a positive infant	Treatment related to HDFN in subsequent pregnancy	NR	7/17 (41)	NR	NR
	N = NR (1 study) (Tovey 1983)			2 x 500 IU at GW 28 and 34 vs no RAADP * all received further dose at or within 72 hrs of delivery of a positive infant	Treatment related to HDFN in subsequent pregnancy	NR	3/11 (27)	NR	NR

Study ID Level of evidence [a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting Location	Comparison	Outcome	Results			
						RAADP n/N (%)	**No RAADP n/N (%)**	**Risk estimate (95% CI)**	***Statistical significance p*-value Heterogeneity [b]**
Koelewijn 2008 Level III-3 *Moderate*	N = 21 221	Non-sensitised Rh D negative pregnant women *primiparae	Obstetrics and maternity (Netherlands)	1 x 1000 IU at GW 30 vs no RAADP *all received further dose after delivery of a positive infant	Prevalence of severe HDFN (detected at Week 12) [c]	12/12576 *0.10% (0.00, 0.15)*	14/8645 *0.16% (0.08, 0.25)*	RR 0.59 (0.00, 1.50)	*No significant difference* *p* = NR
	N = 20 330				Prevalence of severe HDFN (detected at Week 30) [c]	1/11 519 *0.01% (0, 0.03)*	6/8811 *0.07% (0.01, 0.12)*	RR 0.13 (0.00, 0.68)	*Favours RAADP* *p* = NR
	N = NR				Prevalence of severe HDFN (detected at Week 12 or Week 30) [c]	13/NR *0.10% (0.05, 0.16)*	20/NR *0.23% (0.13, 0.33)*	RR 0.45 (0.10, 1.08)	*No significant difference* *p* = NR
	N = NR					0.11%	0.22%	RR 0.51 (0.09, 0.92)	*Favours RAADP* *p* = NR
						excluding cases with postnatal (n = 13) and antenatal (n = 6) prophylaxis status unknown			
Koelewijn 2008 Level III-3 *Moderate*	N = 21 221	Non-sensitised Rh D negative pregnant women *primiparae	Obstetrics and maternity (Netherlands)	1 x 1000 IU at GW 30 vs no RAADP *all received further dose after delivery of a positive infant	Risk of developing HDFN once sensitisation occurred (detected at GW 12)	12/39 (30.8%)	14/58 (24.1%)	M-H Fixed RR 1.27 (0.66, 2.46)[d]	*No significant difference* *p* = NR
	N = 20 330				Risk of developing HDFN once sensitisation occurred (detected at GW 30)	1/29 (3.45%)	6/21.5 (27.9%)	M-H Fixed RR 0.13 (0.02, 0.98)[d]	*Favours RAADP* *p* = 0.02
	N = NR				Risk of developing HDFN once sensitisation occurred (detected at GW 12 or GW 30)	13/68 (19%)	20/79.5 (25%)	M-H Fixed RR 0.76 (0.41, 1.42)[d]	*No significant difference* *p* = NR

CI, confidence interval; GW, gestational week; hrs, hours; HDFN, haemolytic disease of the fetus and newborn; IU, international units; IUT, intrauterine transfusion; M-H, Mantzel-Hentzel; NHMRC, National Health and Medical Research Council; NR, not reported; OR, odds ratio; RAADP, routine antenatal anti-D prophylaxis; RCT, randomised controlled trial; RR, relative risk; UK, United Kingdom; vs, versus

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The risk of bias of the included primary studies is based on the quality assessment reported by the systematic review authors.

b. Only applicable to Level I studies with formal meta-analysis. Heterogeneity defined as follows: (i) no significant heterogeneity if P_{het} >0.1 and I^2 <25%; (ii) mild heterogeneity if I^2 <25%; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity I^2 >50%.

c. Defined as perinatal mortality, the need for IUT, and/or the need for exchange transfusion attributed to anti-D immunisation.

d. Calculated post-hoc using RevMan 5.3.

5.2.4.4 Adverse maternal events attributed to Rh D immunoglobulin

No studies identified.

Table 5-11 Results for *routine* antenatal prophylaxis with Rh D immunoglobulin versus placebo or no *routine* antenatal prophylaxis: Rh D negative women with no anti-D antibodies – Adverse maternal events

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting Location	Comparison	Outcome	Results			
						RAADP n/N (%)	No RAADP n/N (%)	Risk estimate (95% CI)	*Statistical significance* *p-value* Heterogeneity[b]
RAADP (1 dose) vs no RAADP									
McBain 2015 Level I *Low*	No studies identified	Rh negative women without anti-D antibodies at 28 weeks' gestation	Obstetric and maternity (any)	RAADP (1 dose) vs no RAADP	Maternal adverse events	No data	No data	No data	No data
RAADP (2 dose) vs no RAADP									
McBain 2015 Level I *Low*	No studies identified	Rh negative women without anti-D antibodies at 28 weeks' gestation	Obstetric and maternity (any)	RAADP (1 or 2 doses) vs no RAADP	Maternal adverse events	No data	No data	No data	No data
Pilgrim 2009 Level III *Moderate*	N = NR (1 study) (MacKenzie 2004)	Rh D negative pregnant women	Obstetric and maternity (UK)	2 x 500 IU at GW 28 and 34 vs no RAADP * all received further dose at or within 72 hrs of delivery of a positive infant	Mild pain, soreness and itching at injection site	A few cases reported			No serious adverse events reported by any studies (qualitative)
Pilgrim 2009 Level III *Moderate*	N = NR (1 study) (Bowman 1987)	Rh D negative pregnant women	Obstetric and maternity (UK)	2 x 500 IU at GW 28 and 34 vs no RAADP * all received further dose at or within 72 hrs of delivery of a positive infant	Marked flushing and mild chest discomfort that disappeared within 30 seconds without the use of medication	2 out of 3733 women given WinRho either antenatally or postpartum. Noted by the study authors that the batch contained an unacceptable level of moisture and aggregated IgG.			No serious adverse events reported by any studies (qualitative)

CI, confidence interval; GW, gestational week; hrs, hours; IU, international units; M-H, Mantzel-Hentzel; NHMRC, National Health and Medical Research Council; NR, not reported; OR, odds ratio; RAADP, routine antenatal anti-D prophylaxis; RR, relative risk; RCT, randomised controlled trial; UK, United Kingdom; vs, versus

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The risk of bias of the included primary studies is based on the quality assessment reported by the systematic review authors.

b. Only applicable to Level I studies with formal meta-analysis. Heterogeneity defined as follows: (i) no significant heterogeneity if P_{het} >0.1 and I^2 <25%; (ii) mild heterogeneity if I^2 <25%; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity I^2 >50%.

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

5.2.5 Summary of evidence

Three systematic reviews (McBain 2015, Pilgrim 2009, Turner 2012) were identified in the systematic review and hand-searching process that searched for head-to-head comparisons of one-dose versus two-dose RAADP regimes. None of the reviews identified any published evidence. Turner 2012 provided an assessment based on expert opinion. McBain 2015 noted an ongoing RCT (ACTRN12613000661774).

Assuming any relevant primary studies had been identified in the included systematic reviews, the systematic screen of Level II and Level III studies was limited to studies published after the review by Pilgrim 2009. The search for identified one conference abstract (Pennell 2017) linked to the ongoing trial identified by McBain 2015. The study authors were contacted to elicit further information, and it was noted that a publication reporting final results is under final review.

5.2.5.1 Level I

None of the included systematic reviews (McBain 2015, Pilgrim 2009, Turner 2012) identified any head-to-head studies comparing one-dose RAADP with two-dose RAADP. Turner 2012 provided an assessment based on expert opinion. The details of these studies are outlined in Section 5.2.3.

5.2.5.2 Level II

One ongoing RCT (Pennell 2017; trial ID: ACTRN12613000661774) was identified that compared a one-dose versus two-dose regime of RAADP. The study aims to evaluate detectable anti-D antibodies at delivery and compliance. The characteristics of this study are summarised in Table 5-12.

Pennell 2017 is an Australian study comparing two doses of Rh(D) Immunoglobulin-VF (625 IU) administered at 28 and 32 weeks' gestation with a single dose (1500 IU) administered at 28 weeks' gestation. Recruitment of pregnant Rh D negative women who have a negative antibody screen has been completed, with the date of last data collection noted on ANZCTR as 30 November 2016. Pennell 2017 did not report data relating to the prespecified outcomes for this review, but, given the lack of available head-to head data a review of the outcomes relating to serum anti-D levels were reviewed.

Table 5-12 Characteristics and quality of Level II evidence: RAADP (1-dose) versus RAADP (2-dose)

Study ID	Study type *Risk of bias*	Population	Intervention	Comparator	Outcomes
Pennell 2017	RCT *High*	Rh D negative pregnant women who are not alloimmunised N=280	Rh(D) Immunoglobulin-VF 2 x 625 IU IM at 28 and 32 weeks' gestation n = NR 2013–2016, Subiaco WA	Rh(D) Immunoglobulin-VF 1 x 1500 IU IM at 28 weeks' gestation n = NR 2013–2016, Subiaco WA	Serum anti-D levels at delivery

IU, international units; NR, not reported; RCT, randomised controlled trial

5.2.5.3 Level III

The literature search did not identify any Level III studies comparing one- or two-dose RAADP regimes that met the PICO criteria for this review. One prospective observational study (MacKenzie

2011) was identified, however, the study only reported compliance to the protocol, and did not report sensitisation rates.

5.2.6 Results

The systematic review did not identify any evidence relating to a comparison of one- versus two-dose treatment regimens with regards to the incidence of Rh D alloimmunisation, the incidence of a positive test for FMH or adverse neonatal or maternal events associated with Rh D IgG administration.

A GRADE evidence profile summarising the evidence for single-dose and two-dose regimens of universal *routine* antenatal prophylaxis with Rh D immunoglobulin is provided in Appendix 6.

5.2.6.1 Incidence of Rh D alloimmunisation

No studies identified

5.2.6.2 Incidence of a positive test for fetomaternal haemorrhage

No studies identified

5.2.6.3 Adverse neonatal events

No studies identified

5.2.6.4 Adverse maternal events attributed to Rh D immunoglobulin

No studies identified

5.2.6.5 Additional outcomes

Serum anti-D antibody levels

One Level II study provided limited data relating to serum anti-D antibody levels in Rh D negative pregnant women. A summary of the results from this study is provided in Table 5-13.

Pennell 2017 observed that the number of women with no anti-D antibody present at birth was higher in those women who received the one-dose regime compared to the two-dose regime (45.2% vs 14.2%; OR 5.0; 95% CI not reported; $p < 0.001$). 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.

Table 5-13 Results for *routine* antenatal prophylaxis with Rh D immunoglobulin (1 dose) versus *routine* antenatal prophylaxis with Rh D immunoglobulin (2 doses): Rh D negative women with no anti-D antibodies

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						RAADP (1 x) n/N (%) *% (95%CI)*	RAADP (2 x) n/N (%) *% (95%CI)*	Risk estimate (95% CI)	*Statistical significance* *p-value* Heterogeneity[b]
RAADP (1 dose) vs RAADP (2 doses)									
Pennell 2017 Level II *High*	N = 280	Rh D negative pregnant women	Obstetric and maternity (Australia)	1 x 1500 IU at Week 28 vs 2 x 625 IU at GW 28 and 34	Proportion with undetectable anti-D (at birth)	NR (45.2)	NR (14.2)	OR 5.0 (NR)	*Favours two-dose RAADP* $p < 0.001$

CI, confidence interval; GW, gestational week; IU, international units; OR, odds ratio; RAADP, routine antenatal anti-D prophylaxis; NHMRC, National Health and Medical Research Council; NR; not reported; RCT, randomised control trial; vs, versus

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The risk of bias of the included primary studies is based on the quality assessment reported by the systematic review authors.

b. Only applicable to Level I studies with formal meta-analysis. Heterogeneity defined as follows: (i) no significant heterogeneity if $P_{het} > 0.1$ and $I^2 < 25\%$; (ii) mild heterogeneity if $I^2 < 25\%$; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity $I^2 > 50\%$.

5.3 Question 2 - Universal sensitising event prophylaxis in the first trimester

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/without a curette) – does universal first trimester sensitising event prophylaxis with Rh D immunoglobulin prevent Rh D alloimmunisation?

5.3.1 Background

Question 2 is intended to examine whether universal administration of sensitising event prophylaxis in the first trimester should include the following additional events: abdominal trauma, molar pregnancy, threatened miscarriage and medical termination of pregnancy (intervention question).

5.3.2 Methods

One comparison was assessed in this review: (1) prophylactic Rh D immunoglobulin in response to a first trimester sensitising event versus placebo or no prophylactic Rh D immunoglobulin in response to a first trimester sensitising event

Specific, potential sensitising events included the following:

- abdominal trauma
- molar pregnancy
- ectopic pregnancy
- spontaneous miscarriage
- threatened miscarriage
- medical termination of pregnancy (with/without a curette)

Because this is an intervention question, the levels of evidence are as described in Section 5.2.1. For this question, Level I, Level II, or Level III evidence was considered appropriate for inclusion. Studies using other designs (i.e. Level IV) were excluded because it is not possible to attribute observed changes in outcomes to the intervention. There was no restriction on publication date for this question.

Assuming any relevant primary studies had been identified in the included Level I studies, the systematic screen of Level II and Level III studies was limited to studies published 2012 onwards.

5.3.3 Summary of evidence

The systematic review and hand-searching process identified two systematic reviews (NCCWCH, 2012, Karanth et al., 2013) that evaluated the effectiveness of prophylactic Rh D immunoglobulin in response to a first trimester sensitising event. The reviews included one Level II study (Visscher et al., 1972) and two Level III studies meeting the PICO criteria (Gavin, 1972, Simonovits et al., 1974).

All three studies identified by the included systematic reviews were published prior to the previous 2003 Anti-D Guidelines. No studies evaluating the use of prophylactic Rh D immunoglobulin in women with first trimester ectopic pregnancy, threatened miscarriage, or molar pregnancy were identified.

A matrix illustrating the overlap of studies included in each review is provided in Table 5-14.

No additional Level II or Level III studies were identified.

Table 5-14 Overlap table showing primary studies in the included Level I reviews: first trimester sensitising event prophylaxis versus placebo or no first trimester sensitising event prophylaxis

	Review ID	
Study ID	**Karanth 2013**	**NCCWCH 2012**
Level II		
Visscher 1972	✓	✓
Level III		
Simonovits 1974		✓
Gavin 1972		✓

NCCWCH, National Collaborating Centre for Women's and Children's Health

5.3.3.1 Level I

Two systematic reviews (NCCWCH, 2012, Karanth et al., 2013) were identified that assessed the evidence relating to sensitising event prophylaxis in Rh D negative women. The characteristics of these studies and the relevant outcomes assessed are summarised in Table 5-15.

Karanth 2013 was a systematic review of Level II studies. The eligible population was Rh D negative mothers who have had spontaneous miscarriage before 24 weeks' gestation, including those who had medical evacuation of the uterus and early pregnancy complications (e.g. ectopic and molar pregnancy). The search date was from database inception to 31 December 2012 and one eligible study was identified (Visscher 1972).

The NCCWCH 2012 review systematically examined the evidence relating to the management of ectopic pregnancy, threatened miscarriage and miscarriage, and included women receiving medical termination of pregnancy. The review has been used to inform NICE Guidance relating to the diagnosis and management of ectopic pregnancy and miscarriage (NG126, updated in 2014 and 2019). The Guidelines identified eight studies, of which three (Visscher 1972, Gavin 1972, Simonovits 1974) met the PICO criteria for this systematic review. The remaining five papers (Walsh et al., 1970, Murray et al., 1971, Murray et al., 1972, Katz et al., 1973, Simonovits et al., 1980) were noncomparative, descriptive studies reporting the incidence of alloimmunisation in women who did not receive Rh D immunoglobulin prophylaxis following first trimester obstetric events. The search was conducted on 8 February 2012 with an updated search conducted on 8 July 2014 (NICE, 2014). No additional citations were identified. No meta-analysis was conducted by the authors.

Table 5-15 Characteristics and quality of Level I evidence: first trimester sensitising event prophylaxis versus placebo or no first trimester sensitising event prophylaxis

Review ID	Study type *Risk of bias*	Population	Intervention	Comparator	Outcomes
NCCWCH 2012	Systematic review of any relevant studies, and clinical practice guidelines *Moderate*	Women with spontaneous miscarriage, ectopic pregnancy or medical termination of pregnancy N = NR (8 studies)	Any timing or dose of Rh D immunoglobulin n = 136 (3 studies)	No therapy or placebo n = 210 (3 studies)	Rh D alloimmunisation
Karanth 2013	Systematic review and meta-analysis of Level II studies *Low*	Rh negative women with spontaneous miscarriage before 24 weeks' gestation, including medical evacuations of the uterus, ectopic and molar pregnancy N = 48 (1 study)	Any timing or dose of Rh D immunoglobulin n = 19	No therapy or placebo n = 29	Rh D alloimmunisation 6 months following spontaneous miscarriage In subsequent pregnancies Adverse neonatal events Positive Kleihauer test

NCCWCH, National Collaborating Centre for Women's and Children's Health; NR, not reported;

5.3.3.2 Level II

No additional Level II studies were identified in the search that examined Rh D immunoglobulin administration following potential first trimester sensitising events.

The RCT by Visscher 1972 compared the effectiveness of 1500 IU Rh immunoglobulin with placebo within 72 hours of spontaneous complete miscarriage or operative termination of an incomplete miscarriage between eight to 24 weeks' gestation. The study details are outlined in Table 5-16

Table 5-16 Characteristics and quality of Level II evidence: first trimester sensitising event prophylaxis versus placebo or no first trimester sensitising event prophylaxis

Review ID	Study type *Risk of bias*	Population	Intervention	Comparator	Outcomes
Identified and assessed by Karanth 2013 and NCCWCH 2012					
Visscher 1972	RCT, MC *High*	Rh D negative women who have had spontaneous miscarriage at 8 to 24 weeks' gestation N = 57[a]	1 x 1500 IU IM within 72 hours of event n = 19[b] 1 Jul 1968–1 Mar 1971	Homogenous gamma globulins IM within 72 hours of event n = 29[c] 1 Jul 1968–1 Mar 1971	Incidence of Rh D alloimmunisation 6 months following spontaneous miscarriage In subsequent pregnancies

GW, gestational week; IM, intramuscular; IU, international units; MC, multicentre; RCT, randomised controlled trial

a. 9/57 women dropped out preintervention.

b. 14/19 had dilation and curettage, 5/19 had spontaneous miscarriage.

c. 25/29 had dilation and curettage, 4/29 had spontaneous miscarriage, an additional nine were included as a case series of women who received no intervention.

5.3.3.3 Level III

No additional Level III studies were identified in the search that examined Rh D immunoglobulin administration following potential first trimester sensitising events.

Two studies were identified by NCCWCH 2012 and are outlined in Table 5-17.

Table 5-17 Characteristics and quality of Level III evidence: first trimester sensitising event prophylaxis versus placebo or no first trimester sensitising event prophylaxis

Review ID	Study type *Risk of bias*	Population	Intervention	Comparator	Outcomes
Identified and assessed by NCCWCH 2012					
Gavin 1972	Prospective Coh, MC *High*	Rh D negative women with no detectable antibodies and have had medical termination of pregnancy or surgical treatment of miscarriage N = 57[a]	Rhogam 1 x dose NR n = 21 1 Nov 1969–15 Aug 1970 USA	1 x placebo n = 29 1 Nov 1969–15 Aug 1970 USA	Incidence of Rh D alloimmunisation 4 months following treatment
Simonovits 1974	Prospective Coh *High*	Rh D negative women secundigravidae, whose previous pregnancy ended by first trimester induced abortion N = 387[b]	1 x 250 IU n = 96[c] 1972–1973 Hungary	n = 301[d] 1972–1973 Hungary	Incidence of Rh D alloimmunisation 6 months following birth

Coh, cohort; IM, intramuscular; IU, international units; MC, multicentre; NR, not reported

a. Of these three women refused to participate, nine were lost to followup and one was found to have an Rh negative husband. The nine that were lost to followup were replaced by women from another facility. Paternal genotypes were obtained in 50% of the couples.

b. 156 of these pregnancies ended in delivery and did not receive Rh D IgG, therefore the population of interest in 241.

c. Of women in their second pregnancy, 53/96 had an induced abortion, 3/96 had a miscarriage, and 39/36 pregnancies ended in delivery.

d. Of women in their second pregnancy, 121/301 had an induced abortion, 24/301 had a miscarriage, and 156/301 pregnancies ended in delivery.

5.3.4 Results

A GRADE evidence profile summarising the evidence for universal sensitising event prophylaxis in the first trimester with Rh D immunoglobulin in Rh D negative pregnant women with no preformed anti-D antibodies is provided in Appendix 6.

5.3.4.1 Incidence of Rh D alloimmunisation

Three studies (Gavin 1972 , Simonovits 1974, Visscher 1972) assessed whether prophylaxis with Rh D immunoglobulin prevented Rh D alloimmunisation after a first trimester sensitising event. A summary of the results from these studies is provide in Table 5-18.

All three studies reported data on women who had either a miscarriage or medical termination of pregnancy, but there was no evidence in women with a threatened miscarriage, ectopic pregnancy, 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 to measure potential Rh D alloimmunisation (Enzyme-Coombs, Indirect Coombs), and different criteria with regards to the included sensitising events (spontaneous miscarriage, therapeutic evacuation). All included studies were small, and unlikely to be sufficiently powered to detect meaningful differences between comparator groups.

Incidence 4-6 months after sensitising event

Two studies (Visscher 1972, Gavin 1972) reported the incidence of Rh D alloimmunisation between 4 and 6 months after miscarriage (spontaneous or incomplete) or medical termination of pregnancy. The RCT by Visscher 1972 reported no evidence of Rh D alloimmunisation six months after the intervention, as measured by an Enzyme-Coombs test (0/19 in the in the intervention group vs 0/29 in the placebo group); *GRADE: very low quality evidence*.

The prospective cohort study by Gavin 1972 report no cases of Rh D alloimmunisation (measured by the Indirect Coombs test) in the intervention group (0/21) compared with two cases in the placebo group (2/36). This did not reach statistical significance (RR 0.34; 95% CI 0.02, 6.69, p = 0.48); *GRADE: very low quality evidence*.

Incidence in a subsequent pregnancy

Two studies (Visscher 1972, Simonovits 1974) reported the incidence of alloimmunisation in a subsequent pregnancy after miscarriage (spontaneous or incomplete) or medical termination of pregnancy.

The study by Visscher 1972 reported no Rh D alloimmunisation in a subsequent nine Rh D positive pregnancies (6/19 from the intervention group, and 3/29 from the placebo group); *GRADE: very low quality evidence*. It was not clear if any of the other participants had delivered an Rh D positive neonate beyond the followup period.

Simonovits 1974 recorded three Rh D alloimmunisations among 241 Rh D negative women after medical termination of pregnancy (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); *GRADE: very low quality evidence*.

Table 5-18 Results for first trimester sensitising event Rh D immunoprophylaxis versus placebo or no first trimester sensitising event Rh D immunoprophylaxis: Rh D negative women with no preformed anti-D antibodies – Incidence of Rh D alloimmunisation (any timepoint)

Study ID Level of evidence [a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						Rh D IgG n/N (%) *% (95%CI)*	Placebo or no Rh D IgG n/N (%) *% (95%CI)*	Risk estimate (95% CI)	*Statistical significance* *p*-value Heterogeneity [b]
Spontaneous miscarriage, incomplete miscarriage, or therapeutic evacuation of the uterus									
NCCWCH 2012 Level II *Moderate*	N = 48 (1 study) (Visscher 1972)[c]	Rh negative women who have experienced spontaneous miscarriage and/or therapeutic evacuation	Obstetrics and maternity (US)	Prophylactic Rh D IgG (1500 IU) after sensitising event vs no Rh D IgG	Incidence of Rh D alloimmunisation (4–6 months following miscarriage/abortion) - measured by Enzyme-Coombs screening procedure	0/19 (0)	0/29 (0)	Not estimable (no events)	Not estimable
NCCWCH 2012 Level III *Moderate*	N = 57 (1 study) (Gavin 1972)	Rh negative women who have experienced incomplete miscarriage or had medical termination of pregnancy	Obstetrics and maternity (US)	Prophylactic Rh D IgG (doe not specified) after sensitising event vs no Rh D IgG	Incidence of Rh D alloimmunisation (4–6 months following miscarriage/abortion) - measured by Indirect Coombs test	0/21 (0)	2/36 (5.6)	RR 0.34 (0.02, 6.69) 37 fewer per 1000 (54 fewer to 316 more)	*No significant difference*
NCCWCH 2012 Level III *Moderate* Karanth 2013 Level III *Low*	N = 9 (1 study) (Visscher 1972)[c]	Rh negative women who have experienced miscarriage or therapeutic evacuation	Obstetrics and maternity (US)	Prophylactic Rh D IgG (1500 IU) after sensitising event vs no Rh D IgG	Incidence of Rh D sensitisation (subsequent pregnancy) - measured by Enzyme-Coombs screening procedure	0/3 (0)	0/6 (0)	Not estimable	Not estimable

Study ID Level of evidence [a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						Rh D IgG n/N (%) *% (95%CI)*	Placebo or no Rh D IgG n/N (%) *% (95%CI)*	Risk estimate (95% CI)	*Statistical significance* *p*-value Heterogeneity [b]
NCCWCH 2012 Level III *Moderate*	N = 241 (1 study) (Simonovitis 1974)	Rh negative women who have experienced medical termination of pregnancy	Obstetrics and maternity (Hungary)	Prophylactic Rh D IgG (250 IU) after sensitising event vs no Rh D IgG	Incidence of Rh D sensitisation (subsequent pregnancy) - measured by papain-treated cells (intervention), Indirect Coombs test and papain-treated cells or not reported (control)	1/96 (1.0)[d]	2/145 (1.4)	RR 0.76 (0.0, 8.21) 3 fewer per 1000 (from 13 fewer to 99 more)	*No significant difference*
Threatened miscarriage, ectopic pregnancy, molar pregnancy, abdominal trauma									
NCCWCH 2012 Level I *Moderate*	N = 0 (No comparative studies found)	Rh negative women who have experienced threatened miscarriage or ectopic pregnancy	No data	No data	No data	No data	No data	No data	No data

CI, confidence interval; IgG, immunoglobulin G; NHMRC, National Health and Medical Research Council; NCCWCH, National Collaborating Centre for Women's and Children's Health; RCT, randomised control trial; RR, relative risk; US, United States; vs, versus

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The risk of bias of included primary studies is based on the quality assessment reported by the systematic review authors.

b. Only applicable to Level I studies with formal meta-analysis. Heterogeneity defined as follows: (i) no significant heterogeneity if Phet >0.1 and I^2 <25%; (ii) mild heterogeneity if I^2 <25%; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity I^2 >50%.

c. Visscher 1972 compared 300 mcg Rh D immunoprophylaxis with placebo (IgG) in 48 women after miscarriage, with additional prospective case series of nine women who did not receive any prophylaxis.

d. Not clear if sensitisation occurred after medical termination of pregnancy. The woman delivered an Rh+ baby at the end of her second pregnancy and tested negative 6 months before birth; therefore she is likely to have been sensitised in her second, full-term pregnancy).

5.3.4.2 Incidence of a positive Kleihauer test

No studies identified

5.3.4.3 Adverse neonatal events

No studies identified

5.3.4.4 Adverse maternal events

No studies identified

Table 5-19 Results for first trimester sensitising event Rh D immunoprophylaxis versus placebo or no first trimester sensitising event Rh D immunoprophylaxis: Rh D negative women with no preformed anti-D antibodies – Incidence of a positive Kleihauer test

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						Rh D IgG n/N (%)	Placebo or no Rh D IgG n/N (%)	Risk estimate (95% CI)	*Statistical significance* *p*-value Heterogeneity[b]
Karanth 2013 Level I *Low*	N = 0 (0 studies)	Rh negative women who have experienced spontaneous miscarriage, therapeutic evacuation of the uterus, early pregnancy complications up to 24 weeks' gestation, irrespective of parity, ABO compatibility or size of fetomaternal haemorrhage.	Obstetrics and maternity (any)	Prophylactic Rh D IgG after sensitising event vs placebo or no Rh D IgG	Positive Kleihauer test (after miscarriage before 14 weeks' gestation)	No studies identified	No data	No data	No data
					Positive Kleihauer test (after miscarriage following 14 weeks' gestation)	No studies identified	No data	No data	No data

CI, confidence interval; IgG, immunoglobulin G; NHMRC, National Health and Medical Research Council; RCT, randomised control trial; vs, versus

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The quality of the included primary studies are based on the quality assessment reported by the systematic review authors.

b. Only applicable to Level I studies with formal meta-analysis. Heterogeneity defined as follows: (i) no significant heterogeneity if P_{het} >0.1 and I^2 <25%; (ii) mild heterogeneity if I^2 <25%; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity I^2 >50%.

Table 5-20 Results for first trimester sensitising event Rh D immunoprophylaxis versus placebo or no first trimester sensitising event Rh D immunoprophylaxis: Rh D negative women with no preformed anti-D antibodies – Adverse neonatal events

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome	Results			
						Rh D IgG n/N (%)	Placebo or no Rh D IgG n/N (%)	Risk estimate (95% CI)	*Statistical significance* *p*-value Heterogeneity[b]
Karanth 2013 Level I *Low*	N = 0 (0 studies)	Rh negative women who have experienced spontaneous miscarriage, therapeutic evacuation of the uterus, early pregnancy complications up to 24 weeks' gestation, irrespective of parity, ABO compatibility or size of fetomaternal haemorrhage.	Obstetrics and maternity (any)	Prophylactic Rh D IgG after sensitising event vs placebo or no Rh D IgG	Health of infant in subsequent pregnancy	No studies identified	No data	No data	No data
					Need for increased fetal surveillance for suspected isoimmunisation in subsequent pregnancy	No studies identified	No data	No data	No data

CI, confidence interval; IgG, immunoglobulin G; NHMRC, National Health and Medical Research Council; RCT, randomised control trial; vs, versus

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The quality of the included primary studies are based on the quality assessment reported by the systematic review authors.

b. Only applicable to Level I studies with formal meta-analysis. Heterogeneity defined as follows: (i) no significant heterogeneity if P_{het} >0.1 and I^2 <25%; (ii) mild heterogeneity if I^2 <25%; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity I^2 >50%.

Table 5-21 Results for first trimester sensitising event Rh D immunoprophylaxis versus placebo or no first trimester sensitising event Rh D immunoprophylaxis: Rh D negative women with no preformed anti-D antibodies – Adverse maternal events

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome	Results			
						Rh D IgG n/N (%)	Placebo or no Rh D IgG n/N (%)	Risk estimate (95% CI)	*Statistical significance* *p*-value Heterogeneity[b]
Karanth 2013 Level I *Low*	N = 0 (0 studies)	Rh negative women who have experienced spontaneous miscarriage, therapeutic evacuation of the uterus, early pregnancy complications up to 24 weeks' gestation, irrespective of parity, ABO compatibility or size of fetomaternal haemorrhage.	Obstetrics and maternity (any)	Prophylactic Rh D IgG after sensitising event vs placebo	Adverse reactions	No studies identified	No data	No data	No data

CI, confidence interval; IgG, immunoglobulin G; NHMRC, National Health and Medical Research Council; RCT, randomised control trial; vs, versus

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The quality of the included primary studies are based on the quality assessment reported by the systematic review authors.

b. Only applicable to Level I studies with formal meta-analysis. Heterogeneity defined as follows: (i) no significant heterogeneity if P_{het} >0.1 and I^2 <25%; (ii) mild heterogeneity if I^2 <25%; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity I^2 >50%.

5.4 Question 3 - *Targeted* routine antenatal or sensitising event prophylaxis

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 a 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 noninvasive prenatal screening to identify fetal Rh D status?

5.4.1 Background

Question 3 was intended to examine whether targeted administration can replace universal administration of Rh D IgG prophylaxis during pregnancy, thereby reducing the number of women who need to receive Rh D immunoglobulin. Because targeted Rh D immunoprophylaxis relies on the identification of an Rh D positive fetus in pregnant women, a subquestion was included that focused on the diagnostic accuracy of noninvasive prenatal tests (NIPT) for screening Rh D negative women with no preformed anti-D antibodies.

5.4.2 Methods

For the main question, one comparison was addressed in this review:

1. targeted Rh D immunoglobulin prophylaxis (routine antenatal or sensitising event) versus universal anti-D prophylaxis (routine antenatal or sensitising event)

The population of interest was Rh D negative women who were to receive Rh D immunoglobulin as part of routine antenatal care or who had experienced a first trimester sensitising event. The critical measure of effectiveness was the incidence of Rh D alloimmunisation.

As this is a screening intervention question, the levels of evidence are as described in Section 5.2.2. For this question, Level I, Level II, or Level III evidence was considered appropriate for inclusion. Studies using other designs (i.e. Level IV) were excluded because it is not possible to reliably attribute observed changes in outcomes to the intervention.

For the diagnostic subquestion the comparison of interest was:

2. any NIPT for fetal Rh D status versus postnatal testing of cord blood (or other neonatal sample) or any other NIPT.

Because the subquestion is a diagnostic accuracy question, the levels of evidence are as follows:

- Level I – a systematic review of two or more Level II studies
- Level II – a study of test accuracy with an independent, blinded comparison with a valid reference standard among consecutive persons with a defined clinical presentation
- Level III-1 – a study of test accuracy with an independent, blinded comparison with a valid reference standard among consecutive persons with a defined clinical presentation among nonconsecutive persons with a defined clinical presentation

- Level III-2 – a comparison with reference standard that does not meet the criteria required for the above study types
- Level III-3 – a diagnostic case-control study
- Level IV – Study of diagnostic yield (no reference standard)

For the subquestion, Level I, Level II, or Level III evidence was considered appropriate for inclusion. Studies using other designs (i.e. Level IV) were excluded.

Targeted Rh D immunoprophylaxis versus universal Rh D immunoprophylaxis

5.4.3 Summary of evidence

The systematic review and hand-searching process identified one systematic review (Saramago et al., 2018) that searched for evidence regarding the comparative effectiveness of targeted antenatal Rh D immunoprophylaxis against universal routine Rh D immunoprophylaxis. The report did not identify any head-to-head studies of targeted versus routine antenatal prophylaxis regimes that met the criteria for this review.

Assuming any relevant primary studies had been identified in Saramago 2018, the systematic screen of Level II and Level III studies was limited to studies published 6 months prior to the literature search date of that review (2015 onwards).

No additional Level II or Level III studies were identified.

5.4.3.1 Level I

One systematic review (Saramago 2018) was identified in the literature review and hand-searching process that examined the effect of targeted RAADP compared with universal RAADP on the incidence of Rh D alloimmunisation. The characteristics of this study and the relevant outcomes assessed are summarised in Table 5-22. Clinical effectiveness results are discussed in Section 5.4.4, and the diagnostic accuracy results are discussed in Section 5.4.6.

Saramago 2018 was a published HTA report conducted for the NHS that examined the diagnostic accuracy of high-throughput NIPT and the clinical impacts of implementation of targeted antenatal prophylaxis to underpin an economic assessment. The literature search was conducted from database inception to February 2016. Seven observational studies were identified in the review of clinical effectiveness. Two studies (Banch Clausen 2016, Tiblad 2013) assessed the incidence of Rh D alloimmunisation in women receiving NIPT compared to controls (women who did not receive RAADP). The remaining five studies were single-armed, noncomparative cohort studies for women receiving NIPT only (Banch Clausen 2012, Damkjaer 2012, de Haas 2012, Grande 2013, Soothill 2015). None of the identified studies provided sufficient information to assess clinical effectiveness, therefore Saramago 2018 conducted a Monte Carlo simulation relevant to the UK health system based on data presented in each of the studies.

Table 5-22 Characteristics and quality of Level I evidence: targeted Rh D immunoprophylaxis versus universal Rh D immunoprophylaxis

Review ID	Study type *Risk of bias*	Population	Intervention	Comparator	Outcomes
Saramago 2018	SR of RCTs and observational studies *Moderate*	Pregnant women who are Rh D negative and not known to be sensitised to Rh D antigen	Targeted RAADP	Universal RAADP (no restrictions on dose, timing or mode of administration) or no antenatal prophylaxis	Incidence of Rh alloimmunisation (Simulation study)

RAADP, routine antenatal anti-D prophylaxis; RCT, randomised controlled trial; SR, systematic review

5.4.3.2 Level II

The literature search did not identify any RCTs comparing targeted routine antenatal or sensitising event Rh D immunoprophylaxis versus universal routine or sensitising event antenatal Rh D immunoprophylaxis.

5.4.3.3 Level III

The literature search did not identify any Level III studies comparing targeted routine antenatal or sensitising event Rh D immunoprophylaxis versus universal routine antenatal or sensitising event Rh D immunoprophylaxis.

5.4.4 Results

A GRADE evidence profile summarising the evidence for *targeted* routine antenatal prophylaxis with Rh D immunoglobulin in Rh D negative pregnant women with no preformed anti-D antibodies is provided in Appendix 6.

Given the limited evidence directly relating to the clinical effectiveness of NIPT and its impact on Rh D sensitisation rates, Saramago 2018 constructed a simulation model to examine the effectiveness of targeted Rh D IgG administration in unsensitised pregnant Rh D negative women. The model is 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 decision and the ultimate effect on health outcomes and costs. Only studies conducted in Bristol were used to estimate diagnostic performance, therefore the sensitivity of NIPT was 99.79% (95% CI 99.52, 99.01) and the specificity was 95.42% (95% CI 95.42, 92.84). The model did not examine the incidence of a positive test for fetomaternal haemorrhage, adverse neonatal events, or adverse maternal events.

The following clinical scenarios were considered[24]:

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

As no additional studies were identified in this review, the results of the model were considered.

The authors noted that the determination of the Rh D status of the fetus through NIPT may impact the administration of Rh D immunoglobulin following potentially sensitising events, routinely and at birth. In addition, NIPT results may affect postpartum testing (for example cord blood typing and FMH). As the test is not perfect, women who receive an inconclusive or false-positive test result will still receive unnecessary Rh D immunoglobulin.

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

5.4.4.1 Incidence of Rh D alloimmunisation

The results from the simulation conducted by Saramago 2018 for the incidence of Rh D alloimmunisation are summarised in Table 5-23.

The model estimated 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 if women would receive Rh D IgG prophylaxis would increase the number of Rh D sensitisations by between 3 and 15 in 100,000 pregnancies if

[24] Assumptions that feed into the model are provided in their report, available at https://www.journalslibrary.nihr.ac.uk/hta/hta22130#/abstract.

postpartum cord blood testing is continued, or between 15 to 28 per 100,000 women if postpartum cord blood testing is withdrawn (and anti-D is given 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.

Table 5-23 Results for *targeted routine* antenatal or sensitising event prophylaxis in women with Rh D positive fetus versus *universal routine* antenatal or sensitising event prophylaxis: Rh D negative women with no preformed anti-D antibodies – Incidence of Rh D alloimmunisation (any timepoint)

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						Targeted RAADP n/N (%)	Universal RAADP n/N (%)	Risk estimate (95% CI)	Statistical significance *p*-value Heterogeneity[b]
Routine antenatal prophylaxis									
Saramago 2018 Level I *Moderate*	0 studies identified Monte Carlo simulation (10 million women)	Rh D negative pregnant women	Obstetrics and maternity (UK)	Target anti-D prophylaxis vs universal RAADP Assumes women who do not receive NIPT would still be offered RAADP[c]	Incidence of Rh D alloimmunisation (assumes cord blood serology used to guide postpartum anti-D)	284 per 100,000	281 per 100,000	3 additional sensitisations per 100,000	Assumes offering NIPT guided prophylaxis at GW28, cord serology at birth (if given) is 100% accurate, and there are no adverse events[d]
					Incidence of Rh D alloimmunisation (assume no postpartum anti-D to test negative women)	294 per 100,000	281 per 100,000	13 additional sensitisations per 100,000	
Saramago 2018 Level I *Moderate*	0 studies identified Monte Carlo simulation	Rh D negative pregnant women	Obstetrics and maternity (UK)	Target anti-D prophylaxis vs universal RAADP Assumes women who do not receive NIPT would NOT receive RAADP[c]	Incidence of Rh D alloimmunisation (assumes cord blood serology used to guide postpartum anti-D)	296 per 100,000	281 per 100,000	15 additional sensitisations per 100,000	Assumes offering NIPT guided prophylaxis at GW28 cord serology at birth (if given) is 100% accurate, and there are no adverse events
					Incidence of Rh D alloimmunisation (assume no postpartum anti-D to test negative women)	309 per 100,000	281 per 100,000	28 additional sensitisations per 100,000	
Sensitising event prophylaxis – 0 studies identified									

CI, confidence interval; NIPT, noninvasive prenatal testing; RAADP, routine antenatal anti-D prophylaxis; UK, United Kingdom;

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The risk of bias of the included primary studies is based on the quality assessment reported by the systematic review authors.

b. Only applicable to Level I studies with formal meta-analysis. Heterogeneity defined as follows: (i) no significant heterogeneity if P_{het} >0.1 and I^2 <25%; (ii) mild heterogeneity if I^2 <25%; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity I^2 >50%.

c. For any reason such as refusal (e.g. partner confirmed Rh D negative, religious belief), not requested or performed (e.g. missed appointment, insufficient sample), woman does not qualify (e.g. delivery/loss prior to GW28).

d. Data based on eight studies that assessed the diagnostic accuracy of high-throughput NIPT, seven studies assessing clinical effectiveness of anti-D prophylaxis, and three noncomparative studies assessing change in management after implementation of NIPT.

5.4.4.2 Utilisation of Rh D immunoglobulin

The results from the simulation conducted by Saramago 2018 and from other studies that reported data relating to the utilisation of Rh D immunoglobulin are summarised in Table 5-24.

Based on an assumed compliance of 99%, the simulation model estimated that use of NIPT to determine RAADP would reduce the number of women receiving Rh D immunoglobulin to between 62.7% and 65.9%. This corresponds to an estimated reduction in utilisation of Rh D immunoglobulin of between 33.1% and 36.9%. These results were sensitive to compliance, with the range in numbers 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 IgG prophylaxis would be reduced from 38.9% to between 4.5% and 5.7% and the number of women who would fail to receive prophylaxis would increase from an estimated 0.6% to between 1.2% and 3.2%.

The estimated one-third reduction in utilisation of Rh D immunoglobulin corresponds with the observed numbers reported by Soothill 2015 (29%) and Banch Clausen 2014 (37.1%), which were used to inform the simulation model; and also corresponds with that reported by Macher 2012, who observed an 38% reduction in utilisation of Rh D immunoglobulin in in a single centre in Spain.

5.4.4.3 Incidence of a positive test for fetomaternal haemorrhage

No studies identified.

5.4.4.4 Adverse neonatal events

No studies identified.

5.4.4.5 Adverse maternal events attributed to Rh D immunoglobulin

No studies identified.

Table 5-24 Results for *targeted routine* antenatal or sensitising event prophylaxis in women with Rh D positive fetus versus *universal routine* antenatal or sensitising event prophylaxis: Rh D negative women with no preformed anti-D antibodies – Utilisation of Rh D immunoglobulin (any timepoint)

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						Targeted RAADP n/N (%)	Universal RAADP n/N (%)	Risk estimate (95% CI)	Statistical significance *p*-value Heterogeneity[b]
Routine antenatal prophylaxis									
Saramago 2018 Level I *Moderate*	0 studies identified Monte Carlo simulation	Rh D negative pregnant women	Obstetrics and maternity (UK)	Target Rh D immunoprophylaxis vs universal RAADP Assumes women who do not receive NIPT would still be offered RAADP[c]	Utilisation of Rh D immunoglobulin (% Rh D negative women who receive)	65.9	99 *assumes 99% compliance	33.1% reduction in Rh D immunoglobulin use	
					Unnecessary administration of Rh D immunoglobulin (% women with Rh D negative fetus)	5.7	38.9		
					Missed beneficial administration of Rh D immunoglobulin (% women with Rh D positive fetus)	1.2	0.6		
Saramago 2018 Level I *Moderate*	0 studies identified Monte Carlo simulation	Rh D negative pregnant women	Obstetrics and maternity (UK)	Target Rh D immunoprophylaxis vs universal RAADP Assumes women who do not receive NIPT would NOT receive RAADP[c]	Utilisation of Rh D immunoglobulin (% Rh D negative women who receive)	62.7	99 *assumes 99% compliance	36.9% reduction in Rh D immunoglobulin use	
					Unnecessary administration of Rh D immunoglobulin (% women with Rh D negative fetus)	4.5	38.9		
					Missed administration of Rh D immunoglobulin (% women with Rh D positive fetus)	3.2	0.6		

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Outcome (timing)	Results			
						Targeted RAADP n/N (%)	Universal RAADP n/N (%)	Risk estimate (95% CI)	Statistical significance *p*-value Heterogeneity[b]
Saramago 2019 Level I *Moderate*	N = 529 (Soothill 2015)	Rh D negative pregnant women GW 15–26	3 maternity hospitals, UK	Noncomparative	Utilisation of Rh D immunoglobulin	A 6% (95% CI 4, 8) reduction per month, equating to 29% reduction in total use within 6 months of implementation of NIPT.			This corresponded to 35% of Rh D negative women not receiving Rh D immunoglobulin unnecessarily.
	N = 2668 (Banch Clausen 2014)	Rh D negative pregnant women GW 25 (median)	Nationwide, Denmark		Utilisation of Rh D immunoglobulin	4706 (37.1%) Rh D negative women avoided unnecessary Rh D immunoglobulin within 2 years of NIPT screening program.			
	N = 302 (Grande 2013)	Rh D negative pregnant women GW 24–26	Single centre, Spain		Utilisation of Rh D immunoglobulin	5% women with Rh D negative fetus requested Rh D immunoglobulin despite NIPT.			
Macher 2012 Level II *High*	N = 2127	Rh D negative pregnant women GW 10–28	Single centre, Spain	Noncomparative	Utilisation of Rh D immunoglobulin	815 (38%) Rh D negative pregnant women avoided unnecessary Rh D immunoglobulin prophylaxis.			
Sensitising event prophylaxis - 0 studies identified									

CI, confidence interval; GW, gestation week; RAADP, routine antenatal anti-D prophylaxis; UK, United Kingdom;

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The risk of bias of the included primary studies is based on the quality assessment reported by the systematic review authors.

b. Only applicable to Level I studies with formal meta-analysis. Heterogeneity defined as follows: (i) no significant heterogeneity if P_{het} >0.1 and I^2 <25%; (ii) mild heterogeneity if I^2 <25%; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity I^2 >50%.

c. for any reason such as refusal (e.g. partner confirmed Rh D negative, religious belief), not requested or performed (e.g. missed appointment, insufficient sample), woman does not qualify (e.g. delivery/loss prior to GW28).

5.4.5 Summary of evidence

The systematic review and hand-searching process identified four systematic reviews that examined the diagnostic accuracy of NIPT to identify fetal Rh D status (Geifman-Holtzman et al., 2006, Zhu et al., 2014, Mackie et al., 2017, Saramago et al., 2018). The reviews included over 90 studies meeting their search criteria. A matrix illustrating the overlap of studies included in each review is provided in Table 5-25.

Assuming relevant primary studies had been identified in the included systematic reviews, the screening of the Level II and Level III citations was limited to those published after the literature search date of Saramago 2018. Studies excluded by the included reviews were also scrutinised for inclusion. Studies that were of small sample size (N<200), conference abstracts that did not provide sufficient data, and those in which the NIPT was not conducted in the context considered similar to Australia were excluded (see **Appendix B, Volume 2** of the technical report).

Five additional Level II studies (Macher et al., 2012, Picchiassi et al., 2015, Moise et al., 2016, Haimila et al., 2017, Manfroi et al., 2018) and six additional Level III study (Orzińska et al., 2015, Papasavva et al., 2016, Hyland et al., 2017, Ryan et al., 2017, Jakobsen et al., 2018, Sorensen et al., 2018) were identified and subsequently included in this review.

Table 5-25 Overlap table showing primary studies included in the Level I studies: noninvasive prenatal testing to determine fetal Rh D status

	Review ID			
Study ID	**Saramago 2018**	**Mackie 2017**	**Zhu 2014**	**Geifman-Holtzman 2006**
Soothill 2015	✓			
Thurik 2015	✓			
Banch Clausen 2014	✓			
Chitty 2014	✓	✓		
Grande 2013	✓			
Manzanares 2013		✓		
Moise 2013		✓		
Polin 2013		✓		
TadejaDovc Drnovsek 2013			✓	
Clausen 2012	[a]	✓	✓	
Han 2012		✓		
Sbarsi 2012		✓		
Wikman 2012	✓			
Achargul 2011		✓		
Akolekar 2011	✓		✓	
Amaral 2011			✓	
Bombard 2011		✓	✓	
Gunel 2011			✓	
Macher 2011			✓	
Scheffer 2011			✓	
Sedrak 2011			✓	
Tynan 2011		✓	✓	
Aykut 2010		✓		

	Review ID			
Study ID	**Saramago 2018**	**Mackie 2017**	**Zhu 2014**	**Geifman-Holtzman 2006**
Cardo 2010			✓	
Gunel 2010		✓	✓	
Mohammed 2010		✓	✓	
Atamaniuk 2009			✓	
Grill 2009		✓	✓	
Hyland 2009		✓	✓	
Sesarini 2009		✓		
Wang 2009		✓	✓	
Finning 2008	✓		✓	
Kimura 2008			✓	
Minon 2008		✓	✓	
Muller 2008			✓	
Al-Yatama 2007		✓	✓	
Rouillac-Le 2007		✓	✓	
Machado 2006		✓	✓	
Brojer 2005			✓	✓
Clausen 2005a			✓	✓
Clausen 2005b			✓	
Cotter 2005				✓
Gautier 2005		✓		✓
Gonzalez 2005				✓
Hromadnikova 2005a		✓	✓	✓
Hromadnikova 2005b			✓	✓
Hromadnikova 2005c			✓	✓
Hromadnikova 2005d			✓	✓
Zhou 2005		✓	✓	✓
Di Simone 2004			✓	
Finning 2004				✓
Harper 2004				✓
Kirstin 2004			✓	
Rijnders 2004		✓		✓
Rouillac-Le Sciellour 2004				✓
Johnson 2003				✓
Randen 2003			✓	✓
Sashi 2003			✓	
Siva 2003		✓		✓
Turner 2003		✓		✓
Costa 2002		✓		✓
Finning 2002			✓	✓
Legler 2002			✓	✓
Nelson 2001				✓
Zhong 2001		✓	✓	✓
Zhang 2000			✓	✓
Zhong 2000				✓
Bischoff 1999			✓[b]	✓
Cunningham 1999				✓
Sekizawa 1999				✓
Al-Mufti 1998				✓

	Review ID			
Study ID	**Saramago 2018**	**Mackie 2017**	**Zhu 2014**	**Geifman-Holtzman 2006**
Faas 1998				✓
Lo 1998		✓		✓
Toth 1998				✓
Hamlington 1997				✓
Geifman 1996				✓
Sekizawa 1996				✓
Lo 1994a				✓
Lo 1994b				✓
Lo 1993				✓

a. Linked to Banch Clausen 2014

b. Cited as Farideh 1999

5.4.5.1 Level I

Four systematic reviews (Geifman-Holtzman et al., 2006, Zhu et al., 2014, Mackie et al., 2017, Saramago et al., 2018) were identified in the literature search that examined the diagnostic accuracy of noninvasive prenatal screening tests to identify fetal Rh D status. The characteristics of these studies and relevant outcomes assessed are summarised in Table 5-26.

Table 5-26 Characteristics and quality of Level I evidence: noninvasive prenatal screening test to determine fetal Rh D status

Review ID	Study type *Risk of bias*	Population	Intervention	Reference Standard	Outcomes
Saramago 2018	SR and meta-analysis of diagnostic accuracy studies *Moderate*	Rh D negative pregnant women who are not known to be sensitised N = 42 491 (8 studies)	High-throughput[a] cffDNA test of maternal plasma	Serological cord blood sampling at birth or other suitable postnatal blood test	Sensitivity, specificity, inconclusive test results
Mackie 2017	SR and meta-analysis of cohort studies *High*	Women with any singleton pregnancy attending for pregnancy due to risk factors N = NR 10 290 tests (30 studies)	NIPT based on cffDNA in maternal blood	Confirmation of blood type at birth[b]	Sensitivity, specificity, inconclusive test results
Zhu 2014	SR and meta-analysis *Serious*	Rh D negative pregnant women at any gestational age N = NR 11 129 tests (37 studies)	NIPT based on cffDNA in maternal whole blood	Determination of fetus (or newborn) Rh D blood type	Sensitivity, specificity
Geifman-Holtzman 2006	SR and meta-analysis *Serious*	Rh D negative pregnant women at any gestational age N = 3261 (31 studies)	NIPT of cffDNA in maternal plasma, serum, or fetal cells	Confirmation of fetus / blood type at birth	Sensitivity, specificity, PPV NPV

ccfDNA, cell-free fetal DNA; CVS, chorionic villus sampling; NIPT, noninvasive prenatal testing; SR, systematic review

a. defined by Saramago 2018 as any NIPT that was conducted using an automated robotic platform including automated DNA extraction and liquid handling for large scale screening purposes.

b. Some included studies reported CVS/amniocentesis as the reference standard

As described in Section 5.4.3.1, Saramago 2018 was a published HTA report conducted for the NHS that examined the diagnostic accuracy of high-throughput NIPT and the clinical impacts of implementation of targeted antenatal prophylaxis to provide an assessment of cost-effectiveness. The report 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 for diagnosis (rather than screening) of sensitised women 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. The characteristics of these studies and relevant outcomes assessed are summarised in Table 5-27.

Mackie 2017 was a systematic review and bivariate meta-analysis that looked at cell-free fetal DNA (cffDNA) NIPT in singleton pregnancies for various conditions including Rh status. The eligible population were women with a singleton pregnancy of any gestation and the test was NIPT based on cffDNA in maternal blood. The meta-analysis was restricted to cohort studies that used outcome at birth for the reference standard, but it was noted in the supplementary table (Table S1) that 12 of the included studies used CVS/amniocentesis results as the reference standard. The literature search was conducted 13 April 2015, with all identified studies published after 1997. Thirty studies (10 290 tests) were identified that had been conducted in various countries including Argentina, Australia, Austria, Belgium, Brazil, China, Czech-Republic, Denmark, France, Germany, Ireland, Italy, Korea, Kuwait, Morocco, Netherlands, Pakistan, Spain, Switzerland, Turkey, UK, USA. The included studies were assessed by Mackie 2017 to be at overall low risk of bias, with key concerns related to selection bias and index test bias. The authors noted that only 13 of 30 studies reported inconclusive test results and explored the diagnostic accuracy of different test platforms (real-time quantitative PCR, conventional PCR, mass spectrometry) where available.

Zhu 2014 conducted a systematic review and meta-analysis of NIPT for fetal Rh D status using cffDNA in Rh D negative pregnant women. The NIPT was to be conducted using maternal whole blood and studies that included less than 10 participants were excluded. The date of the literature search was not provided but all included studies were published between 1999 and 2013. Zhu 2014 identified 41 publications including 11 129 tests, but no details regarding the included studies or assessment of bias was provided. It is unclear if any effort was made to ensure duplicate sample results are not included. The diagnostic accuracy of testing was assessed by gestational age at time of sampling.

Geifman-Holtzman 2006 was a systematic review and meta-analysis of NIPT for fetal Rh D status in Rh D negative women. There was no restriction on alloimmunisation status or gestational age. The sample was maternal blood, including maternal plasma, serum or fetal cells. The literature search date was not provided but all included studies were published between 1999 and 2005. The search 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 included studies risk of bias assessment was not included but the authors noted that 16 included studies reported 100% diagnostic accuracy in their fetal *RHD* genotyping, and many authors excluded samples because of absence of detectable DNA or 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.

Table 5-27 Characteristics and quality of studies included in Saramago 2018: noninvasive prenatal testing to determine fetal Rh D status

Study ID	Study type *Risk of bias* [a]	Population [b]	Intervention	Reference standard	Outcomes
Akolekar 2011	Prospective Coh *High*	Rh D negative pregnant women at 11–14 weeks' gestation and not known to be alloimmunised N = 586	NIPT of cffDNA in maternal plasma exons 5 and 7	Serological newborn typing	Sensitivity Specificity Inconclusive results
Banch Clausen 2014	Prospective Coh *Low*	Rh D negative pregnant women at 23–28 weeks' gestation and not known to be alloimmunised N = 12 668	NIPT of cffDNA in maternal plasma targeting two exons (5 and 7; 5 and 10; 7 and 10)	Serological newborn typing	Sensitivity Specificity Inconclusive results
Chitty 2014	Prospective Coh *Low*	Rh D negative pregnant women at 5–35 weeks' gestation and not known to be alloimmunised N = 4913	NIPT of cffDNA in maternal plasma exons 5 and 7	Serological newborn typing	Sensitivity Specificity Accuracy by gestational age Inconclusive results
Finning 2008	Prospective Coh *Low*	Rh D negative pregnant women at 8–38 weeks' gestation and not known to be alloimmunised N = 1869	NIPT of cffDNA in maternal plasma exons 5 and 7	Serological newborn typing	Sensitivity Specificity Inconclusive results
Grande 2013	Prospective Coh *Low*	Rh D negative pregnant women at 24–26 weeks' gestation and not known to be alloimmunised N = 282	NIPT of cffDNA in maternal plasma exons 5 and 7	Serological newborn typing	Sensitivity Specificity
Soothill 2015	Prospective Coh *Low*	Rh D negative pregnant women at 15–17 weeks' gestation (mostly) and not known to be alloimmunised N = 499 [c]	NIPT of cffDNA in maternal plasma exons 5 and 7	Serological newborn typing	Sensitivity Specificity Inconclusive results
Thurik 2015	Prospective Coh *High*	Rh D negative pregnant women at 26 weeks' gestation and not known to be alloimmunised N = 18 383 [c]	NIPT of cffDNA in maternal plasma exons 5 and 7	Serological newborn typing	Sensitivity Specificity
Wikman 2012	Prospective Coh *Low*	Rh D negative pregnant women at 8–40 weeks' gestation and not known to be alloimmunised N = 3291 [d]	NIPT of cffDNA in maternal plasma exon 4	Serological newborn typing	Sensitivity Specificity Inconclusive results

cff-DNA, cell-free fetal DNA; Coh, cohort; NIPT; noninvasive prenatal testing

a. Study quality was assessed by Saramago 2018 using a modified version of the QUADAS-2 tool containing 14 items.

b. As reported by Saramago 2018. N = number of samples unless otherwise specified.

c. Number of participants.

d. Excludes pre 8 weeks' gestation pregnancies.

5.4.5.2 Level II

Five additional Level II studies (Macher et al., 2012, Picchiassi et al., 2015, Moise et al., 2016, Haimila et al., 2017, Manfroi et al., 2018) assessing the diagnostic accuracy of NIPT for fetal Rh D status were identified for inclusion in this review. The characteristics of these studies and the relevant outcomes assessed are summarised in Table 5-28.

Table 5-28 Characteristics and quality of Level II evidence: noninvasive prenatal testing to determine fetal Rh D status

Study ID	Study type *Risk of bias*	Population	Intervention	Reference standard	Outcomes
Manfroi 2018	Prospective Coh, MC *High*	Rh D negative pregnant women at 11–30 weeks' gestation with Rh positive or unknown partners N = 455	RT-qPCR of cffDNA in maternal plasma exons 5, 7 and 10 internal controls not described	Serological typing of newborn (cord blood)	Sensitivity Specificity PPV NPV Inconclusive results
Haimila 2017	Prospective Coh, *Low*	Rh D negative pregnant women at 24–26 weeks' gestation who are not Rh D alloimmunised participating in a national screening program N = 10 814	RT-qPCR of cffDNA in maternal plasma exons 5 and 7 no internal control	Serological typing of newborn (postnatal cord or heel stick)	Sensitivity Specificity False-negative rate False-positive rate Inconclusive results
Moise 2016	Prospective Coh, MC *High*	Rh D negative pregnant women at 10–32 weeks' gestation who are not Rh D alloimmunised N = 522	PCR and MALDI-TOF of cffDNA in maternal plasma exons 4, 5, and 7, plus 37 bp insert at exon 4 TGIF internal control	Serological typing of newborn (cord blood)	Sensitivity Specificity PPV NPV Inconclusive results
Picchiassi 2015	Prospective Coh, SC *Unclear*	Rh D negative pregnant women at 10–14 weeks' gestation N = 216	RT-qPCR of cffDNA in maternal plasma exons 5 and 7 TERT internal control	Serological typing of newborn	Sensitivity Specificity PPV NPV
Macher 2012	Prospective Coh, SC *High*	Rh D negative pregnant women at 10–28 weeks' gestation N = 2127	RT-PCR of cffDNA in maternal plasma[b] exons 5 and 7 SRY internal control	Serological typing of newborn (cord blood)	Sensitivity Specificity

bp, base pairs; cffDNA, cell-free fetal DNA; Coh, cohort; MALDI-TOF, matrix-assisted laser desorption/ionisation-time-of-flight mass spectrometry; MC, multicentre; NIPT, noninvasive prenatal testing; NPV, negative predictive value; PPV, positive predictive value; RT-qPCR, real-time quantitative polymerase chain reaction; SC, single centre

a. 32 622 pregnancies, 62 women were pregnant twice during the study period. 382 samples were from Rh D positive women, 18 samples carried variant maternal alleles.

b. Cohort 1, single TaqMan PCR; Cohort 2, multiplex TaqMan PCR

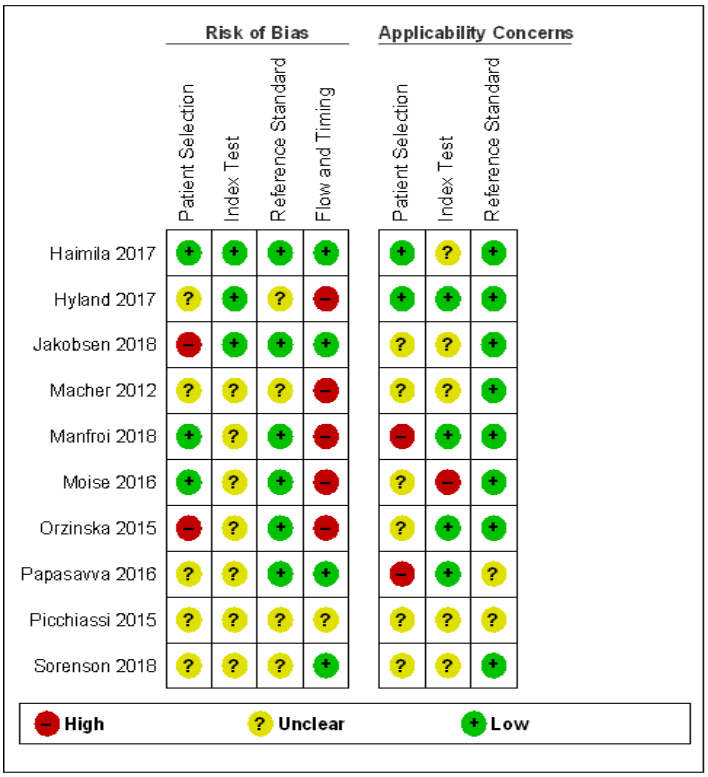
Three studies (Haimila 2017, Manfroi 2018, Moise 2016) were published after the search date of Saramago 2018, whereas two studies (Macher 2012 and Picchiassi 2015) were identified by Saramago 2018 but excluded as not high-throughput NIPT. Three studies (Macher 2012, Moise 2016, Picchiassi 2015) were also published prior to the literature search date of Mackie 2017, but Macher 2012 and Moise 2016 permitted the inclusion of multiple gestations and were likely excluded for this reason. It is unclear if Picchiassi 2015 included only singleton pregnancies.

The studies were performed in a variety of countries including Finland, Italy, Spain, US and Canada, and used NIPT of cffDNA in maternal plasma targeting exons 5 and 7 of the *RHD* gene (Haimila 2017,

Macher 2012, Picchiassi 2015), exons 5, 7, and 10 (Manfroi 2018) or exons 4, 5, and 7 as well as probes for the 37-base pair insertion in exon 4 (*RHD* pseudogene) (Moise 2016). The reference standard used in all studies was serological testing at birth. The studies enrolled between 216 to 32 560 Rh D negative pregnant women with gestational ages ranging between 9 and 37 weeks' gestation. Participants were predominantly of European ethnicity. Rh D alloimmunised were explicitly excluded in two studies (Haimila 2017, Moise 2016) and no studies reported if there were any adverse events associated with NIPT or conduct of the reference standard.

A summary figure illustrating of the risk of bias assessment for each of the included studies is provided in Figure 5.8. Further details are provided in **Appendix D, Volume 2** of the technical report.

Figure 5.8 Summary of risk of bias assessments of additional included diagnostic accuracy studies



Note: Risk of bias of Ryan 2017 not assessed due to insufficient information

In brief, the NIPT test was conducted independently of the reference standard in most studies, but given the nature of the testing, the result of the NIPT was not considered to likely influence the result of the neonate serology typing, so the risk of incorporation bias was considered low. Multiple gestation pregnancies may pose an issue for NIPT (if, for example, twin fetuses have discordant Rh D status), exclusion of multiple pregnancies therefore may introduce selection bias. Multiple pregnancies were included in four studies (Manfroi 2018, Haimila 2017, Moise 2016, Macher 2012) and their inclusion or exclusion was not stated in one (Picchiassi 2015).

Manfroi 2018 recruited women who had partners known to be Rh D positive, or partners of unknown Rh D phenotype (ie excluded partners known to be Rh D negative); therefore the study is likely to have a higher prevalence of Rh D positive newborns. The study was rated as having a high risk of bias for selection bias and applicability to the Australian healthcare system as the women

were considered sufficiently different to the intended Guidelines population. Inconclusive results were reported in only three studies (Manfroi 2018, Haimila 2017, Moise 2016). 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 one study, which may also have introduced bias (Macher 2017). Other studies used internal controls to account for the total genomic DNA. In the nationwide screening programs, no internal control was used (Haimila 2017).

5.4.5.3 Level III

Six additional Level III-1 studies (Orzińska et al., 2015, Papasavva et al., 2016, Hyland et al., 2017, Ryan et al., 2017, Jakobsen et al., 2018, Sorensen et al., 2018) assessing the diagnostic accuracy of NIPT for fetal Rh D status were identified for inclusion in this review. The characteristics of these studies and the relevant outcomes assessed are summarised in Table 5-29.

Table 5-29 Characteristics and quality of Level III evidence: noninvasive prenatal testing to determine fetal Rh D status

Study ID	Study type *Risk of bias*	Population	Intervention	Reference standard	Outcomes
Jakobsen 2018	All or none, MC *Unclear*	Rh D negative, pregnant women at 25 weeks' gestation N = 1588	NIPT of cffDNA in maternal plasma exons 5 and 10 CCR5 internal control	Serological typing of newborn	Sensitivity Specificity
Sørenson 2018	Non-consecutive Coh, SRC *Unclear*	Rh D negative, pregnant women at 16-–36 weeks' gestation N = 373	RT-PCR of cffDNA in maternal plasma exons 7 and 10 GADPH internal control	Serological typing of newborn	Sensitivity Specificity Inconclusive results
Hyland 2017	Prospective Coh, SRC *High*	Rh D negative pregnant women at 9–37.5 weeks' gestation N = 665	qPCR of cffDNA in maternal plasma collected in EDTA tubes or BCT[a] tubes exons 5 and 10 CCR5 internal control n = 647	Serological typing of newborn (cord blood) n = 599	Sensitivity Specificity False-negative rate False-positive rate Inconclusive results
Ryan 2017	Non-consecutive Coh, MC *Not assessed* [b]	Rh D negative pregnant women at mean 13 weeks' gestation N = 232	RT-PCR of cffDNA in maternal plasma exons 7 and 10 GADPH internal control	(not stated)	Sensitivity Specificity Inconclusive results
Papasavva 2016	Non-consecutive Coh, SRC *Unclear*	Rh D negative, pregnant women at ≥ 16 weeks' gestation N = 73	RT-PCR of cffDNA in maternal plasma exons 4, 5 and 10 SYR and CCR5 internal control	Serological typing of newborn	Sensitivity Specificity
Orzińska 2015	Non-consecutive Coh, SRC *High*	Rh D negative pregnant women at 5–39 weeks' gestation with suspected alloimmunisation N = 536	RT-PCR of cffDNA in maternal plasma exon 5 and exon 7 SYR and CCR5 internal control	Serological typing of newborn (cord blood or swabs)	Sensitivity Specificity

BCT, blood collection tube; cffDNA, cell-free fetal DNA; Coh, cohort; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; MC, multicentre; NIPT, noninvasive prenatal testing; qPCR, quantitative polymerase chain reaction; RT-PCR, real-time polymerase chain reaction; SRC, single referral centre

a. Cell-free DNA BCT tubes (Streck, USA) contain a preservative to prevent further maternal DNA release, conserving the cffDNA fraction.
b. Insufficient information to assess risk of bias.

Five studies (Jakobsen 2018, Hyland 2017, Papasavva 2016, Ryan 2017, Sørenson 2018) were published after the search date of the systematic review of Saramago 2018. Orzińksa 2015 was identified by Saramago 2018 but had been excluded due to an ineligible reference standard.

The studies were conducted in Australia, Denmark, Norway, Ireland, Cyprus and Poland and used NIPT of cffDNA in maternal plasma targeting exons 5 and 7 of the *RHD* gene (Orzińska 2015), exons 5 and 10 (Jakobsen 2018, Hyland 2017, Sørenson 2018, Ryan 2017), or exons 5, 7 and 10 (Papasavva 2016). The reference standard used was serological testing at birth, except Ryan 2017 (not stated). The sample size of the included studies ranged from 73 to 1,588, with recruited pregnant women being of gestational age between 5 and 39 weeks' gestation. Most participants were of white European ethnicity. None of the studies reported if there were any adverse events associated with NIPT or conduct of the reference standard. The SRY gene was used as an internal control for male fetal DNA in two studies (Papasavva 2016, Orzińska 2015). Other studies used internal controls to account for the total genomic DNA (Jakobsen 2018, Sørenson 2018, Ryan 2017).

A summary figure illustrating of the risk of bias assessment for each of the included studies is provided in Figure 5.8. Further details are provided in **Appendix D, Volume 2** of the technical report.

Many of the studies were rated as having overall unclear risk of bias, as they did not contain sufficient information to make a judgement. As described for the Level II studies (see Section 5.4.5.2) the result of the NIPT was not considered likely to influence the result of the neonate serology, thus the risk of incorporation bias was considered low. Rh D alloimmunised women were not explicitly excluded in any study; however, the study by Orzińksa 2015 only included women with suspected red cell alloimmunisation. The study by Papasavva 2016was conducted in a Cypriot population, where the prevalence of Rh D negative serology was estimated to be 7.2% (95%CI 5, 10). The study enrolled pregnant women with Rh D positive partners, thus the overall proportion of neonates who would be Rh D positive is higher than the target population in Australia. The applicability and risk of selection bias was therefore considered high in Orzińksa 2015 and Papasavva 2016.

One study (Jakobsen 2018) included multiple pregnancies, whereas the other five studies did not state whether only singleton pregnancies were permitted. The exclusion of multiple pregnancies may introduce selection bias. Inconclusive results were reported in only four studies (Jakobsen 2018, Hyland 2017, Papasavva 2016, Sørenson 2018). Exclusion of inconclusive results would also introduce bias in favour of the index test.

5.4.6 Results

5.4.6.1 Diagnostic performance

Four systematic reviews presented meta-analyses on the diagnostic accuracy of NIPT for fetal Rh D status. Five additional Level II studies and six additional Level III-1 studies were also identified that assessed the diagnostic accuracy of NIPT. A summary of the result from these studies is presented in Table 5-30.

Each of the included studies varied with regards to 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, source of fetal DNA sample); therefore several analyses were conducted to assess the implications for diagnostic performance (see Subgroup analyses.

Saramago 2018 conducted a bivariate meta-analysis of eight studies that were considered most applicable to the UK healthcare 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 indicate any clinically meaningful differences between studies. This is because I^2 increases as the average within-study variance declines.

Saramago 2018 also conducted a sensitivity analyses to adjust for potential bias associated with two of the studies (Thurik 2015 and Grande 2013) 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 2017 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 thirty studies included the 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) was: 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/incorrect neonatal blood testing.

The meta-analysis by Zhu 2014 (random effects) included 44 studies, many of which likely overlapped with that included by Mackie 2017, but full details regarding the included studies were not provided. It is likely 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. It is likely this is due to small within-study variance rather than representing a clinically meaningful differences between studies.

Geifman-Holtzman 2006 conducted two meta-analysis 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 on the included studies were not provided but it is likely that inconclusive results and substandard samples were not included in the analysis.

Among the 13 protocols (10 studies) identified in this review, 12 showed a sensitivity of 100%, meaning all women with an Rh D positive fetus would be correctly identified. Picchiassi 2015 reported a sensitivity of 92.8 (95% CI 86.9, 96.2), which is notably lower than the other studies and is likely due to the small sample size and the early gestational age (GW 10 to 15) 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 (Papasavva 2016) 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 RT-PCR protocol reported by Macher 2012 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) (Jakobsen 2018) and 100 (95% CI 81, 100) (Papasavva 2016); meaning up to 8.4% (between 11% and 6%) of women with an Rh D negative fetus would be incorrectly identified. The heterogeneity in specificity is likely to be a consequence of differing reporting and handling of inconclusive tests.

A forest plot of the studies included in this review is shown in Figure 5.9, noting that studies from two of the systematic reviews (Zhu 2014 and Geifman-Holtzman 2006) were not available for inclusion (although 24 studies in Zhu 2014 likely overlapped with Mackie 2017). 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 a test positive.

A bivariate meta-analysis of included studies[25] revealed a pooled sensitivity of 99.7 (95% CI 99.4, 99.9) and specificity of 98.3 (95% CI 97.4, 98.9), with a false positive rate of 0.017 (95% CI 0.011, 0.026) (random effects correlation 0.412). In a sensitivity analysis involving only studies published after 2008, the pooled sensitivity was 99.8 (95% CI 99.4, 99.9) and specificity was 98.4 (95% CI 97.5, 99.0), with a false positive rate of 0.016 (95% CI 0.01, 0.025) (random effects correlation 0.523). Summary ROC curves are shown in Figure 5.10.

A GRADE evidence profile summarising the evidence for diagnostic accuracy of noninvasive prenatal screening tests for fetal Rh D status in Rh D negative pregnant women with no preformed anti-D antibodies is provided in Appendix 6.

[25] Freeman SC, Kerby CR, Patel A, Cooper NJ, Quinn T, Sutton AJ. Development of an interactive web-based tool to conduct and interrogate meta-analysis of diagnostic test accuracy studies: MetaDTA. BMC Medical Research Methodology 2019; 19: 81 https://doi.org/10.1186/s12874-019-0724-x

Figure 5.9 Forest plot of tests: 1 Mackie 2017, 2 Saramago 2018, 3 Additional studies identified.

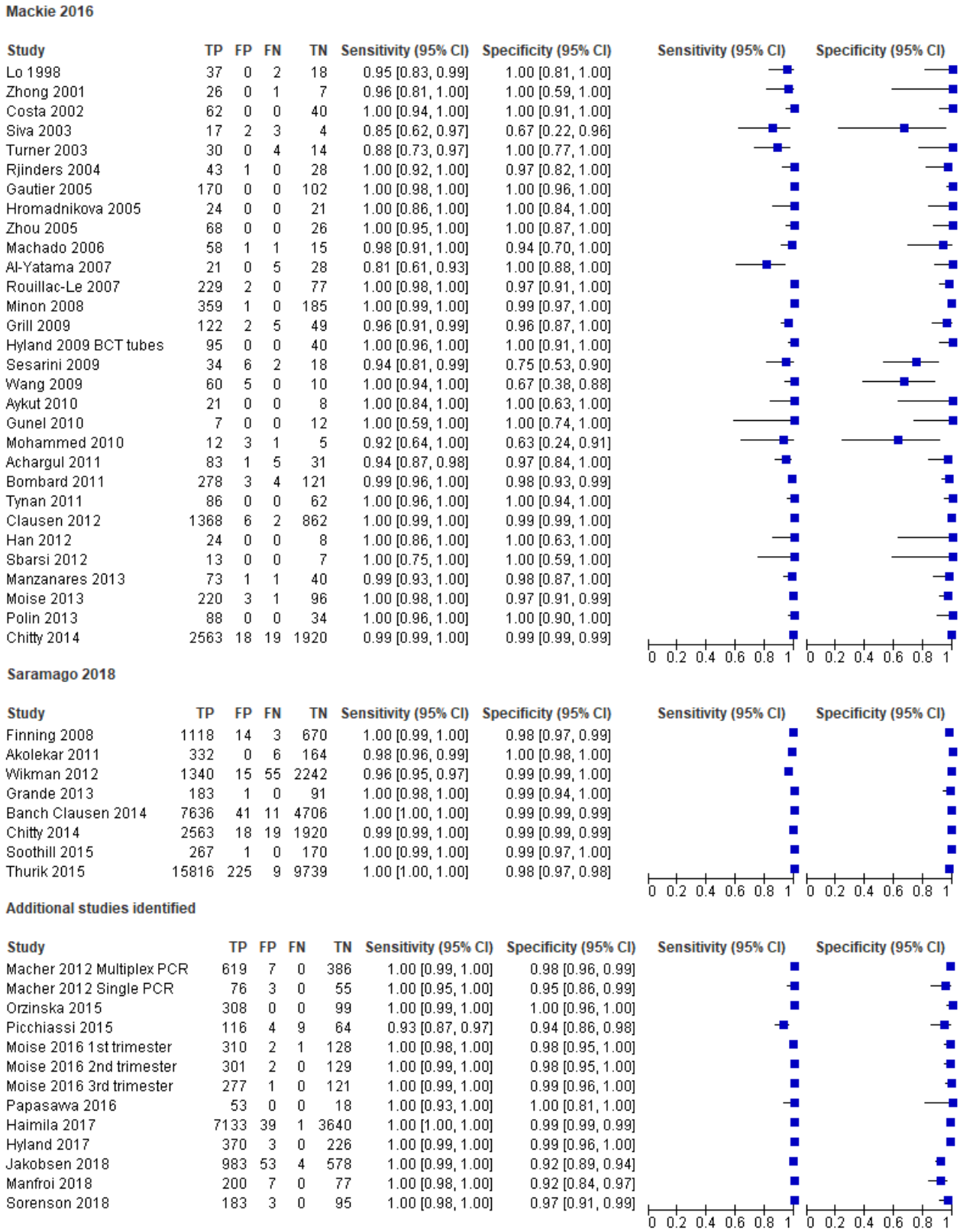


Figure 5.10 Summary receiver operation characteristic curve: sensitivity analysis

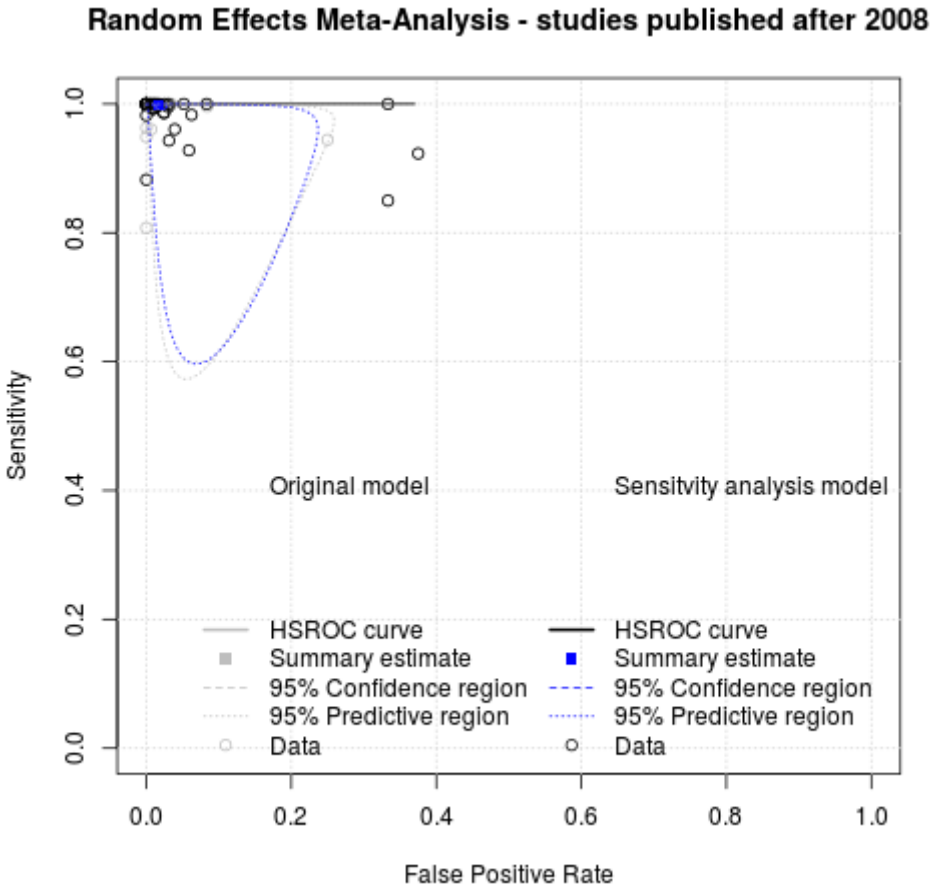


Table 5-30 Results for the diagnostic performance of noninvasive prenatal screening tests to identify fetal Rh D status against reference standard of postnatal cord blood testing: Rh D negative pregnant women with no preformed anti-D antibodies – diagnostic performance (any timepoint)*

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials in analysis)	Population	Setting (Location)	Comparison	Outcome (timing/source)	Results						
						Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR+ (95% CI)	LR– (95% CI)	Accuracy % (95% CI) n/N (%)
Level I												
Saramago 2018 Level I *Moderate*	N = 42 491 (8 studies)	Rh D negative pregnant women	Obstetrics and maternity (Denmark, The Netherlands, Spain, Sweden, UK)	NIPT against birth serology	Diagnostic performance [b] (median GW 10–28) Bivariate meta-analysis	99.66 (99.24, 99.85)	96.14 (94.18, 97.46)	NR	NR	NR	NR	NR
						Sensitivity analysis (2 studies with high risk of bias excluded): sensitivity 99.62 (99.06, 99.85); specificity 95.63 (93.22, 97.21)						
Mackie 2017 Level I *High*	N = 10 290 (30 studies)	Rh D negative pregnant women Singletons only	Obstetrics and maternity (Argentina, Australia, Austria, Belgium, Brazil, China, Czech-Republic, Denmark, France, Germany, Ireland, Italy, Korea, Kuwait, Morocco, Netherlands, Pakistan, Spain, Switzerland, Turkey, UK, USA)	NIPT against birth serology	Diagnostic performance (any timepoint) bivariate logistic regression model	99.3 (98.2, 99.7)	98.4 (96.4, 99.3)	NR	NR	61 (22, 167)	0.007 (0.003, 0.186)	OR 8466 (1877, 38 183)
Zhu 2014 Level I *Serious*	N = 11 129 (46 studies)	Rh D negative pregnant women	Obstetrics and maternity (Not stated)	NIPT against birth serology	Diagnostic performance (any timepoint)	--	--	--	--	--	--	10611/11129 (95.3)
	N = 10 777 (44 studies)[c]				Random effects model	99 (99, 99) I^2 = 80.5%	98 (97, 98) I^2 = 78.0%	98.7 (NR)	98.0 (NR)	--	--	10611/10777 (98.5)
	N = 3261 (44 studies)			NIPT against birth serology	Diagnostic performance	--	--	--	--	--	--	NR (91.4)

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials in analysis)	Population	Setting (Location)	Comparison	Outcome (timing/source)	Results						
						Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR+ (95% CI)	LR– (95% CI)	Accuracy % (95% CI) n/N (%)
Geifman-Holtzman 2006 Level I *Serious*	N = 3184 (no. studies not reported)[d]	Rh D negative pregnant women who could be alloimmunised	Obstetrics and maternity (Not stated)		(any timepoint)	--	--	--	--	--	--	NR (91.7)
	N = 3078 (no. studies not reported)[e]				Random effects model	95.4 (90.6, 97.8)	98.6 (96.4, 99.5)	99.0 (97.9, 99.6)	92.1 (80.9, 97.0)	--	--	NR (94.8)
					Bayesian model	96.7 (92.5, 98.9)	98.9 (96.7, 99.9)	99.4 (98.4, 99.9)	92.7 (81.8, 97.9)	--	--	NR (94.8)
Level II												
Manfroi 2018 Level II *High*	N = 455 [f] (N = 284 included in the analysis)	Rh D negative pregnant women	Obstetrics and maternity (Italy)	RT-qPCR of cffDNA (exons 5, 7, 10) against birth serology	Diagnostic performance (GW 24–28)	100 (98, 100)	91.67 (84, 97)	96.62	100	12.00	0.0000	96.1 (93.9, 98.4)
						Including 31 samples collected prior to GW 24, the sensitivity of the test was 99.6 (98.7, 100) and diagnostic accuracy was 95.5 (93.3, 97.8).						
Haimila 2017 Level II *Low*	N = 10 814 *birth serology missing in one sample	Rh D negative pregnant women with no preformed anti-D antibodies	Obstetrics and maternity (Finland)	RT-qPCR of cffDNA (exons 5, 7) against birth serology	Diagnostic performance [g] (GW 24–26)	100 (100, 100)	99 (99.0, 99.0)	99.46 (NR)	99.97 (NR)	94.3201	0.0001	10773/10813 (99.63)
De Haas 2016 Level II *Unclear*	N = 25 789	Rh D negative pregnant women Singletons only	Obstetrics and maternity (The Netherlands)	RT-qPCR of cffDNA (exons 5, 7) against birth serology	Diagnostic performance [h] (GW 27–29)	99.94% (99.89-99.97)	97.74 (97.43, 98.02)	98.60 (98.40, 98.77)	99.91 (99.82, 99.95)	44.2593	0.0006	99.09 (NR)
Moise 2016 Level II	N = 441	Rh D negative pregnant women	Obstetrics and maternity (United States)		Diagnostic performance [i] (GW 10.7–14.7)	99.68 (98.22, 99.94)	98.46 (94.56, 99.58)	99.36	99.22	64.791	0.0033	99.32 (98.03, 99.77)

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials in analysis)	Population	Setting (Location)	Comparison	Outcome (timing/source)	Results						
						Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR+ (95% CI)	LR– (95% CI)	Accuracy % (95% CI) n/N (%)
High	N= 432			MALDI-TOF of cffDNA (exons 4, 5, 7, & *RHD* pseudogene) against birth serology	(GW 15.1–24.4)	100 (98.74, 100)	98.47 (94.60, 99.58)	99.34	100	65.50	0.0000	99.53 (98.33, 99.87)
	N= 399				(GW 26.0–32.4)	100 (98.63, 100)	99.18 (95.50, 99.96)	99.64	100	122.0	0.0000	99.75 (95.50, 99.96)
Picchiassi 2015 Level II *Unclear*	N = 216 *birth serology missing 23 samples	Rh D negative pregnant women	Obstetrics and maternity, SC (Italy)	RT-qPCR of cffDNA (exons 5, 7) against birth serology	Diagnostic performance* (GW 10–15) *not clear if inconclusive results included	92.8 (86.9, 96.2)	94.1 (85.8, 97.7)	96.7 (93.5, 99.9)	87.7 (80.1, 95.2)	15.7760	0.0765	93.3 (88.8, 96.0)
Macher 2012 Level II *High*	N = 136 *birth serology missing in two samples (aborted)	Rh D negative pregnant women	Obstetrics and maternity (Spain)	RT-PCR (single) of cffDNA (exons 5, 7) against birth serology	Diagnostic performance* (GW 10–28) *not clear if inconclusive results included	100 (95, 100)	94.8 (89, 99)	96.2 (NR)	100 (NR)	19.3333	0.0000	131/134 (97.8)
	N = 1993 *birth serology missing in 981 samples (not yet born)	Rh D negative pregnant women	Obstetrics and maternity (Spain)	RT-PCR (multiplex) of cffDNA (exons 5, 7) against birth serology	Diagnostic performance* (GW 10–28) *not clear if inconclusive results included	100 (99, 100)	98.2 (96, 99)	98.9 (NR)	100 (NR)	56.1429	0.0000	1005/1012 (99.3)
Level III												
Jakobsen 2018 Level III-1 *Unclear*	N = 1618	Rh D negative pregnant women	Obstetrics and maternity (Denmark)	RT-qPCR of cffDNA (exons 5, 10) against birth serology	Diagnostic performance (GW 25)	99.59 (99, 100)	91.60 (89, 94)	94.88	99.31	11.8574	0.0044	1561/1618 (96.48)

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials in analysis)	Population	Setting (Location)	Comparison	Outcome (timing/source)	Results						
						Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR+ (95% CI)	LR– (95% CI)	Accuracy % (95% CI) n/N (%)
Sørenson 2018 Level III-1 *Unclear*	N = 281	Rh D negative pregnant women	Obstetrics and maternity (Norway)	RT-qPCR of cffDNA (exons 7, 10) against birth serology	Diagnostic performance (GW 16–36, median 24)	100 (98, 100)	96.94 (91, 99)	98.39	100.00	32.6667	0.0000	278/281 (98.93)
Hyland 2017 Level III-1 *High*	N = 647 *birth serology missing in 48 samples	Rh D negative pregnant women Singletons only	Obstetrics and maternity (Australia)	RT-qPCR of cffDNA (exons 5, 10) against birth serology	Diagnostic performance (GW 9–37.1, median 19.29)	100 (99, 100)	98.69 (96, 100)	99.20	100	76.33	0.000	596/599 (99.5)
Ryan 2017 Level III-1 [h] *Not assessed*	N = 323	Rh D negative pregnant women	Obstetrics and maternity (Ireland)	RT-PCR of cffDNA (exons 7, 10) against birth serology	Diagnostic performance* (GW 13) *not clear if inconclusive results included	100 (98.87, 100)	97.59 (95.26, 99.92)	NR	NR	NR	NR	NR
Papasavva 2016 Level III-1 *Unclear*	N = 73 *birth serology not reported in two samples	Rh D negative pregnant women with Rh D positive partners	Obstetrics and maternity (Cyprus)	RT-PCR of cffDNA (exons 4, 5, 10) against birth serology	Diagnostic performance* (after GW 16) *inconclusive results not included	100 (93, 100)	100 (81, 100)	100.00	100.00	Not calculable	0.0000	100 (95.3, 100)
Orzińska 2015 Level III-1 *High*	N = 407 *birth serology missing or data not reported in 129 (24%) samples	Rh D negative pregnant women with and without preformed anti-D antibodies 72.95% sensitised	Obstetrics and maternity (Poland)	RT-qPCR of cffDNA (against birth serology exon 7, 10, intron 4 (between 2000–2011) the exon 5, 7 (from 2012)	Diagnostic performance* (GW 5–39, median 19) *inconclusive results not included	100 (100, 100)	100 (100, 100)	100.00	100.00	not calculable	0.0000	407/407 (100)

--, data not reported; CI, confidence interval; cff, cell-free fetal DNA; hrs, hours; IU, international units; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NIPT, noninvasive prenatal testing; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value; PCR, polymerase chain reaction; RCT, randomised controlled trial; RR, relative risk; RT-qPCR, real-time quantitative PCR; UK, United Kingdom; US, United States

* All data reported with inconclusive results considered as Rh D positive unless otherwise indicated. (calculated post-hoc using RevMan 5.3.

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The risk of bias of the included primary studies is based on the quality assessment reported by the systematic review authors.

b. Number of participants and/or samples ranged from 282 to 18 383. Not clear number of women included in the meta-analysis. Data converted from FNR (at risk of sensitisation) and FPR (unnecessary Rh D IgG). PPV and NPV were not provided.

c. Zhu (2014) stated they removed substandard samples. It is assumed this means the 352 inconclusive samples were excluded from the analysis; however, only 44 studies listed in the meta-analysis, it is not clear which two protocols were not included.

d. Studies with less than ten women and subjects with more than one sample excluded.

e. Excluding 183 (5.6%) samples from the meta-analysis: 49 were duplicates (1.5%), 28 (0.86%) were reported in studies of less than 10 women. Of these 77 excluded, 79.22% (61/77) were correctly diagnosed. There were also 106 (3.3%) samples excluded by study authors that were not included in the meta-analysis; 6 not enough specimen, 56 no DNA detected, 44 results unable to be verified of *RHD* gene rearrangements were suspected.

f. 31 samples were not tested due to various reasons; 31 samples excluded as these were in women before GW 24 and a further 26 excluded, with reasons not specified. One sample excluded as cord blood not available after stillbirth and a further 82 pregnancies were ongoing at the time of reporting.

g. Includes 85 inconclusive results (53 positive, 32 negative) counted at Rh D positive; 69% (60/86) were due to mothers' *RHD* null variants, 15% (13/86) were due to fetal *RHD* variants, and 15% (13/86) due to a haemolytic sample and weak or variable amplification.

h. Inconclusive results were minimised by reporting Rh D positive results if any *RHD* sequences were detected in maternal plasma, and in cases in which a pregnant woman was suspected of carrying an *RHD* variant allele. Inconclusive fetal RHD test results issued only when the presence of an *RHD* variant gene in the mother was suggested.

i. Data as reported by Moise 2016. Not including 26 inconclusive results. PPV, NPV, LR+, LR– calculated post-hoc using RevMan 5.3.

Subgroup analyses

A summary of the subgroup analysis from the included systematic reviews is presented in Table 5-31 and Table 5-32.

Method of detection

Mackie 2017 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) over conventional PCR (sensitivity of 92.4; specificity of 95.4). Saramago 2018 noted that, because each country used a different machine to perform NIPT, a subgroup analysis by type of NIPT method was not feasible, as it would be confounded by study location.

Sample source

Geifman-Holtzman 2006 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%) than using DNA or RNA from fetal cells within maternal blood (diagnostic accuracy between 67.7% and 76.3%).

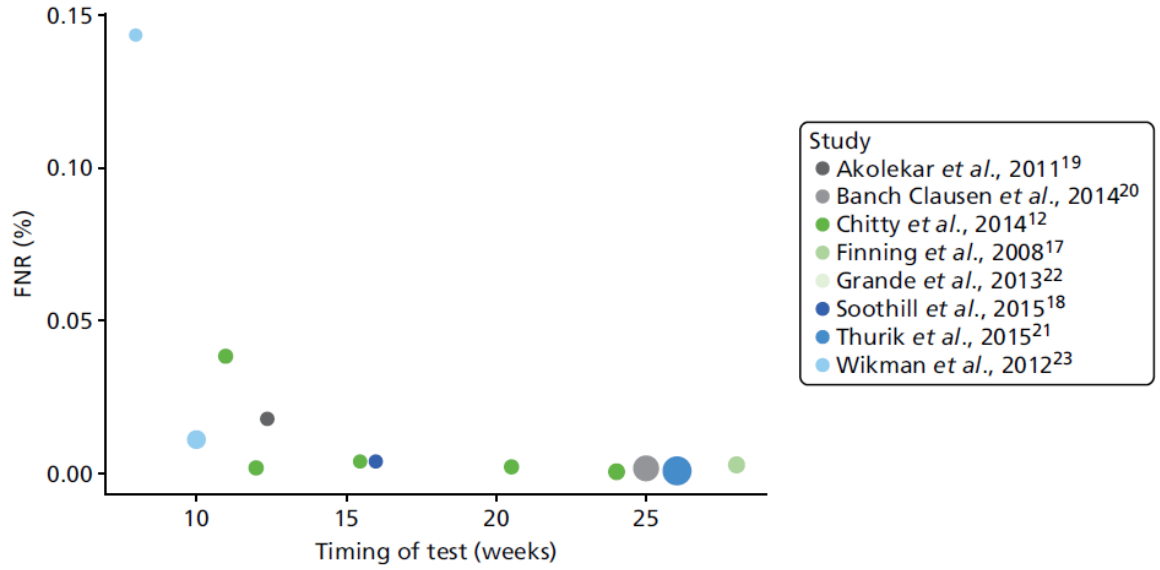
Alloimmunised women

Geifman-Holtzman 2006 also performed a subgroup analysis of the diagnostic performance of NIPT in Rh D negative pregnant women who were alloimmunised and showed diagnostic accuracy to be 91.8% in this group.

Gestational age

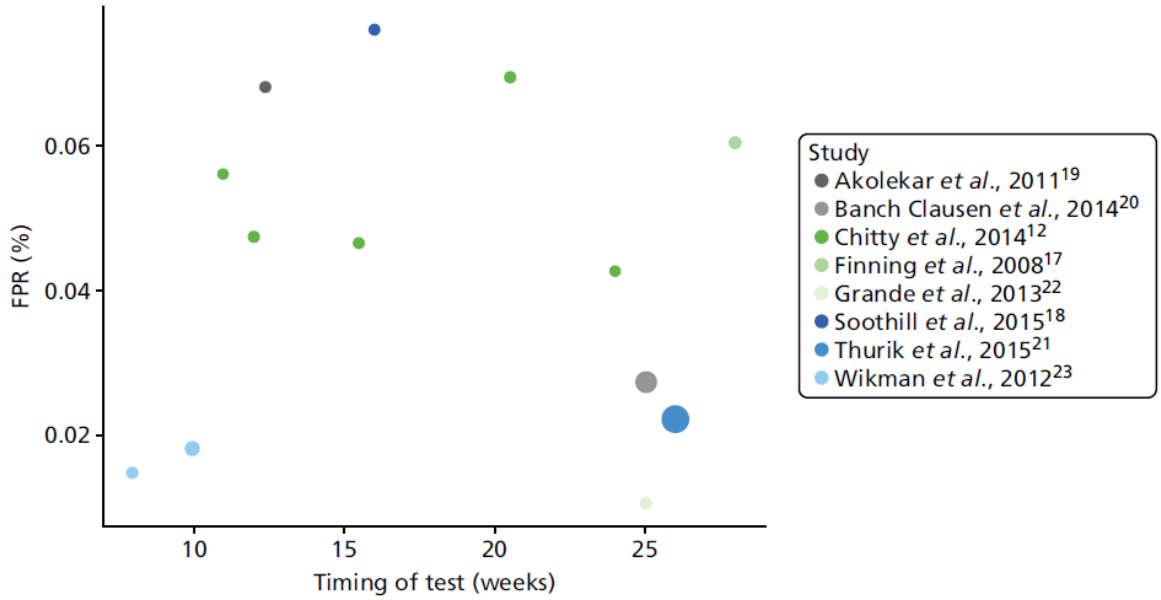
Saramago 2018 performed a subgroup analysis to determine the significance of gestational age on false-negative rate (FNR), false-positive rate (FPR) and number of inconclusive results in the included studies. This is because of concerns that diagnostic sensitivity and specificity is worse in samples collected before 11 weeks' gestation (due to low number of fetal cells). The study authors plotted FNR against gestational age of the included studies (see Figure 5.11), which indicated that FNRs were higher before 11 weeks' gestation but were consistent after 11 weeks' gestation. No obvious relationship between gestational age and FPR (Figure 5.12) or number of inconclusive results was observed (Figure 5.13).

Figure 5.11 False-negative rate by gestational age at time of NIPT.



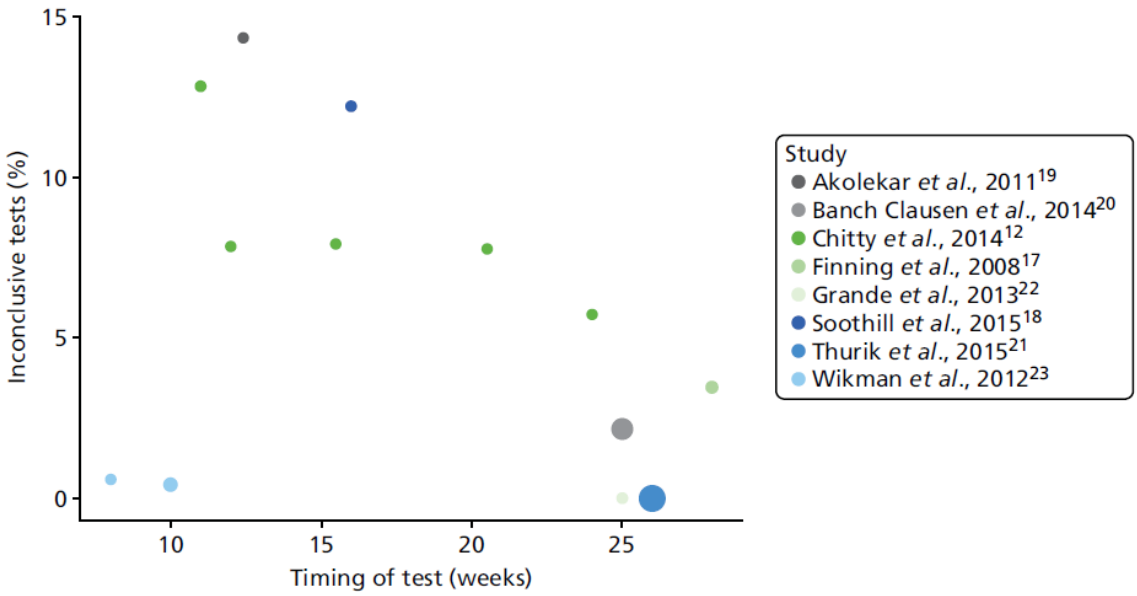
Source: (Saramago et al., 2018)
FNR, false-negative rate

Figure 5.12 False-positive rate by gestational age at time of NIPT.



Source: (Saramago et al., 2018)
FPR, false-positive rate

Figure 5.13 Inconclusive test rate result by gestational age at time of NIPT.



Source: (Saramago et al., 2018)

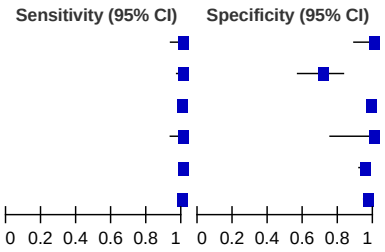
Ethnicity

Saramago 2018 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, therefore, numbers of participants of non-white ethnicity were likely to be few.

Supplementary data provided in the study reported by De Haas 2016[26] (see Figure 5.14) 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).

Figure 5.14 Forest plot of tests: Diagnostic performance subgroup (ethnicity)

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
De Haas 2016 Asian	54	0	0	31	1.00 [0.93, 1.00]	1.00 [0.89, 1.00]
De Haas 2016 Creole	116	15	0	37	1.00 [0.97, 1.00]	0.71 [0.57, 0.83]
De Haas 2016 European	11839	147	6	7486	1.00 [1.00, 1.00]	0.98 [0.98, 0.98]
De Haas 2016 Hindustani	53	0	0	13	1.00 [0.93, 1.00]	1.00 [0.75, 1.00]
De Haas 2016 Mediterranean	602	14	0	263	1.00 [0.99, 1.00]	0.95 [0.92, 0.97]
De Haas 2016 Other	540	10	1	309	1.00 [0.99, 1.00]	0.97 [0.94, 0.98]



Source: (De Haas et al., 2016)

[26] This study population overlaps with the population reported by Thurik 2015 and De Haas 2012 that was included in Saramago 2018.

Table 5-31 Results for the diagnostic performance of noninvasive prenatal screening tests to identify fetal Rh D status against reference standard of postnatal cord blood testing: Rh D negative pregnant women with no preformed anti-D antibodies – diagnostic performance (any timepoint), subgroup analyses

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials in analysis)	Population	Setting (Location)	Comparison	Outcome (timing/source)	Results						
						Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR+ (95% CI)	LR– (95% CI)	Accuracy % (95% CI)
Diagnostic performance, by method of detection against birth blood sample												
Mackie 2017 Level I *High*	N = 9295 (22 studies)	Rh D negative pregnant women Singletons only	Obstetrics and maternity (Argentina, Australia, Austria, Belgium, China, Czech-Republic, Denmark, France, Germany, Ireland, Italy, Korea, Netherlands, Pakistan, Spain, Turkey, UK, USA)	NIPT against birth serology	Diagnostic performance RT-qPCR	99.7 (98.7, 99.9)	98.9 (96.4, 99.7)	NR	NR	90 (20, 383)	0.003 (0.001, 0.013)	OR 25 978 (3125, 215980)
	N = 275 (4 Studies)		(Australia, Brazil, Kuwait, Morocco)		Conventional PCR	92.4 (83.2, 96.8)	95.4 (80.4, 99.1)	NR	NR	20 (4, 96)	0.079 (0.034, 0.1883)	OR 254 (41, 1576)
	N = 1052 (4 studies)		(Germany, Switzerland, USA)		Mass spectrometry	Not calculable	Not calculable	Not calculable	Not calculable	Not calculable	Not calculable	OR will not converge[d]
Diagnostic performance, by source of fetal DNA/RNA												
Geifman-Holtzman 2006 Level I *Serious*	N = 3078 (no. studies not reported)[b]	Rh D negative pregnant women who could be alloimmunised	Obstetrics and maternity (Not stated)	NIPT against birth serology	Diagnostic performance, n/N Maternal blood, fetal cells, DNA 42/62	--	--	--	--	--	--	67.7 (54.5, 78.7)
					Maternal blood, fetal cells, RNA 100/131	--	--	--	--	--	--	76.3 (68.0, 83.1)
					Maternal blood, free DNA 90/98	--	--	--	--	--	--	91.8 (84.1, 96.2)

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials in analysis)	Population	Setting (Location)	Comparison	Outcome (timing/source)	Results						
						Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR+ (95% CI)	LR– (95% CI)	Accuracy % (95% CI)
					Maternal plasma free DNA 2293/2377	--	--	--	--	--	--	96.5 (95.6, 97.2)
					Maternal serum, free DNA 394/410	--	--	--	--	--	--	96.1 (93.6, 97.7)
						A significant improvement (z-test) in diagnostic performance using cffDNA from maternal serum, plasma or blood compared to using DNA or RNA from fetal cells in maternal blood ($p < 0.001$)						
Diagnostic performance in alloimmunised pregnant women												
Geifman-Holtzman 2006 Level I *Serious*	N = 3078 (no. studies not reported)[b]	Rh D negative pregnant women who were alloimmunised	Obstetrics and maternity (Not stated)	NIPT against birth serology	Diagnostic performance, n/N 25.44% of total included women 783/3078	--	--	--	--	--	--	91.8 (NR)

--, data not reported; CI, confidence interval; cff, cell-free fetal DNA; hrs, hours; IU, international units; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NIPT, noninvasive prenatal testing; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value; PCR, polymerase chain reaction; RCT, randomised controlled trial; RR, relative risk; RT-qPCR, real-time quantitative PCR; UK, United Kingdom; US, United States

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The risk of bias of the included primary studies is based on the quality assessment reported by the systematic review authors.

b. Excluding 183 (5.6%) samples from the meta-analysis: 49 were duplicates (1.5%), 28 (0.86%) were reported in studies of less than 10 women. Of these 77 excluded, 79.22% (61/77) were correctly diagnosed. There were also 106 (3.3%) samples excluded by study authors that were not included in the meta-analysis; 6 not enough specimen, 56 no DNA detected, 44 results unable to be verified of *RHD* gene rearrangements were suspected.

Table 5-32 Results for the diagnostic performance of noninvasive prenatal screening tests to identify fetal Rh D status against reference standard of postnatal cord blood testing: Rh D negative pregnant women with no preformed anti-D antibodies – diagnostic performance (by gestational age)

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials in analysis)	Population	Setting (Location)	Comparison	Outcome (timing/source)	Results						
						Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR+ % (95% CI)	LR- % (95% CI)	Diagnostic accuracy % (95% CI)
Diagnostic performance, by gestational age												
Saramago 2018 Level I *Moderate*	N = 42 491 (8 studies)	Rh D negative pregnant women	Obstetrics and maternity (Denmark, The Netherlands, Spain, Sweden, UK)	NIPT against birth serology	Diagnostic performance [b] (median GW 10–28) Bivariate meta-analysis	99.66 (99.24, 99.85)	96.14 (94.18, 97.46)	NR	NR	NR	NR	NR
						Subgroup analysis (timing of NIPT): Meta-regression not performed as no linear trend observed. FNR after 11 weeks' gestations were consistent, irrespective of timing, but were higher before 11 weeks' gestation. No consistent pattern observed with FPRs. *Subgroup analysis (timing of NIPT, number of inconclusive results):* Meta-regression not performed as no linear trend observed. A trend towards reduced number of inconclusive results after GW 11. *Subgroup analysis (ethnicity):* Not feasible as relevant data not reported. *Subgroup analysis (type of machine used to perform NIPT):* Not feasible a relevant data confounded by study location.						
Zhu 2014 Level I *Serious*	N = 6670 (no. not reported)[b]	Rh D negative pregnant women	Obstetrics and maternity (Not stated)	NIPT against birth serology	Diagnostic performance, n/N (1st trimester) 882/898	--	--	--	--	--	--	99.0 (NR)
					(2nd trimester) 282/3322	--	--	--	--	--	--	98.3 (NR)
					(3rd trimester) 2418/2450	--	--	--	--	--	--	96.4 (NR)
Geifman-Holtzman 2006 Level I *Serious*	N = 3078 (no. not reported)[c]	Rh D negative pregnant women who could be alloimmunised	Obstetrics and maternity (Not stated)	NIPT against birth serology	Diagnostic performance, n/N (1st trimester) 218/240	--	--	--	--	--	--	90.8 (86.3, 94.0)
					(2nd trimester) 350/412	--	--	--	--	--	--	85.0 (81.1, 88.2)

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials in analysis)	Population	Setting (Location)	Comparison	Outcome (timing/source)	Results						
						Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR+ % (95% CI)	LR- % (95% CI)	Diagnostic accuracy % (95% CI)
					(3rd trimester) 232/272	--	--	--	--	--	--	85.3 (80.4, 89.2)
						The diagnostic accuracies in the first trimester compared with the accuracies of the second trimester and third trimester (z-test) were significantly different (p = 0.041). There was no statistically significant difference between second and third trimesters (p > 0.05)						

--, data not reported; CI, confidence interval; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NIPT, noninvasive prenatal testing; NPV, negative predictive value; PPV, positive predictive value

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The risk of bias of the included primary studies is based on the quality assessment reported by the systematic review authors.

b. Zhu (2014) stated they removed substandard samples. It is assumed this means inconclusive samples were excluded from the analysis. Only 44 studies listed in the meta-analysis, it is not clear which two protocols were not included.

c. Excluding 183 (5.6%) samples from the meta-analysis: 49 were duplicates (1.5%), 28 (0.86%) were reported in studies of less than 10 women. Of these 77 excluded, 79.22% (61/77) were correctly diagnosed. There were also 106 (3.3%) samples excluded by study authors that were not included in the meta-analysis; 6 not enough specimen, 56 no DNA detected, 44 results unable to be verified of *RHD* gene rearrangements were suspected.

5.5 Question 4 - Risk of failure of Rh D immunoprophylaxis due to increased BMI

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 Rh D immunoglobulin administration?

5.5.1 Background

Question 4 aimed to investigate whether an increasing BMI, maternal weight or any other weight-related factors impacted the effectiveness of Rh D immunoglobulin dosing.

5.5.2 Methods

The population of interest was Rh D negative pregnant OR postpartum women with no preformed anti-D antibodies receiving prophylactic Rh D immunoglobulin. Additional information such as product type, mode of administration, dose and number of doses, timing and technique were also of interest.

As this is a prognostic question the levels of evidence are as follows:

- Level I – a systematic review of two or more Level II studies
- Level II – a prospective cohort study
- Level III-1 – all or none
- Level III-2 – analysis of prognostic factors among persons in a single arm of an RCT
- Level III-3 – a retrospective cohort study

There was no restriction on publication date for this question. Level III studies were assessed from 2014 onwards.

5.5.3 Summary of evidence

The systematic review and hand-searching process identified no Level I studies, two Level II studies (MacKenzie et al., 2004, Woelfer et al., 2004) and two Level III studies (Bichler J. et al., 2003, Koelewijn et al., 2009) that provided some evidence relating maternal body weight to Rh D immunoglobulin administration.

5.5.3.1 Level I

No Level I evidence was identified that examined the effect of increasing BMI on the effectiveness of Rh D immunoglobulin in reducing the incidence of Rh D alloimmunisation in Rh D negative pregnant or postpartum women with no preformed anti-D antibodies.

5.5.3.2 Level II

Two Level II studies (MacKenzie et al., 2004, Woelfer et al., 2004) were identified that informed on the effect of BMI on the risk of failure of Rh D immunoglobulin administration. The characteristics of these studies and the relevant outcomes assessed are summarised in Table 5-33.

Table 5-33 Characteristics and quality of Level II evidence: effect of increasing BMI on risk of failure of Rh D IgG administration

Study ID	Study type *Risk of bias*	Population	Intervention	Prognostic factor	Outcomes
MacKenzie 2006	Prospective Coh, SC *Serious*	Rh D negative pregnant women with possible Rh D positive fetus, nulliparous and multiparous and not known to be sensitised N=45[a]	500 IU Rh D IgG im[b] at 28 and 34 weeks' gestation n = 43[c] D-Gam Oxford, UK	Maternal weight at 28 weeks' gestation BSA	Serum anti-D concentration
Woelfer 2004	Consecutive Coh, SC *Moderate*	Rh D negative women, primiparae and multigravidae N=26[d]	1500 IU Rh D IgG IM within 72 hours postpartum Rhesogam Austria	BMI	Serum anti-D concentration

BMI, body mass index; BSA, body surface area; Coh, cohort; IgG, immunoglobulin; im, intramuscular; IU, international units; iv, intravenous; NR, not reported; SC, single centre

a. One woman withdrew after entry and one woman was given an alternative preparation in error.

b. Two women delivered before the second injection at 34 weeks' gestation. An additional 500 IU Rh D IgG was given postpartum, with additional doses pending the result of the Kleihauer test.

c. 26 of the babies delivered were positive, and 17 were negative.

d. All women gave birth to Rh+ babies, 15 had a normal vaginal delivery, 11 had caesarean section.

MacKenzie 2006 was a prospective cohort study set in Oxford, UK, that recruited 45 Rh D negative pregnant nulliparous and multiparous women who were not known to be sensitised. Two doses of 500 IU Rh D immunoglobulin (D-Gam) were administered antenatally into the deltoid muscle. An additional dose of 500 IU was administered to all mothers postpartum, with additional doses provided pending the result of a maternal Kleihauer test. Serum levels of Rh D immunoglobulin administered antenatally were measured via flow cytometry. The authors evaluated serum concentration with respect to BMI and body surface area (BSA). The study was assessed to have an overall serious risk of bias due insufficient reporting of outcome data. The cohort was too small to provide any useful information relating to the association between BMI and persistence of anti-D antibodies.

Woelfer 2004 was cohort study conducted in Austria that recruited 26 consecutive Rh D negative pregnant women, who were both nulliparous and multigravidae. None of the women had received antenatal Rh D immunoglobulin. Rh D immunoglobulin (1500 IU) was administered in the bottom muscle within 72 hours after delivery and anti-D serum levels were determined by flow cytometry. The effect of BMI on serum levels was evaluated, and a multivariate linear regression model was constructed to extrapolate the effect of increasing BMI on Rh D immunoglobulin serum concentrations. The study was assessed to have a moderate risk of bias, but there was insufficient longer term data to provide useful information relating to association between BMI and incidence of Rh D alloimmunisation in a subsequent pregnancy.

5.5.3.3 Level III

Two Level III studies (Bichler J. et al., 2003, Koelewijn et al., 2009) were identified that informed on the effect of BMI on the risk of failure of Rh D immunoglobulin administration. The characteristics of these studies and the relevant outcomes assessed are summarised in Table 5-34.

Table 5-34 Characteristics and quality of Level III evidence: effect of increasing BMI on anti-D antibodies

Study ID	Study type *Risk of bias*	Population	Intervention	Prognostic factor	Outcomes
Koelewijn 2009	Case-control, MC *Moderate*	Rh D negative women, primiparae N = 381 [a]	1000 IU Rh D IgG [b] at 30 weeks' gestation n = 190 The Netherlands	BMI Body weight	Incidence of Rh D alloimmunisation
Bichler 2003	RCT, open label, MC *Critical*	Rh D negative pregnant women, primigravidae and unsensitised multigravidae N = 18 [c]	1500 IU Rh D IgG IV or IM at 28 weeks' gestation [d] n = 15 [e] Rhophylac Bavaria, Germany	Body weight	Serum anti-D concentration Adverse events

BMI, body mass index; Coh, cohort; IM, intramuscular; IV, intravenous; IU international units; MC, multicentre, RCT, randomised controlled trial; Rh D IgG, Rh D immunoglobulin; SC, single centre

a. 42 women who were sensitised (cases) and 339 controls.

b. Administration route not specified.

c. 18 women with Rh+ partners were screened, two had previously received Rh D IgG, 15 women were treated.

d. If the child was Rh+ a dose of 1500 IU Rh D IgG was administered postnatally.

e. One woman was retested following low Rh D IgG serum concentration and found to be Rh D^{weak} + and excluded. Of the 14 women treated, eight women gave birth to Rh+ babies.

Koelewijn 2009 was a case-control study set in The Netherlands examining 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 covering the years 1999, 2000, 2002, 2003 and 2004. Controls were selected over a 10 month period between September 2002 and June 2003 who had registered a negative red cell antibody screening results in their first trimester (includes Rh D positive and Rh D negative parae-1). RAADP had been available in The Netherlands since 1 July 1998. One dose of 1000 IU Rh D immunoglobulin is administered at 30 weeks' gestation to women pregnant with their first child. The study excluded women who were sensitised between first trimester screening and had a positive screening tests on or after Week 30 of screening. The study was assessed to have an overall moderate risk of bias, with a key concern being confounding and selection bias. The study authors acknowledged an over-representation of women from the primary care setting (midwives, GPs) in the control group (as compared to the obstetric setting) compared with cases. To compensate, weighted data was used in the analysis.

Bichler 2003 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 (IM versus IV route). Rh D negative pregnant women who were nulliparous and multigravidae and were not known to be sensitised were given a dose of 1500 IU Rhophylac at 28 weeks' gestation. Serum Rh D immunoglobulin was measured by flow cytometry and 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.

5.5.4 Results

A GRADE evidence profile summarising the evidence for weight-related factors associated with risk of failure of antenatal or postnatal prophylaxis with Rh D immunoglobulin in Rh D negative pregnant women with no preformed anti-D antibodies are provided in Appendix 6.

5.5.4.1 Incidence of Rh D alloimmunisation (any timepoint)

One study (Koelewijn 2009) was identified that considered whether increasing BMI increased the risk of failure of Rh D immunoprophylaxis (measured by the incidence of Rh D alloimmunisation in a second pregnancy). A summary of the result from this study is presented in Table 5-35.

Koelewijn 2009 examined various risk factors for Rh D alloimmunisation in Dutch primiparae 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) on the incidence of Rh D alloimmunisation; *GRADE: very low quality evidence*.

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 (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 (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 indicated when maternal body weight was measured. Also, the antenatal dose of Rh D immunoglobulin used in this study (1000 IU at 30 weeks' gestation) is different to the current Australian context (625 IU at 28 and 34 weeks' gestation).

Table 5-35 Results for weight-related risk factors for failure of Rh D immunoprophylaxis: Rh D negative women with no preformed anti-D antibodies – Incidence of Rh D alloimmunisation (any timepoint)

Study ID Level of evidence [a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Comparison	Weight-related risk factor	Results			
						Cases Mean ± SD n/N (%)	Controls Mean ± SD n/N (%)	Risk estimate (95% CI)	*Statistical significance* p-value Heterogeneity [b]
Koelewijn 2009 Level III *Moderate*	Cases: N = 42 Controls: N = 339 weighted controls n = 146	Cases: Rh D negative parae-1 women with anti-D antibodies despite RAADP Controls: Rh D positive and negative parae-1 women with no red cell antibodies	Obstetric and maternity (The Netherlands)	Women with newly detected Rh D alloimmunisations vs women with no red cell antibodies	Body mass index	23.8 ± 4.5	24.0 ± 4.5	MD –0.20 (–1.74, 1.34) [c]	*No significant difference* $p = 0.80$ [c]
					Body weight in kg	67.6 ± 11.5	69.6 ± 13.3	MD –2.00 (–6.09, 2.09) [c]	*No significant difference* $p = 0.34$ [c]
					Body weight > 75 kg	NR (21.9)	NR (23.8)	NR	*No significant difference* $p = 0.82$
					Body weight > 100 kg	NR (3.1)	NR (3.3)	NR	*No significant difference* $p = 0.71$
					The multivariate analysis found the following factors to be significantly associated with Rh D alloimmunisation despite RAADP: - post-maturity (≥ 42 weeks' gestation of completed gestation) OR 3.07 (1.02, 9.20) - caesarean section or assisted vaginal delivery OR 2.23 (1.04, 4.74) - maternal age at delivery (years) OR 0.89 (0.80, 0.98)				

CI, confidence interval; kg, kilogram; NHMRC, National Health and Medical Research Council; NR, not reported; OR, odds ratio; RCT, randomised control trial; RAADP, routine anti-D antenatal prophylaxis; SD, standard deviation; vs, versus

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The quality of the included primary studies are based on the quality assessment reported by the systematic review authors.

b. Only applicable to Level I studies with formal meta-analysis. Heterogeneity defined as follows: (i) no significant heterogeneity if $P_{het} > 0.1$ and $I^2 < 25\%$; (ii) mild heterogeneity if $I^2 < 25\%$; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity $I^2 > 50\%$.

c. Calculated post-hoc using RevMan 5.3.

5.5.4.2 Anti-D levels (at any timepoint)

Three studies (Woelfer 2004, MacKenzie 2006, Bichler 2003) were identified that considered whether increasing maternal body weight increased the risk of failure of Rh D immunoprophylaxis, as measured by anti-D serum levels following administration of Rh D immunoglobulin. The studies identified a correlation between higher maternal body weight and lower peak serum anti-D levels; however, sample sizes were small and the evidence was of very low quality. Table 5-36 summarises the results from these studies.

Woelfer 2004 assessed the influence of BMI on measurable anti-D levels after delivery at one, two, and three days, and two weeks after injection and estimated women with a BMI less than or equal to 27 kg/m^2 had significantly higher concentrations of serum anti-D (ng/mL) than women with a BMI greater 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 Rh D Ig G serum concentration 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 week two); *GRADE: very low quality evidence*. The authors note that further research is needed to determine if lower levels of measurable anti-D in obese women correlates to higher rates of Rh D alloimmunisation.

MacKenzie 2006 reported a significant inverse relationship between peak serum concentration of anti-D (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 (28 weeks' gestation); *GRADE: very low quality evidence*. However, the author noted this did not significantly influence duration of persistence of Rh D IgG at 12 weeks after first dose when women with a maternal BSA less than 1.80 m^2, a BSA between 1.8 to 1.99 m^2, and a BSA greater than 2.00 m^2 were compared (p = not reported).

The study by Bichler 2003 estimated the bioavailability of intramuscular Rh D IgG to be 77.8% (95% CI 41% to 96%), noting that the wide confidence interval was influenced by low results observed in two women who weighed more than 80 kg. The six women with a maternal body weight less than 80 kg had a mean anti-D 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 anti-D serum levels, the two women had quantifiable anti-D IgG levels up to last scheduled blood sample (weeks 9 and 11, respectively). The authors suggested that, for overweight women, the administration of IV Rhophylac may be more advantageous. There were no women who were over 80 kg in the IV group.

Table 5-36 Results for weight-related risk factors for failure of Rh D immunoprophylaxis: Rh D negative women with no preformed anti-D antibodies – Anti-D levels (any timepoint)

Study ID Level of evidence [a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Intervention	Outcome (timing)	Results			
						Low BMI	High BMI	Risk estimate	*Statistical significance* *p*-value Heterogeneity [b]
Woelfer 2004 Level II *Moderate*	26	Rh D negative women who had delivered an Rh D positive child	Obstetrics and maternity (Austria)	Rh D IgG within 72 hours of delivery (1500 IU)	Rh D IgG serum concentration, ng/mL, mean (95% CI)	BMI ≤ 27 kg/m^2 (n=12)	BMI > 27 kg/m^2 (n=14)	Estimated [d] for each kg/m^2 higher than 27 kg/m^2	
					(1 day after dose) (2 days after dose) (3 days after dose) (2 weeks after dose)	64.3 (46.7, 81.8) 109.3 (87.2, 131.4) 154.4 (118.8, 190.1) 158.0 (100.9, 215.1)	NR [c]	MD 4.2 (6.4, 2.0) MD 6.0 (10.1, 1.8) MD 7.6 (14.0, 1.2) MD 8.4 (15.8, 1.1)	p = 0.002 p = 0.011 p = 0.025 p = 0.030
					Rh D IgG serum concentration, ng/mL, median (1IQR, 3IQR)	BMI ≤ 27 kg/m^2 (n=12)	BMI > 27 kg/m^2 (n=14)		
					(1 day after dose) (2 days after dose) (3 days after dose) (2 weeks after dose)	39.6 (30.0, 64.3) 99.6 (77.5, 152.5) 114.3 (91.1, 128.9) 143.6 (99.3, 70.7)	32.9 (16.4, 45.7) 54.6 (34.6, 92.5) 94.3 (55.7, 113.9) 96.4 (61.4, 111.4)	NR [d]	*Increasing BMI associated with lower serum anti-D over time* p < 0.001
MacKenzie 2006 Level II *Serious*	45	Rh D negative nulliparae and multiparae women with no preformed anti-D antibodies	Obstetrics and maternity (UK)	Rh D IgG at 28 and 34 weeks' gestation (500 IU)	Persistence of Rh D IgG, n/N (%) (12 weeks [84 days or more] after first dose)	BSA < 1.80 m^2 5/9 (56%)	BSA 1.80–1.99 m^2 3/6 (50%)	BSA > 2.00 m^2 3/6 (50%)	*No significant difference* p = NR
					Rh D IgG peak serum concentration, ng/mL (7 days after first dose)	Correlation with BSA at GW28 Correlation with maternal body weight at GW28		R^2 = 0.299 R^2 = 0.171	*Significant inverse relationship favours low BSA and low maternal body weight* p = 0.002 p = 0.006

Study ID Level of evidence [a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting (Location)	Intervention	Outcome (timing)	Results			
						Low BMI	**High BMI**	**Risk estimate**	***Statistical significance* *p*-value Heterogeneity [b]**
Bichler 2003 Level III *Critical*	14	Rh D negative women with no preformed anti-D antibodies	Obstetrics and maternity (Germany)	Rh D IgG at 28 weeks' gestation (1500 IU)	Rh D IgG peak serum concentration (C_{max}), ng/mL (up to 11 weeks after RAADP)	Body weight < 80 kg (n=6) Mean: 26.6	Body weight > 80 kg (n=2) Pt 9: 6.9 Pt 12: 10.0	Not assessed	Not assessed
						Despite low peak Rh D IgG serum the two women (> 80 kg) had quantifiable Rh D IgG levels up to last scheduled blood sample (weeks 9 and 11 respectively). BMI of subject 9 = 32.29; subject 12 = 26.79			

BMI, body mass index; BSA, body surface area; C_{max}, maximum pharmacokinetic serum concentration; GW, gestational week; IgG, immunoglobulin G; im, intramuscular; IQR, interquartile range; IU, international units; kg, kilogram; MD, mean difference; mL, millilitre; NR, not reported; ng, nanogram; NHMRC, National Health and Medical Research Council; R^2, correlation coefficient; RAADP, routine antenatal anti-D prophylaxis; RCT, randomised control trial; UK, United Kingdom

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The quality of the included primary studies are based on the quality assessment reported by the systematic review authors.

b. Only applicable to Level I studies with formal meta-analysis. Heterogeneity defined as follows: (i) no significant heterogeneity if P_{het} >0.1 and I^2 <25%; (ii) mild heterogeneity if I^2 <25%; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity I^2 >50%.

c. Mean Rh D Ig G levels were skewed to underestimate in women with BMI < 32 kg/m^2 and overestimate in women with BMI > 36 kg/m^2.

d. Based on the general linear model the study authors found each kg/m^2 BMI higher than 27 kg/m^2 reduced the Rh D Ig G serum concentration by the calculated value.

5.5.4.3 Incidence of a positive Keilhauer tests

No studies identified

5.5.4.4 Adverse neonatal events

No studies identified

5.5.4.5 Maternal adverse events

One study was identified that reported maternal adverse events associated with administration of Rh D immunoglobulin (Bichler 2003). A summary of the results from this study is provided in Table 5-37.

Bichler 2003 compared the pharmacokinetics of antenatal Rh D IgG (1500 IU) administered either IV or IM to 14 women. Two of six women in the IV group experienced three adverse events, and three of eight women in the IM group experienced four adverse events. All adverse events were considered not related to the study drug. One woman experienced oesophagitis, three women experienced influenza-like symptoms. One of these women also suffered from neuritis (following postpartum administration of 1500 IU Rh D IgG, route unknown). All adverse events were of mild to moderate severity and resolved completely within 13 days. No statistical analysis was performed.

Table 5-37 Results for weight-related risk factors for failure of Rh D immunoprophylaxis: Rh D negative women with no preformed anti-D antibodies – Adverse maternal events

Study ID Level of evidence[a] *Risk of bias*	Sample size (no. of trials) included in analysis	Population	Setting Location	Comparison	Outcome (timing)	Results			
						Rh D IgG 1500 IU i.v. n/N (%)	Rh D IgG 1500 IU i.m. n/N (%)	Risk estimate (95% CI)	*Statistical significance* p-value Heterogeneity[b]
Bichler 2003 Level III *Critical*	N = 14	Rh D negative women with no preformed anti-D antibodies	Obstetrics and maternity (Germany)	Rh D IgG at GW 28 (1500 IU) IV vs IM	Maternal adverse events	Three events in two women.	Four events in three women.	Not assessed	Events considered not related to the study drug

CI, confidence interval; GW, gestational week; IM, intramuscular; IV, intravenous; IU, international units; NHMRC, National Health and Medical Research Council; RCT, randomised controlled trial; vs, versus

a. NHMRC evidence hierarchy (Merlin et al., 2009). Where only one RCT is identified in a Level I review, the evidence has been considered as Level II. Where the systematic review assesses Level III studies, the evidence has been considered as Level III. The quality of the included primary studies are based on the quality assessment reported by the systematic review authors.

b. Only applicable to Level I studies with formal meta-analysis. Heterogeneity defined as follows: (i) no significant heterogeneity if P_{het} >0.1 and I^2 <25%; (ii) mild heterogeneity if I^2 <25%; moderate heterogeneity if I^2 between 25–50%; substantial heterogeneity I^2 >50%.

6 Cost considerations

The 2003 *Guidelines on the prophylactic use of Rh D immunoglobulin (anti-D) in obstetrics* (NBA, 2003) included a cost-effectiveness analysis Rh D Immunoprophylaxis in both the antenatal and postpartum settings. The model investigated varying costs of Rh D immunoglobulin (imported and domestic supply) and was based on that developed for the previous NHMRC guidelines (NHMRC, 1999). Results were provided as cost per life-year saved with and without any treatment cost savings deducted. Treatment costs were those incurred due to management of a sensitised Rh D negative women during pregnancy and of the management of a neonate at birth[27]. No costs relating to years of disability due to long-term sequelae of HDFN were included.

Cost-effectiveness or resource implications for Rh D immunoprophylaxis were not included as part of the systematic review of the eivdence for the updated 2019 guidelines. However, when developing recommendations, discussions surrounding the issue of costs brought forth the need to identify available evidence relating to this issue. A systematic search specific for cost-effectiveness was not conducted but studies identified in literature search were identified and reviewed. The reference lists of identified studies were also retrieved and reviewed.

Analysis was undertaken on cost-effectiveness studies published since the release of the 2003 Guidelines (see Table as well as those assessing the cost-effectiveness of non-invasive prenatal testing (NIPT). A summary of each study and the issues or constraints identified in those studies are provided in Table 6-1 (RAADP) and Table 6-2 (NIPT).

All identified studies concluded that routine antenatal prophylaxis with Rh D immunoglobulin is cost-effective using one or two doses (Chilcott et al., 2003, NICE, 2008, Pilgrim et al., 2009). Whereas the evidence for cost-effectiveness of targeted antenatal Rh D immunoprophylaxis (subsequent to NIPT) varied (Teitelbaum et al., 2015, Neovius et al., 2016, NICE, 2016, Gordon et al., 2017, Darlington et al., 2018, Saramago et al., 2018). Here, the studies were sensitive to unit costs of the test and additional pathway costs (including supply chain and implementation costs).

[27] The cost of additional maternity and neonatal resources related to Rh D sensitisation were based on a retrospective dataset of 338 pregnancies in Scotland ending in the years 1987 to 1991.

Table 6-1 Summary of studies evaluating cost-effectiveness of routine antenatal anti-D prophylaxis

Study ID	Highlights	Constraints
NICE (2008). Routine antental anti-D prophylaxis for women who are rhesus D negative. NICE technology appraisal guidance [TA156]. 2008.	• The guidance replaces 'The clinical effectiveness and cost effectiveness of routine anti-D prophylaxis for RhD-negative women in pregnancy' (NICE technology appraisal guidance 41) issued in May 2002. The assessment report was prepared by the University of Sheffield, School of Health and Related Research (ScHARR) Pilgrim H, Lloyd-Jones M, Rees A. Routine antenatal anti-D prophylaxis for RhD-negative women (review), November 2007. • Modelled a cohort of Rh D negative primigravidae and multigravidae and conducted additional analyses that combined primigravidae and multigravidae into one group. Treating the combined group with RAADP was compared with giving no RAADP. RAADP can be given as one or two doses in the UK. The comparison resulted in ICERs of £21,156 for Rhophylac, £27,810 for D-Gam, £36,326 for Partobulin SDF and £163,268 for WinRho SDF per QALY gained. (Based on D-Gam costs of £54 per two-dose course per 500IU vial, Partobulin SDF costs £70 two dose course of 1250IU prefilled syringe, Rhophylac costs £46.50 per 1500IU prefilled syringe, WinRho SDF costs £313.50 per 1500IU vial). Concluded that: • RAADP is a cost effective use of NHS resources. • Although it was not possible to recommend a particular product, individual purchasers should use the product with the lowest available local cost, taking into account the acquisition cost as well as the costs associated with administration.	• Age of the study • Not in the Australian context • Cost of differing products • Costs used in managing a pregnancy in a sensitised mother maybe understated • Costs for managing a severe disability were derived from a study may have been limited and that children with a severe disability resulting from HDN were likely to require more NHS resources and a greater range of services than those provided to the young adults in the study • The differences in cost effectiveness of a one or two dose RAADP regime were a result of the differences in price of the products and administration costs
Pilgrim H, Lloyd-Jones M, Rees A (2009). Routine antenatal anti-D prophylaxis for RhD-negative women: a systematic review and economic evaluation. Health Technology Assessment. 2009;13(37): 1–126	• To identify any evidence for advances in the use of RAADP since the 2002 NICE appraisal, and to assess the current clinical effectiveness and cost-effectiveness of RAADP for Rh D negative women. This report updates the assessment of RAADP undertaken on behalf of the NICE by Chilcott and colleagues in 2001. • The health economic model developed for the 2002 NICE appraisal of RAADP was modified to assess the cost-effectiveness of different regimens of RAADP. Of the nine studies in the cost-effectiveness review, only two described a model that could be applicable to the NHS. The economic model modified from the 2002 appraisal suggests that: • the cost per QALY gained of RAADP given to RhD-negative primigravidae versus no treatment is between £9000 and £15,000, and for RAADP given to all RhD-negative women rather than to RhD-negative primigravidae only is between £20,000 and £35,000 depending upon the regimen.	• Refer to NICE 2008
Chilcott J, Lloyd Jones M, Wight J, Forman K, Wray J, Beverley C et al. (2003). A review of the clinical effectiveness and cost-effectiveness of routine anti-D prophylaxis for pregnant women who are rhesus-negative. Health Technol Assess. 2003;7(4): iii–62	• A model-based economic evaluation of offering RAADP to all pregnant women who are Rh D negative, and to Rh D negative primigravidae only, in addition to conventional AADP applicable to the NHS, was performed. This economic evaluation assessed the cost per fetal loss, stillbirth, neonatal or post neonatal death avoided, the cost per life-year gained (LYG) and the cost per QALY gained as a result of disabilities avoided. • The drug costs of treating one pregnancy with two doses of 500 IU are £54.00, and with two doses of 1250 IU are £47.80, at NHS list prices and added an estimated cost of administration of £10. Concluded that: • RAADP given to primigravidae has a cost per LYG that is very good in comparison to other interventions routinely funded by the NHS. • The incremental cost per LYG of giving RAADP to all pregnant women who are Rh D negative is not as good, but there is still roughly a 90% chance of the cost-effectiveness being better than £30,000 per LYG.	• Replaced by Pilgrim et al (2009)

NHS, National Health Service; NICE, National Instituted for Clinical and Care Excellence; RAADP, routine antenatal anti-D prophylaxis; QALY, quality-adjusted life-year; UK, United Kingdom

Table 6-2 Summary of studies assessing cost-effectiveness pf non-invasive prenatal testing for fetal *RHD* status

Study ID	Highlights	Issues
Darlington et al. (2018) Effectiveness and costs of non-invasive foetal *RHD* genotyping in rhesus-D negative mothers: a French multicentric two-arm study of 850 women. BMC Pregnancy and Childbirth 18:496 https://doi.org/10.1186/s12884-018-2114-5	• The paper evaluated the impact of non-invasive fetal *RHD* status determination on the costs of managing Rh D negative pregnant women and on the appropriate use of anti-D prophylaxis in a large sample of Rh D negative pregnant women using individual prospectively collected clinical and economic data. • Non-invasive fetal *RHD* genotyping was performed before 26 weeks' gestation in the experimental arm whereas the control arm participants received usual care. The costs associated with management of mothers in relation to their Rh D negative status (biological tests, anti-D prophylaxis and visits) were calculated from inclusion to the end of the postpartum period. The costs of hospital admissions during pregnancy and delivery were also determined. • A total of 949 women were included by 11 centres between 2009 and 2012, and 850 completed followup, including medical and biological monitoring. Using the costs related to *RHD* status (biological tests, anti-D immunoglobulin injections and visits) the incremental cost-effectiveness ratio (ICER) was calculated to be €578 for each percentage gain in women receiving appropriate management. • Early knowledge of the *RHD* status of the foetus using non-invasive foetal *RHD* genotyping significantly improved the management of Rh D negative pregnancies with a small increase in cost.	• Age of the study • Not in the Australian context (France) • Cost assumptions and inclusion of specific costs need to be validated for the Australian setting • Single 1500 IU dose • Unsure if a centralised or decentralised supply chain or testing facilities
Neovius M, Tiblad E, Westgren M, Kublickas M, Neovius K, Wikman A (2016). Cost-effectiveness of first trimester non-invasive fetal *RHD* screening for targeted antenatal anti-D prophylaxis in RhD-negative pregnant women: a model-based analysis. BJOG.123(8): 1337–46	• The study was undertaken to estimate the cost-effectiveness of first trimester non-invasive fetal *RHD* screening for targeted antenatal versus no RAADP or versus non-targeted RAADP. • Compared with RAADP, targeted prophylaxis was associated with fewer alloimmunisations and lower costs. The savings were from lower costs during pregnancy and delivery, and lower costs of future pregnancies through fewer immunisations. • Based on data from 2008 to 2009 for historical comparators and 2010 to 2011 for Rh D negative pregnant women receiving first trimester fetal *RHD* screening followed by targeted anti-D. • Based on effect data from a population-based cohort study, targeted prophylaxis was associated with lower immunisation risk and costs versus no RAADP. • Based on effect data from theoretical calculations, non-targeted RAADP was predicted to result in lower costs and immunisation risk compared with targeted prophylaxis.	• Age of the study • Not in the Australian context (The Swedish Health Service) • Cost assumptions and inclusion of specific costs need to be validated for the Australian setting • Single 1500 IU dose • Unsure if a centralised or decentralised supply chain or testing facilities
NICE - High-throughput non-invasive prenatal testing for fetal *RHD* genotype Diagnostics guidance Published: 9 November 2016 nice.org.uk/guidance/dg25	• The diagnostics assessment report was prepared by the Centre for Reviews and Dissemination/Centre for Health Economics Technology Assessment Group, University of York. (Yang H, Saramago Goncalves P, Llewellyn A, et al. High-throughput, non-invasive prenatal testing for fetal rhesus D status in RhD-negative women not known to be sensitised to the Rh D antigen: a systematic review and economic evaluation. May 2016.) • Seven studies were identified in a review of existing studies on the cost effectiveness of high-throughput NIPT to determine fetal *RHD* genotype in pregnant women who are D negative and are not sensitised to the D antigen. Results across the existing economic studies were conflicting. • The external assessment group developed a *de novo* economic model designed to assess the cost effectiveness of high-throughput NIPT to determine fetal *RHD* genotype in pregnant women who are D negative and are not sensitised to D antigen. The committee concluded that: • Although the ICER appears to be large, at £1,269,100 saved for each QALY lost, it is very sensitive to changes in the numerator (change in cost) or denominator (change in QALYs), and is therefore subject to substantial uncertainty.	• Age of the study (2015 prices) • Not in the Australian context (The UK National Health Service) • Centralised supply chain and testing facility - The model assumed testing in a facility that is dealing with at least 100,000 samples per year. • Cost assumptions and inclusion of specific costs need to be validated for the Australian setting

Study ID	Highlights	Issues
	• The total costs for using high throughput NIPT for fetal *RHD* genotype to guide antenatal anti-D prophylaxis are not substantially different from the total costs for the current practice of offering antenatal anti-D prophylaxis to all D-negative women, provided that there are no changes to postpartum practice. • High-throughput NIPT for fetal *RHD* genotype is recommended as a cost-effective option to guide antenatal prophylaxis with anti-D immunoglobulin, provided that the overall cost of testing is £24 or less. This will help reduce unnecessary use of a blood product in pregnant women, and conserve supplies by only using anti-D immunoglobulin for those who need it. • Cost savings associated with high-throughput NIPT for fetal *RHD* genotype are sensitive to the unit cost of the test, additional pathway costs and implementation costs. Trusts adopting NIPT should collect and monitor the costs and resource use associated with implementing testing to ensure that cost savings are achieved.	
Saramago, P., Yang, H.et al (2018). 'High-throughput, non-invasive prenatal testing for fetal Rhesus D genotype to guide antenatal prophylaxis with anti-D immunoglobulin: a cost-effectiveness analysis'. BJOG.	• The study evaluated the cost-effectiveness of high-throughput NIPT for fetal *RHD* genotype to guide antenatal prophylaxis with anti-D immunoglobulin compared to RAADP. They used a simulated population of 100,000 RhD negative women not known to be sensitised to the RhD antigen. • A decision tree model was used to characterise the antenatal care pathway in England and the long-term consequences of sensitisation events. The diagnostic accuracy of NIPT was derived from a systematic review and bivariate meta-analysis; estimates of other inputs were derived from relevant literature sources and databases. Women in whom the NIPT was positive or inconclusive continued to receive RAADP, while women with a negative result received none. Five alternative strategies in which the use of NIPT may affect the existing post-partum care pathway were considered. The results suggested that: • NIPT appears cost saving but also less effective than current practice, irrespective of the post-partum strategy evaluated. A post-partum strategy in which inconclusive test results are distinguished from positive results performed best. NIPT is only cost-effective when the overall test cost is £26.60 or less. • NIPT would reduce unnecessary treatment with routine anti-D immunoglobulin and is cost saving when compared to current practice. The extent of any savings and cost-effectiveness is sensitive to the overall test cost.	• Age of the study (2015 prices) • Not in the Australian context (The UK National Health Service) • Centralised supply chain and testing facility • Cost assumptions and inclusion of specific costs need to be validated for the Australian setting
Teitelbaum, L., Metcalfe, A.et al (2015). 'Costs and benefits of non-invasive fetal RhD determination'. Ultrasound Obstet Gynecol, 45 (1), 84-8.	• The purpose of the study was to determine the costs and benefits of implementing targeted antenatal anti-RhD prophylaxis based on non-invasive fetal *RHD* genotyping in unsensitized Rh D negative pregnant women and compare these with the current RAADP program in the Canadian province of Alberta. • A decision analysis model based on a theoretical population representing the total number of pregnancies in Alberta over a 1-year period (n=69 286). A decision tree was created that outlined targeted prophylaxis for unsensitized RhD-negative pregnant women screened for cffDNA (targeted group) vs routine prophylaxis for all unsensitized RhD-negative pregnant women (routine group). • Probabilities at each decision point and costs associated with each resource were calculated from local clinical and administrative data. • Outcomes measured were cost, number of women sensitized and doses of Rh immunoglobulin (RhIG) administered. The authors concluded: • The estimated cost per pregnancy for the routine group was $71.43 compared with $67.20 Canadian dollars in the targeted group. • The sensitization rates per Rh D negative pregnancy were equal, at 0.0012, for the current and targeted programs. • Implementing targeted antenatal anti-RhD prophylaxis would save 4072 doses (20.1%) of Rh D immunoglobulin G over a 1-year period in Alberta when compared to the current program. • The data support the feasibility of a targeted antenatal anti-RhD prophylaxis program, at a lower cost than that of the existing routine prophylaxis program, with no increased risk of sensitization.	• Age of the study • Not in the Australian context (Alberta, Canada) • Cost assumptions and inclusion of specific costs need to be validated for the Australian setting • Single 1500 IU dose • Unsure if a centralised or decentralised supply chain or testing facilities

Study ID	Highlights	Issues
Saramago P, Yang H, Llewellyn A, Walker R, Harden M, Palmer S et al. (2018). 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. 22(13): (https://njl-admin.nihr.ac.uk/document/download/2013309).	• The study was conducted to systematically review the evidence on the diagnostic accuracy, clinical effectiveness and implementation of high-throughput NIPT and to develop a cost-effectiveness model. • They developed a *de novo* probabilistic decision tree-based cohort model that considered four alternative ways in which the results of NIPT could guide the use of Rh D immunoglobulin antenatally and post-partum. Sensitivity analyses were conducted to address key uncertainties and model assumptions. • The *de novo* model suggested that high-throughput NIPT is likely to be cost saving compared with the current practice of providing RAADP to all women who are RhD negative. The extent of the cost saving appeared to be sufficient to outweigh the small increase in sensitisations. However, the magnitude of the cost saving is highly sensitive to the cost of NIPT itself. Concluded: • High-throughput NIPT is sufficiently accurate to detect fetal *RHD* status in RhD-negative women from 11 weeks' gestation and would considerably reduce unnecessary treatment with routine anti-D immunoglobulin, potentially resulting in cost savings of between £485,000 and £671,000 per 100,000 pregnancies if the cost of implementing NIPT is in line with that reflected in the evaluation.	• Centralised supply chain and testing facility • Not in the Australian context (UK) • Cost assumptions and inclusion of specific costs need to be validated for the Australian setting
Gordon LG, Hyland CA, Hyett JA, O'Brien H, Millard G, Flower RL et al. (2017). Noninvasive fetal *RHD* genotyping of RhD negative pregnant women for targeted anti-D therapy in Australia: A cost-effectiveness analysis. Prenat Diagn. 37(12): 1245-53	• The study undertook a cost-effectiveness analysis of non-invasive fetal *RHD* genotyping to target pregnant women for antenatal anti-D prophylaxis therapy. • A decision-analytic model was constructed to compare fetal *RHD* testing and targeted Rh D immunoprophylaxis, with current universal Rh D immunoprophylaxis among pregnant women with Rh D negative blood type. • Model estimates were derived from national perinatal statistics, published literature, donor program records, and national cost sources. One-way sensitivity analyses addressed the uncertainty of variables on the main findings. • The study took a health system perspective including direct costs incurred by hospitals, the NBA, the Australian Red Cross Blood Service but it did not include other third-party or personal out-of-pocket costs. The additional cost of non-invasive *RHD* genotyping is offset by the lower demand and use of anti-D related resources. Some costs were broadly determined in the absence of patient-level data (e.g. costs relating to deliveries of babies with HDFN complications and antenatal hospitalizations) while a few assumptions were necessary such as transportation/packaging costs and compliance levels with anti-D care. Concluded: • Non-invasive *RHD* fetal genotyping for targeted Rh D immunoprophylaxis use in Australia was estimated to generate cost savings for the NBA from reduced use of anti-D products during pregnancy. For the health system, to avoid unnecessary anti-D injections using valuable donated blood, non-invasive *RHD* fetal genotyping produced small cost savings representing 0.05% of the full cost of pregnancy and birth among Rh D negative women. • Given the vulnerable supply of donor plasma and other health concerns, *RHD* genotyping is an economically sound option for Australia.	• Cost assumptions need to be tested • Central supply chain and testing facility - Model assumed 2 fully equipped laboratories capable of 46,000 tests per annum • In Australian context but not a Health Technology Assessment • Did not include other third party costs

HDFN, haemolytic disease of the fetus and newborn; ICER, incremental cost-effectiveness ratio; NBA, National Blood Authority; NICE, National Instituted for Clinical and Care Excellence; non-invasive prenatal testing; RAADP, routine antenatal anti-D prophylaxis; QALY, quality-adjusted life-year; UK, United Kingdom

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Appendix 1 Summary of International Guidance

Summary of International Guidance on the prophylactic use of Rh D Immunoglobulin

SOGC, Canada (Fung et al., 2018)	BCSH, UK and N Ireland (Qureshi et al., 2014)	NICE, UK (NICE, 2008)	Sweden (Tiblad et al., 2013, Neovius et al., 2016)	The Netherlands (de Haas et al., 2014)	Denmark (Clausen et al., 2012)	Italy (Bennardello et al., 2015)	CNGOF, France (Cortey et al., 2006)	WHO, International (WHO)	ACOG, US (Committee on Practice Bulletins-Obstetrics, 2017)
Antenatal prophylaxis									
Universal A single dose of 1500 IU (300 µg) at 28 weeks OR Two doses of 500–600 IU (100–120 µg) may be given; one at 28 weeks and one at 34 weeks (120 µg being the lowest currently available dose)	*Universal* A single dose of 1500 IU (300 µg) should be administered between 28 and 30 weeks OR A minimum dose of 500 IU (100 µg) given at 28 and 34 weeks The single dose regimen may be more cost effective, potentially enabling better compliance and providing logistic benefits.	*Universal* Two doses of 500 IU Rh D IgG, one at 28 weeks and one at 34 weeks gestation OR Two doses of 1000–1650 IU Rh D IgG, one at 28 weeks and one at 34 weeks gestation OR A single dose of 1500 IU Rh D Ig G either at 28 weeks or between 28 and 30 weeks gestation.	*Targeted* A single dose of 1250–1500 IU Rh D IgG given at 28–30 weeks to women for whom a fetal *RHD* typing predicts the presence of an Rh D positive child (replaces no RAADP)	*Targeted* A single dose of 1000 IU Rh D IgG given at 30 weeks to women for whom a fetal *RHD* typing predicts the presence of an Rh D positive child	*Targeted* A single dose of 1250–1500 IU Rh D IgG given at 29 weeks to women for whom a fetal *RHD* typing predicts the presence of an Rh D positive child (replaces no RAADP)	*Universal* A single dose of 1500 IU (300 µg) Rh D IgG offered at 27–29 weeks	*Universal* A single dose of 1500 IU (300 µg) Rh D IgG offered at 28 weeks (+/− 1 week) Abstention of Rh prophylaxis is possible if the alleged father is certified RhD negative or if the fetal RhD genotype is confirmed negative	*Not recommended* Antenatal prophylaxis with anti-D immunoglobulin in non-sensitised Rh-negative pregnant women at 28 and 34 weeks of gestation to prevent RhD alloimmunisation is recommended only in the context of rigorous research.	*Universal* A single dose of 1500 IU (300 µg) Rh D IgG should be offered at 28 weeks of gestation. [a]

SOGC, Canada (Fung et al., 2018)	BCSH, UK and N Ireland (Qureshi et al., 2014)	NICE, UK (NICE, 2008)	Sweden (Tiblad et al., 2013, Neovius et al., 2016)	The Netherlands (de Haas et al., 2014)	Denmark (Clausen et al., 2012)	Italy (Bennardello et al., 2015)	CNGOF, France (Cortey et al., 2006)	WHO, International (WHO)	ACOG, US (Committee on Practice Bulletins-Obstetrics, 2017)
A repeat antepartum dose of Rh Ig G is generally not required at 40 weeks, provided that the antepartum injection was given no earlier than 28 weeks									
Sensitising event prophylaxis in the first 12 weeks									
Minimum 600 IU (120 µg) given for: · miscarriage, · threated abortion, · induced abortion, · ectopic pregnancy, · molar pregnancy (may be withheld if complete mole is certain), · chorionic villous sampling Minimum 1200 IU (240 µg) given for:	Minimum 250 IU (50 µg) given following: · ectopic pregnancy, · molar pregnancy, · therapeutic termination of pregnancy and · cases of uterine bleeding where this is repeated, heavy or associated with abdominal pain. A test for FMH is not required.	Offer 250 IU (50 µg) to all Rh D negative women who have a surgical procedure to manage an ectopic pregnancy or a miscarriage.	*NA*	*NA*	*NA*	May give Rh D IgG following: · spontaneous abortion without dilation and curettage and · pharmacologically induced abortions. A minimum dose of Rh D IgG is sufficient, i.e. from 250 to 600 IU (50-120 µg)	Delay should be less than 72 hours sensitising event. IV administration of Rh D IgG allows immediate neutralization of D positive fetal red blood cells and should be, if possible, preferred to IM administration.	*NA*	At least 50 µg Rh D IgG should be considered after spontaneous first-trimester miscarriage, especially those that are later in the first trimester. 250–600 IU (50 µg–120 µg) Rh D IgG should be given after pregnancy termination, either medical or surgical. Rh D IgG should be given to

SOGC, Canada (Fung et al., 2018)	BCSH, UK and N Ireland (Qureshi et al., 2014)	NICE, UK (NICE, 2008)	Sweden (Tiblad et al., 2013, Neovius et al., 2016)	The Netherlands (de Haas et al., 2014)	Denmark (Clausen et al., 2012)	Italy (Bennardello et al., 2015)	CNGOF, France (Cortey et al., 2006)	WHO, International (WHO)	ACOG, US (Committee on Practice Bulletins-Obstetrics, 2017)
· amniocentesis and cordocentesis If Rh D IgG is not given within 72 hours, it should be given as soon as the need is recognised, for up to 28 days after delivery or other potentially sensitising event	Rh D IgG should be administered as soon as possible and always within 72 hrs of the event. If this deadline has not been met, some protection may be offered if Rh D IgG is given up to 10 days after the sensitising event.	Do not offer Rh D IgG to women who receive solely medical management for an ectopic pregnancy or miscarriage or have a threatened miscarriage or have a complete miscarriage or have a pregnancy of unknown location. Do not use a Kleihauer test for quantifying FMH.				If not administered within 72 hours, an attempt to avoid immunisation must be made with the administration of Rh D IgG up to 10 days (and as many as 28 days) after the event.	After a first injection of Rh D IgG, if repetition of potential sensitising events occurs, abstention of prophylaxis is possible depending on the previous administrated dose (protection lasts 6 weeks for 200 µg and 9 weeks for 300 µg) and the amount of FMH.		· women who are suspected of molar pregnancy and undergo a uterine evacuation. · all invasive diagnostic procedures such as chorionic villus sampling or amniocentesis There is insufficient evidence regarding threatened pregnancy loss at or before 12 weeks gestation.
Postpartum dose									
Targeted 1500 IU (300 µg) IM or IV should be given within 72 hours to a non-sensitised Rh D negative woman who delivers an Rh D positive baby	*Targeted* At least 500 IU Rh D IgG should be given within 72 hours to a non-sensitised Rh D negative woman who delivers an Rh D positive baby			*Targeted* 1000 IU (200 µg) given to women for whom a fetal *RHD* typing predicts the presence of an Rh D positive child.	A single dose of 1250–1500 IU (250 to 300 µg) be given within 72 hours to a non-sensitised Rh D negative woman who delivers an Rh D positive baby.	*Targeted* All non-immunised Rh D negative women who have delivered a Rh D positive (or weak D) neonate (or stillborn baby) Must be given a within 72 hours of delivery.	NA	Rh D IgG given within 72 hours after childbirth reduces the risk of alloimmunisation in Rh-negative women who have given birth to an Rh-positive infant. The evidence on the optimal dose is limited.	Prophylactic Rh D IgG should be offered to unsensitized Rh D-negative women if the infant is confirmed to be Rh D positive within 72 hours of delivery.

SOGC, Canada (Fung et al., 2018)	BCSH, UK and N Ireland (Qureshi et al., 2014)	NICE, UK (NICE, 2008)	Sweden (Tiblad et al., 2013, Neovius et al., 2016)	The Netherlands (de Haas et al., 2014)	Denmark (Clausen et al., 2012)	Italy (Bennardello et al., 2015)	CNGOF, France (Cortey et al., 2006)	WHO, International (WHO)	ACOG, US (Committee on Practice Bulletins-Obstetrics, 2017)
Alternatively, give 600 IU (120 µg) Rh D IgG IM or IV within 72 hours of delivery, with testing and additional Rh D IgG given for FMH over 6 mL of fetal red blood cells.	Maternal samples should be tested for FMH and additional dose(s) given as guided by FMH tests.			No cord serology is conducted except in cases of a twin pregnancy (if both are Rh D positive, two anti-D dosages are administered), or the fetal *RHD* status could not be determined / is inconclusive.					

µg, micrograms; ACOG, The American College of Obstetricians and Gynecologists; BCSH, British Committee for Standards in Haematology; CNGOF, French College of Obstetricians and Gynaecologists; FMH, fetomaternal haemorrhage; IU, international units; IgG; immunoglobulin; IM, intramuscular; IV, intravenous; NA, not available; NICE, The National Institute for Health and Care Excellence; RAADP, Routine antenatal anti-D prophylaxis; SOGC, The Society of Obstetricians and Gynaecologists of Canada; US, United States; WHO, World Health Organisation

a. Although administration of anti-D immune globulin at 28 weeks of gestation is highly effective, pharmacokinetic studies suggest that levels of anti-D vary between women and some may not have adequate anti-D levels at delivery (28). In the past, some authorities advised giving a second dose of Rh D immune globulin in women who have not given birth 12 weeks after receiving their antenatal dose (29). However, the vast majority of women who give birth more than 12 weeks after receiving antenatal Rh D immune globulin do not become alloimmunised. Because of this low risk of alloimmunisation and the fact that 40% of infants of Rh D-negative women will be Rh D negative, most guidelines do not recommend that a second dose of anti-D immune globulin be given until after delivery when newborn Rh D typing becomes available.

Appendix 2 Research questions

Question number	**1**				**Notes**
Date of consideration	5 October 2017				
New Question (in full)	**In Rh D negative pregnant women with no preformed anti-D, does *universal*[a] routine antenatal prophylaxis with Rh D immunoglobulin (1 or 2 doses) prevent Rh D alloimmunisation?**				
Subquestions (in full)	**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?**				The evidence for this question will come from the same evidence base as identified for the above question – so it is included as a subquestion rather than a separate question
Question type	*Population*	*Intervention*	*Comparator*	*Outcome*	*Importance of outcome*[b]
Main Question (Intervention)	Rh D negative pregnant women with no preformed anti-D	Routine antenatal prophylactic Rh D immunoglobulin Stratify by: • 1 or 2 doses • 1 dose only • 2 doses only	Placebo or no routine antenatal prophylactic Rh D immunoglobulin	• incidence of Rh D alloimmunisation[c] • incidence of a positive test for fetomaternal haemorrhage[d] (e.g. Keilhauer test, flow cytometry) • adverse neonatal events (e.g. jaundice) • adverse maternal events attributed to anti-D (e.g. allergic response, infection)	Critical Not Important If available[e] If available[e]
Subquestion *(Intervention)*	Rh D negative pregnant women with no preformed anti-D	1-dose routine antenatal prophylactic Rh D immunoglobulin	2-dose routine antenatal prophylactic Rh D immunoglobulin	• incidence of Rh D alloimmunisation • adverse neonatal events (e.g. jaundice) • adverse maternal events (e.g. allergic response, infection)	Critical If available[e] If available[e]

[a] Includes all pregnant women who are Rh D negative with no preformed anti-D.

[b] Critical, important or resource use.

[c] Also known as Rh D sensitisation. Defined as the presence of antibody to D antigen in maternal serum detected during the current pregnancy, postpartum or a subsequent pregnancy. Measured as a dichotomous outcome (present or not present).

[d] The ERG debated whether to include 'incidence of a positive Kleihauer test' as an outcome. Given its inclusion in the 2015 Cochrane review (http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD000020.pub3/full) the ERG agreed to include in this review, but have noted the outcome as not important.

[e] Data will be extracted for these outcomes if they are available in the studies included for the critical outcome – Rh D alloimmunisation. Additional searches to identify studies for these outcomes will not be conducted.

Additional information					
Data to extract	Number of pregnancies	Product type Mode of administration Number of doses Dosage Timing	Subquestion only Product type Mode of administration Dosage Timing	Rh D alloimmunisation Timing (i.e., during pregnancy, postpartum [after birth of a Rh positive infant up to 12 months] and subsequent pregnancy) Kleihauer test / flow cytometry At potentially sensitising events and postpartum [after birth of a Rh positive infant]) Adverse neonatal events Timing (current or subsequent pregnancy) and severity Adverse maternal events Timing and severity	

Source: Anti-D scoping report (Health Research Consulting, November 2017)

Question number	**2**				**Notes**
Date of consideration	05 October 2017				
New Question (in full)	**In Rh D negative women with no preformed anti-D who have experienced one of the following first trimester[f] sensitising events – abdominal trauma, molar pregnancy, ectopic pregnancy, spontaneous miscarriage, threatened miscarriage or medical termination of pregnancy (with/without a curette), does *universal*[g] first trimester sensitising event prophylaxis with Rh D immunoglobulin prevent Rh D alloimmunisation?**				
Subquestions (in full)	--				
Question type	*Population*	*Intervention*	*Comparator*	*Outcome*	*Importance of outcome*[h]
Main Question *(intervention)*	Rh D negative women with no preformed anti-D with a first trimester sensitising event, specifically: • abdominal trauma • molar pregnancy • ectopic pregnancy • spontaneous miscarriage • threatened miscarriage • medical termination of pregnancy (with/without a curette)	First trimester sensitising event prophylactic Rh D immunoglobulin	Placebo or no first trimester sensitising event prophylactic Rh D immunoglobulin	• incidence of Rh D alloimmunisation[i] • incidence of a positive test for fetomaternal haemorrhage[j] (e.g. (Kleihauer test, flow cytometry) • adverse neonatal events (e.g. jaundice) • adverse maternal events attributed to anti-D (e.g. allergic response, infection)	Critical Not important If available[k] If available[k]
Additional information					
Data to extract	Number of pregnancies Timing of sensitising event Nature of sensitising event Use of curette	Product type Mode of administration Number of doses Dosage Timing		<u>Rh D alloimmunisation</u> Timing (i.e., during pregnancy, postpartum [after birth of a Rh positive infant up to 12 months] and subsequent pregnancy) <u>Kleihauer test / flow cytometry</u> At potentially sensitising events and postpartum [after birth of a Rh positive infant]) <u>Adverse neonatal events</u> Timing (current or subsequent pregnancy) and severity <u>Adverse maternal events</u> Timing and severity	

Source: Anti-D scoping report (Health Research Consulting, November 2017)

[f] The definition of first trimester varies across countries and for this review will be defined by the literature. The definition used by each included study should be extracted.

[g] Includes all pregnant women who are Rh D negative with no preformed anti-D.

[h] Critical, important or resource use.

[i] Also known as Rh D sensitisation. Defined as the presence of antibody to D antigen in maternal serum detected during the current pregnancy, postpartum or a subsequent pregnancy. Measured as a dichotomous outcome (present or not present).

[j] The ERG debated whether to include 'incidence of a positive Kleihauer test' as an outcome. Given its inclusion in the 2015 Cochrane review (http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD000020.pub3/full) the ERG agreed to include in this review, but have noted the outcome as not important.

[k] Data will be extracted for these outcomes if they are available in the studies included for the critical outcome – Rh D alloimmunisation. Additional searches to identify studies for these outcomes will not be conducted.

Question number	**3**				**Notes**
Date of consideration	05 October 2017				
New Question (in full)	**In Rh D negative pregnant women with no preformed anti-D, does *targeted*[l] routine antenatal or sensitising event prophylaxis to women with a Rh D positive fetus increase the incidence of Rh D alloimmunisation compared with *universal*[m] routine antenatal or sensitising event prophylaxis?**				
Subquestions (in full)	**In Rh D negative pregnant women with no preformed anti-D, what is the diagnostic accuracy of noninvasive prenatal screening to identify fetal Rh D status?**				
Question type	*Population*	*Intervention/Test*	*Comparator and/or reference standard*	*Outcome*	*Importance of outcome*[n]
Main Question *(Screening)*	Rh D negative pregnant women with no preformed anti-D	*Targeted* administration of prophylactic Rh D immunoglobulin (based on noninvasive prenatal screening) Stratify by: • any prophylaxis • routine antenatal prophylaxis • sensitising event antenatal prophylaxis	*Universal* administration of prophylactic Rh D immunoglobulin Stratify by: • any prophylaxis • routine antenatal prophylaxis • sensitising event antenatal prophylaxis	• incidence of Rh D alloimmunisation[o] • utilisation of anti-D • incidence of a positive test for fetomaternal haemorrhage[p] (e.g. Kleihauer test, flow cytometry) • adverse neonatal events (e.g. jaundice) • adverse maternal events attributed to anti-D (e.g. allergic response, infection)	Critical Resource use Not important If available[q] If available[q]
Subquestion *(Diagnostic)*	Rh D negative pregnant women with no preformed anti-D	Noninvasive prenatal testing for fetal Rh D status	Postnatal cord blood testing (or other neonatal sample) for fetal Rh D status Other noninvasive fetal RhD determination	• sensitivity • specificity • false positives • false negatives • positive likelihood ratio • negative likelihood ratio	Critical Critical Critical Critical Important Important
Additional information					

[l] Includes pregnant women who are Rh D negative with no preformed anti-D with a Rh D positive fetus identified via first trimester noninvasive prenatal screening.

[m] Includes all pregnant women who are Rh D negative with no preformed anti-D.

[n] Critical, important or resource use.

[o] Also known as Rh D sensitisation. Defined as the presence of antibody to D antigen in maternal serum detected during the current pregnancy, postpartum or a subsequent pregnancy. Measured as a dichotomous outcome (present or not present).

Data to extract	Number of pregnancies BMI	<u>Screening question</u> Product type Number of doses Dosage Timing Testing methodology Timing <u>Diagnostic question</u> Testing methodology Timing	<u>Screening question</u> Product type Number of doses Dosage Timing <u>Diagnostic question</u> Testing methodology Timing	<u>Rh D alloimmunisation</u> Timing (i.e., during pregnancy, postpartum [after birth of a Rh positive infant up to 12 months] and subsequent pregnancy) <u>Utilisation</u> Rates <u>Kleihauer test /flow cytometry</u> At potentially sensitising events and postpartum [after birth of a Rh positive infant]) <u>Adverse neonatal events</u> Timing (current or subsequent pregnancy) and severity <u>Adverse maternal events</u> Timing and severity <u>Diagnostic accuracy</u> Timing of test	

Source: Anti-D scoping report (Health Research Consulting, November 2017)

[p] The ERG debated whether to include 'incidence of a positive Kleihauer test' as an outcome. Given its inclusion in the 2015 Cochrane review (http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD000020.pub3/full), the ERG agreed to include in this review, but have noted the outcome as not important.

[q] Data will be extracted for these outcomes if they are available in the studies included for the critical outcome – Rh D alloimmunisation. Additional searches to identify studies for these outcomes will not be conducted.

Question number	**4**			**Notes**
Date of consideration	05 October 2017			
New Question (in full)	**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?**			
Subquestions (in full)	--			
Question type	*Population*	*Prognostic/Risk factor*	*Outcome*	*Importance of outcome*[r]
Main Question *(prognostic)*	Rh D negative pregnant or postpartum women with no preformed anti-D receiving prophylactic Rh D immunoglobulin Stratify by • pregnant women • postpartum women	• BMI (dichotomous or continuous) • weight • any other weight-related factors examined	• incidence of Rh D alloimmunisation[s] • anti-D levels [t] • incidence of a positive test for fetomaternal haemorrhage (e.g. (Kleihauer test, flow cytometry)[u] • adverse neonatal events (e.g. jaundice) • adverse maternal events (e.g. allergic response, infection)	Critical Critical (if data for Rh D alloimmunisation is not available) Not important If available If available (particularly if increased dose or different mode of administration/technique used)
Additional information				
Data to extract	Product type Mode of administration Number of doses/dosage Timing of administration Administration technique	Specific details of weight-related risk factors	<u>Rh D alloimmunisation</u> Timing (i.e., during pregnancy, postpartum [after birth of a Rh positive infant] up to 12 months and subsequent pregnancy) <u>Anti-D levels</u> Timing <u>Kleihauer test /flow cytometry</u> At potentially sensitising events and postpartum [after birth of a Rh positive infant]) <u>Adverse neonatal events</u> Timing (current or subsequent pregnancy) and severity <u>Adverse maternal events</u> Timing and severity	

Source: Anti-D scoping report (Health Research Consulting, November 2017)

[r] Critical, important or resource use.

[s] Also known as Rh D sensitisation. Defined as the presence of antibody to D antigen in maternal serum detected during the current pregnancy, postpartum or a subsequent pregnancy. Measured as a dichotomous outcome (present or not present).

[t] This is a surrogate outcome. Measured as a continuous outcome (actual anti-D Level In maternal blood). If this is used instead of Rh D alloimmunisation will need background research to look for evidence of link between lower anti-D levels and alloimmunisation.

[u] The ERG debated whether to include 'incidence of a positive Kleihauer test' as an outcome. Given its inclusion in the 2015 Cochrane review (http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD000020.pub3/full), the ERG agreed to include in this review, but have noted the outcome as not important.

Appendix 3 Sample data extraction forms

STUDY DETAILS: SR/MA			
Citation			
Affiliation/Source of funds			
Study design	**Level of evidence**	**Location**	**Setting**
Intervention		**Comparator**	
Population characteristics			
Length of followup		**Outcomes measured**	
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			
Rating: Description:			
RESULTS:			

Outcome **No. trials (No. subjects)**	**[intervention]** **n/N (%)** **Mean ± SD**	**[comparator]** **n/N (%)** **Mean ± SD**	**Risk estimate (95% CI)**	***Statistical significance*** ***P-value*** **Heterogeneity** ***P-value (I^2)***
[intervention 1] versus [intervention 2]				
[outcome 1]			RR 1.00 [0.68, 1.48]	*Favours <intervention/comparator> or No significant difference* P = X <Substantial/moderate/mild> or No significant heterogeneity P = X (I^2 = X)
[outcome 2]			MD	
[outcome 3]				
[intervention 1] versus [intervention 3]				
[outcome 1]				
[outcome 2]				
[outcome 3]				

EXTERNAL VALIDITY
Generalisability (relevance of the study population to the Guidelines target population)
Applicability (relevance of the evidence to the Australian health care system)
Additional comments

CI, confidence interval; ITT, intention-to-treat; MD, mean difference; PP, per protocol; RCT, randomised controlled trial; RR, relative risk; SD, standard deviation

STUDY DETAILS: RCT				
Citation				
Affiliation/Source of funds				
Study design	**Level of evidence**		**Location**	**Setting**
Intervention			**Comparator**	
Population characteristics				
Length of followup			**Outcomes measured**	
INTERNAL VALIDITY				
Overall risk of bias (descriptive)				
Rating: Description:				
RESULTS				
Population analysed	**Intervention**		**Comparator**	
Randomised				
Efficacy analysis (ITT)				
Efficacy analysis (PP)				
Safety analysis				
Outcome	**Intervention** **n/N (%)** **Mean ± SD**	**Comparator** **n/N (%)** **Mean ± SD**	**Risk estimate (95% CI)**	***Statistical significance*** ***P-value***
[intervention 1] versus [intervention 2]				
			RR	*Favours <intervention/ comparator> or No significant difference* *P = X*
			MD	
[intervention 1] versus [intervention 3]				
EXTERNAL VALIDITY				
Generalisability (relevance of the study population to the Guidelines target population)				
Applicability (relevance of the evidence to the Australian health care system)				
Additional comments				

CI, confidence interval; ITT, intent-to-treat; NA, not applicable; NR, not reported; PP, per protocol; RBC, red blood cell; RCT, randomised controlled trial;

STUDY DETAILS: Cohort / Case-control			
Citation			
Affiliation/Source of funds			
Study design	**Level of evidence**	**Location**	**Setting**
Intervention		**Comparator**	
Population characteristics			
Length of followup		**Outcomes measured**	
Method of analysis			
INTERNAL VALIDITY			
Overall risk of bias (descriptive)			
Rating: Description:			
RESULTS			

Population analysed	**Intervention**		**Comparator**	
Available				
Analysed				
Outcome	**Intervention** **n/N (%)** **Mean ± SD**	**Comparator** **n/N (%)** **Mean ± SD**	**Risk estimate (95% CI)**	***Statistical significance*** ***P-value***
[intervention] vs [comparator]				
				Favours <intervention/ comparator> or No significant difference P = X
				Favours <intervention/ comparator> or No significant difference P = X

EXTERNAL VALIDITY
Generalisability (relevance of the study population to the Guidelines target population)
Applicability (relevance of the evidence to the Australian health care system)
Additional comments

CI, confidence interval; ITT, intention-to-treat; PP, per protocol; RCT, randomised controlled trial; SD, standard deviation

Appendix 4 Sample risk of bias forms

Level I – Systematic review of RCTs

Question	Comments	Judgement
1. Was an 'a priori' design provided?	The research question and inclusion criteria should be established before the conduct of a review.	Yes No Can't answer Not applicable
2. Was there duplicate study selection and data extraction?	There should be at least two independent data extractors and a consensus procedure for disagreements should be in place.	Yes No Can't answer Not applicable
3. Was a comprehensive literature search performed?	At least two electronic sources should be searched. The report must include years and databases used (e.g. Central, EMBASE, and MEDLINE). Key words and/or MESH terms must be stated and where feasible the search strategy should be provided. All searches should be supplemented by consulting current contents, reviews, textbooks, specialised registers, or experts in the particular field of study, and by reviewing the references in the studies found.	Yes No Can't answer Not applicable
4. Was the status of publication (i.e. grey literature) used as an inclusion criterion?	The authors should state that they searched for reports regardless of their publication type. The authors should state whether or not they excluded any reported (from the systematic review), based on their publication status, language, etc.	Yes No Can't answer Not applicable
5. Was a list of studies (included and excluded) provided?	A list of included and excluded studies should be provided	Yes No Can't answer Not applicable
6. Were the characteristics of the included studies provided?	In an aggregated form such as a table, data from the original studies should be provided on the participants, interventions and outcomes. The ranges of characteristics in all the studies analysed e.g. age, race, sex, relevant socioeconomic data, disease status, duration, severity, or other diseases should be reported.	Yes No Can't answer Not applicable
7. Was the scientific quality of the included studies assessed and documented?	'A priori' methods of assessment should be provided (e.g. for effectiveness studies if the author(s) chose to include only randomised, double-blind, placebo controlled studies, or allocation concealment as inclusion criteria); for other types of studies alternative items will be relevant.	Yes No Can't answer Not applicable
8. Was the scientific quality of the included studies used appropriately in formulating conclusions?	The results of the methodological rigour and scientific quality should be considered in the analysis and the conclusions of the review, and explicitly stated in formulating recommendations.	Yes No Can't answer Not applicable
9. Were the methods used to combine the findings of studies appropriate?	For the pooled results, a test should be done to ensure the studies were combinable, to assess their homogeneity (i.e. Chi-squared test for homogeneity, I^2). If heterogeneity exists a random effects model should be used and/or the clinical appropriateness of combining should be taken into consideration (i.e. is it sensible to combine?).	Yes No Can't answer Not applicable
10. Was the likelihood of publication bias assessed?	An assessment of publication bias should include a combination of graphical aids (e.g. funnel plot, other available tests) and/or statistical tests (e.g. Egger regression test).	Yes No Can't answer Not applicable
11. Was the conflict of interest stated?	Potential sources of support should be clearly acknowledged in both the systematic review and the included studies.	Yes No Can't answer Not applicable
Overall risk of bias		

Source: Shea et al. 2007. BMC Medical Research Methodology 7:10 doi:10.1186/1471-2288-7-10 http://amstar.ca/Amstar_checklist.php

Level I - Systematic review of Observational studies

Question	Comments	Judgement
1. Did the research questions and inclusion criteria for the review include the components of the PICO?	The research question and inclusion criteria should be established before the conduct of a review.	Yes No Partial yes
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?		Yes No Partial yes
3. Did the review authors explain their selection of the study designs for inclusion in the review?	The authors should state that they searched for reports regardless of their publication type. The authors should state whether or not they excluded any reported (from the systematic review), based on their publication status, language, etc.	Yes No Partial yes
4. Did the review authors use a comprehensive literature search strategy?	At least two electronic sources should be searched. The report must include years and databases used (e.g. Central, EMBASE, and MEDLINE). Key words and/or MESH terms must be stated and where feasible the search strategy should be provided. All searches should be supplemented by consulting current contents, reviews, textbooks, specialised registers, or experts in the particular field of study, and by reviewing the references in the studies found.	Yes No Partial yes
5. Did the review authors perform study selection in duplicate?	There should be at least two independent reviewers and a consensus procedure for disagreements should be in place.	Yes No Partial yes
6. Did the review authors perform data extraction in duplicate?	There should be at least two independent data extractors and a consensus procedure for disagreements should be in place.	Yes No Partial yes
7. Did the review authors provide a list of excluded studies and justify the exclusions?	A list of included and excluded studies should be provided	Yes No Partial yes
8. Did the review authors describe the included studies in adequate detail?	In an aggregated form such as a table, data from the original studies should be provided on the participants, interventions and outcomes. The ranges of characteristics in all the studies analysed e.g. age, race, sex, relevant socioeconomic data, disease status, duration, severity, or other diseases should be reported.	Yes No Partial yes
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	'A priori' methods of assessment should be provided.	Yes No Partial yes
10. Did the review authors report on the sources of funding for the studies included in the review?	Potential sources of support should be clearly acknowledged in both the systematic review and the included studies.	Yes No Partial yes
11. If meta-analysis was performed, did the review authors use appropriate methods for statistical combination of results?	For the pooled results, a test should be done to ensure the studies were combinable, to assess their homogeneity (i.e. Chi-squared test for homogeneity, I^2). If heterogeneity exists a random effects model should be used and/or the clinical appropriateness of combining should be taken into consideration (i.e. is it sensible to combine?).	Yes No Partial yes
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the		Yes No Partial yes

meta-analysis or other evidence synthesis?		
13. Did the review authors account for RoB in individual studies when interpreting/discussing the results of the review?	The results of the methodological rigour and scientific quality should be considered in the analysis and the conclusions of the review, and explicitly stated in formulating recommendations.	Yes No Partial yes
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?		Yes No Partial yes
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?		Yes No Partial yes
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Potential sources of support should be clearly acknowledged in both the systematic review and the included studies.	Yes No Partial yes
Overall risk of bias		

Source: Shea et al. 2017. BMJ 358: j4008 doi:10.1136 (https://doi.org/10.1136/bmj.j4008)

Level II - Randomised controlled trials

Domain	Judgement	Description	Source
Random sequence generation (selection bias)	High risk Unclear risk Low risk	Describe the method used to generate the allocation of sequence in sufficient detail to allow an assessmenet of whether it should produce comparable groups.	p. XX
Allocation concealment (selection bias)	High risk Unclear risk Low risk	Describe the method used to conceal the allocation of sequence in sufficient detail to determine whether intervention allocation could have been foreseen in advance of, or during, enrolment.	p. XX
Blinding of participants and personnel (performance bias)	High risk Unclear risk Low risk	Describe all measures used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective. Should include an assessment for each outcome.	p. XX
Blinding of outcome assessment (detection bias)	High risk Unclear risk Low risk	Describe all measures used, if any, to blind outcome assessors from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective.	p. XX
Incomplete outcome data addressed (attrition bias)	High risk Unclear risk Low risk	Describe the completeness of outcome data for each primary/secondary outcome, including attrition (missing data) and exclusions from the analysis. State whether attritions and exclusions were reported, if they match number of total randomised participants, and if review authors have re-included any participants in the analysis.	p. XX
Selective reporting (reporting bias)	High risk Unclear risk Low risk	State how the possibility of outcome reporting examined by the review authors, and what was found (e.g. protocol of clinical trial available and matched to study report).	p. XX
Other sources of bias	High risk Unclear risk Low risk	State any concerns about bias not addressed elsewhere. (e.g. source of funding, crossover design, cluster randomised trial).	p. XX

Source: Chapter 8 of the Cochrane Handbook for Systematic Reviews of Interventions, version 5.1.0

Level III - Observational studies

Domain	Judgement	Description	Source
Bias due to failure to develop and apply appropriate eligibility criteria (inclusion of control population)	Low risk Moderate risk Serious risk Critical risk No information	Under- or over-matching in case-control studies OR Selection of exposed and unexposed in cohort studies from different populations.	
Bias due to flawed measurement of both exposure and outcome	Low risk Moderate risk Serious risk Critical risk No information	Differences in measurement of exposure (e.g. recall bias in case-control studies) OR Differential surveillance for outcome in exposed and unexposed in cohort studies.	
Bias due to failure to adequately control confounding	Low risk Moderate risk Serious risk Critical risk No information	Failure of accurate measurement of all known prognostic factors OR Failure to match for prognostic factors and/or adjustment in statistical analysis.	
Bias due to incomplete or inadequately short followup	Low risk Moderate risk Serious risk Critical risk No information	Especially within prospective cohort studies, both groups should be followed for the same amount of time.	

Source: Table 5.5 GRADE handbook http://gdt.guidelinedevelopment.org/app/handbook/handbook.html#h.m9385o5z3li7

Diagnostic accuracy studies

Study ID	Risk of bias					Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Overall risk of bias	Patient selection	Index test	Reference standard
Author, date	☺	?	☺	☺	Unclear	☺	☹	?

☺ Low Risk; ☹ High Risk; ? Unclear Risk>

Domain	Risk of bias	Applicability
Patient selection	Describe patient sampling (Free text): e.g clinic, sampling, recruitment, exclusion criteria Could the selection of patients have introduced bias? Was a consecutive or random sample of patients enrolled? If yes, low risk Was a case-control design avoided? Did the study avoid inappropriate exclusions? (e.g. difficult to diagnose)	Describe patient characteristics and setting (free text): e.g. Gender, age, genetics, clinical test results Do the included patients and settings match the review question?
Index test	Could the conduct or interpretation of the index test have introduced bias? Were the index test results interpreted without knowledge of the results of the reference standard? Was the pathogenicity of identified mutations analysed? if a threshold was used, was it prespecified?	Are there concerns that the index test, its conduct, or its interpretation differ from the review question? Could variations in test technology affect the accuracy, execution or interpretation of the test results? Will all patients be tested using the same technology?
Reference standard	Could the reference standard, its conduct, or its interpretation have introduced bias? Is the reference standard likely to correctly classify the target condition? Were the reference standard results interpreted without knowledge of the results of the indext test ?	Are There Concerns That the Target Condition as Defined by the Reference Standard Does Not Match the Question? Was the execution of the reference standard described in sufficient detail to permit its replication? Were the reference standard results interpreted without knowledge of the results of the index test ? Is the target condition that the reference standard defines the same as the target condition specified in the review question?
Patient flow	Could the Patient Flow Have Introduced Bias? Was there an appropriate interval between the index test and reference standard? Did all patients receive the same reference standard? Were all patients included in the analysis?	

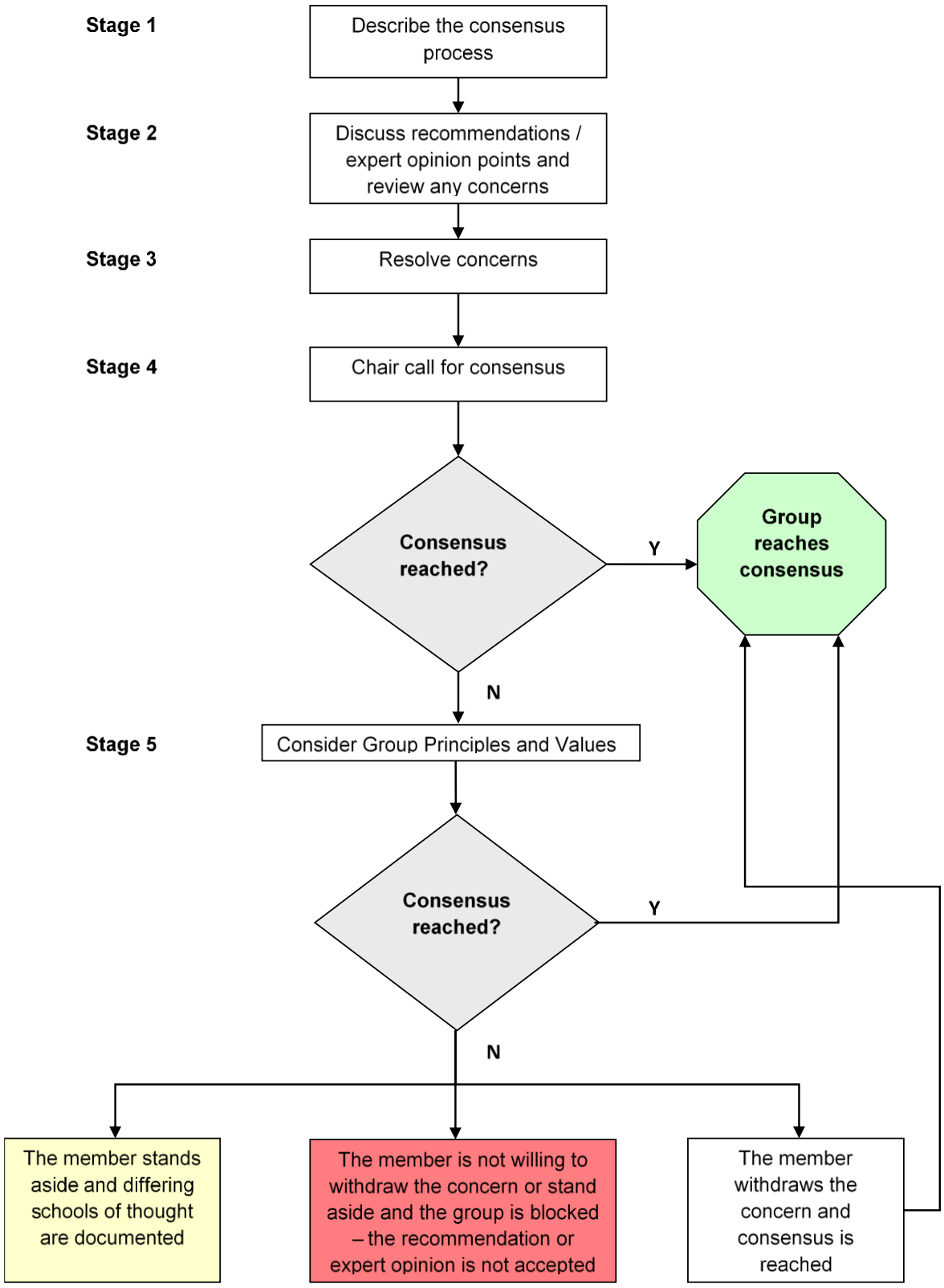
Source: (Whiting et al., 2011)

Appendix 5 Consensus process

The consensus process for developing evidence-based recommendation and expert opinion points is illustrated in Figure 7.1.

- *Stage 1 – Introduction.* The Chair describes the consensus process, participants' roles and responsibilities, ground rules and the guiding principles.
- *Stage 2 – Open discussion.* The Chair opens the floor to a general discussion and suggestions for recommendation / expert opinion wording, noting that recommendations will be based on the GRADE framework. 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.
- *Stage 5 – Second call for consensus.* If consensus is not reached, the Expert Reference Group (ERG) will consider the consensus process guiding principles and values, and:
    - 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 recommendation or expert opinion is not accepted
    - the member withdraws their concern and consensus is reached

Figure 7.1 Consensus process flow chart



Consensus guiding principles and values

- Consensus is reached where all members agree with the recommendation / expert opinion point. Consensus is not achieved on the basis of a 'majority'.

- The opinions of all members of the group are equally valid/important, notwithstanding that some members may have discipline-specific expert opinion.
- Where consensus is not reached, the dissenting members may present their case. This may be done immediately in the current meeting, or be carried over to the subsequent meeting to allow the members to succinctly formulate their concerns or provide other documentation/ research.
- Issues of semantics, language or content, while recognised as important, should preferably not absorb discussion time within the meetings.
- Members are respectfully asked to reflect upon their own values and conflicts of interests, and be mindful of the extent to which these may influence their opinions.

Consensus ground rules

- Members agree to take turns speaking and not interrupt each other.
- Members agree to stay away from establishing hard positions or express themselves in terms of personal needs and interests and the outcomes that they wish to realise.
- Members recognise that, even if they do not agree with it, each of them is entitled to their own perspective.
- Members will not dwell on things that did not work in the past, but instead will focus on the future they would like to create.
- Members agree to make a conscious, sincere effort to refrain from unproductive arguing, venting, or narration, and agree to use their time to work towards what they perceive to be their fairest and most constructive agreement possible.
- Members will speak up if something is not working for them during the consensus process.
- Members will request a break when they need to.
- Members will point out if they feel the Chair is not being impartial.

Appendix 6 GRADE profiles

Question 1 – *Routine* antenatal Rh D immunoprophylaxis

Question: Universal RAADP (1 or 2 doses) compared to placebo or no universal RAADP in Rh D negative pregnant women with no preformed anti-D antibodies
Setting: Obstetrics and maternity, primary
Bibliography: [1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16]

Certainty assessment							№ of women		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Universal RAADP (1 or 2 doses)	Placebo or no universal RAADP	Relative (95% CI)	Absolute (95% CI)		
Incidence of Rh D alloimmunisation (any timepoint)												
2	randomised trials	serious [a,b,c]	serious [d]	not serious [e]	serious [f]	dose response gradient	6/1112 (0.5%)	16/1185 (1.4%)	**RR 0.39** (0.09 to 1.63)	**8 fewer per 1,000** (from 12 fewer to 9 more)	⊕⊕◯◯ LOW	CRITICAL
8	observational studies	serious [b,g,h]	serious [i]	not serious [e]	not serious	dose response gradient	132/32162 (0.4%)	222/19825 (1.1%)	**RR 0.31** (0.18 to 0.54)	**8 fewer per 1,000** (from 9 fewer to 5 fewer)	⊕◯◯◯ VERY LOW	CRITICAL
Incidence of Rh D alloimmunisation (in subsequent pregnancy)												
6	observational studies	serious [b,g,h]	not serious [j]	not serious [e]	not serious	dose response gradient	57/18028 (0.3%)	111/13798 (0.8%)	**RR 0.43** (0.31 to 0.59)	**5 fewer per 1,000** (from 6 fewer to 3 fewer)	⊕⊕◯◯ LOW	IMPORTANT
Incidence of Rh D alloimmunisation (during pregnancy)												
4 [k]	observational studies	serious [a,b,c,g,h]	serious [i]	not serious [e]	serious [f]	dose response gradient	35/14755 (0.2%)	80/13602 (0.6%)	**RR 0.33** (0.08 to 1.37)	**4 fewer per 1,000** (from 5 fewer to 2 more)	⊕◯◯◯ VERY LOW	IMPORTANT
Incidence of Rh D alloimmunisation (at birth of Rh positive newborn or within three days of delivery)												

Certainty assessment							№ of women		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Universal RAADP (1 or 2 doses)	Placebo or no universal RAADP	Relative (95% CI)	Absolute (95% CI)		
8 [l]	observational studies	serious [a,b,c,e,g,h]	serious [i]	not serious [e]	not serious	dose response gradient	33/16053 (0.2%)	117/8569 (1.4%)	**RR 0.19** (0.08 to 0.45)	**11 fewer per 1,000** (from 13 fewer to 8 fewer)	⊕◯◯◯ VERY LOW	IMPORTANT
Incidence of Rh D alloimmunisation (up to 12-months postnatal followup)												
8 [m]	observational studies	serious [a,b,c,e,g,h]	not serious [j]	not serious [e]	not serious	dose response gradient	32/11999 (0.3%)	80/5373 (1.5%)	**RR 0.19** (0.13 to 0.29)	**12 fewer per 1,000** (from 13 fewer to 11 fewer)	⊕⊕◯◯ LOW	IMPORTANT
Incidence of a positive test for fetomaternal haemorrhage (assessed with: Kleihauer test at 32 to 35 weeks gestation)												
1	randomised trials	serious [a,b]	not serious [n]	not serious [e]	not serious	none	39/927 (4.2%)	67/957 (7.0%)	**RR 0.60** (0.41 to 0.88)	**28 fewer per 1,000** (from 41 fewer to 8 fewer)	⊕⊕⊕◯ MODERATE	NOT IMPORTANT
Incidence of a positive test for fetomaternal haemorrhage (assessed with: Kleihauer test at birth of Rh positive newborn)												
1	randomised trials	serious [a,b,c]	not serious [n]	not serious [e]	not serious	none	73/599 (12.2%)	119/590 (20.2%)	**RR 0.60** (0.46 to 0.79)	**81 fewer per 1,000** (from 109 fewer to 42 fewer)	⊕⊕⊕◯ MODERATE	NOT IMPORTANT
Adverse neonatal events: jaundice												
1	randomised trials	serious [a,b,c]	not serious [n]	not serious [e]	serious [f]	none	1/927 (0.1%)	4/955 (0.4%)	**RR 0.26** (0.03 to 2.30)	**3 fewer per 1,000** (from 4 fewer to 5 more)	⊕⊕◯◯ LOW	NOT IMPORTANT
Adverse neonatal events: prevalence of severe HDFN (perinatal mortality, need for IUT and/or exchange transfusion)												

Certainty assessment							№ of women		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Universal RAADP (1 or 2 doses)	Placebo or no universal RAADP	Relative (95% CI)	Absolute (95% CI)		
1	observational studies	serious [o,p]	not serious [n]	not serious	not serious	none	13/12576 (0.1%)	20/8645 (0.2%)	**RR 0.51** (0.09 to 0.92)	**1 fewer per 1,000** (from 2 fewer to 0 fewer)	⨁◯◯◯ VERY LOW	IMPORTANT
Adverse maternal events attributed to Rh D immunoglobulin - not measured												
-	-	-	-	-	-	-	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. [1,2]				-	IMPORTANT

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. One or more randomised studies with plausible bias that raises serious doubts about the results.
b. Missing data and exclusion of some women may overestimate the clinical effectiveness of RAADP.
c. Includes one quasi-randomised trial with high risk of selection bias.
d. No significant heterogeneity, with variability in effect estimates assessed as moderate (I^2 statistic between 25-50%). Does not reduce confidence in results to inform decision-making.
e. Obstetric practice and the baseline characteristics of the population may not be reflective of current practice, but this was considered to not seriously affect the confidence in the observed effect and could be sensibly applied.
f. Low event rate and/or wide confidence intervals that cross the line of no effect. Confidence in the results is weak.
g. One or more comparative observational studies with some important problems that seriously weaken the confidence in the results.
h. Studies include historical and/or geographic controls and it is not clear if intervention and control groups are comparable at baseline.
i. Significant heterogeneity with substantial variability in effect estimates (I^2 statistic greater than 50%). Reduces confidence in the results to inform decision-making.
j. No significant heterogeneity. I^2 statistic equals 0%.
k. Includes one RCT and one quasi-RCT.
l. Includes one RCT, one quasi-RCT and six observational studies. One observational study does not contribute any data.
m. Includes one RCT, one quasi-RCT and six observational studies. Two observational studies do not contribute any data.
n. One study only. Heterogeneity not assessed.
o. 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.
p. Some concerns with reporting bias and missing data.

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Subquestion 1 – One-dose RAADP versus two-dose RAADP

Question: 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?

Setting: Obstetrics and maternity, primary setting

Bibliography: [1,2,3,4,5,6]

Certainty assessment							№ of women		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	RAADP (single-dose)	RAADP (two-dose)	Relative (95% CI)	Absolute (95% CI)		
Incidence of Rh D alloimmunisation - not data												
-	-	-	-	-	-	-	No evidence found.				-	CRITICAL
Incidence of a positive test for fetomaternal haemorrhage - not reported												
-	-	-	-	-	-	-	No evidence found.				-	NOT IMPORTANT
Adverse neonatal events - not reported												
-	-	-	-	-	-	-	-	-	-	-	-	IMPORTANT
Adverse maternal events - not reported												
-	-	-	-	-	-	-	-	-	-	-	-	IMPORTANT
Serum anti-D levels at birth												
1	randomised trials	very serious a	serious [b]	not serious	not serious	none	Complete data not available (reported as abstract only). The proportion of women with undetectable anti-D antibodies was 45.2% vs 14.2%; OR 5.0; 95% CI NR; p<0.001. Favouring the two-dose regimen.				⨁◯◯◯ VERY LOW	NOT IMPORTANT
Incidence of Rh D alloimmunisation (1 dose, any timepoint)												
4	observational studies	serious [c,d,e,f]	serious [g]	not serious [h]	not serious	dose response gradient	109/24023 (0.5%)	148/12532 (1.2%)	**RR 0.31** (0.12 to 0.80)	**8 fewer per 1,000** (from 10 fewer to 2 fewer)	⨁◯◯◯ VERY LOW	IMPORTANT

Certainty assessment							№ of women		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	RAADP (single-dose)	RAADP (two-dose)	Relative (95% CI)	Absolute (95% CI)		
Incidence of Rh D alloimmunisation (2 dose, any timepoint)												
6 [i]	observational studies	serious [c,d,e,f]	not serious [j]	not serious [h]	serious [k]	dose response gradient	23/7381 (0.3%)	81/7883 (0.6%)	**RR 0.32** (0.20 to 0.51)	**7 fewer per 1,000** (from 8 fewer to 5 fewer)	⨁◯◯◯ VERY LOW	IMPORTANT
Incidence of Rh D alloimmunisation (1 dose, estimated)												
10	observational studies	serious [c,d,e,f,l]	not serious [b]	not serious [h]	not serious	dose response gradient	In a meta-regression model, Turner 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. [1] Using only studies relevant to the UK health system Pilgrim 2009 estimated the risk of sensitisation using a single dose to be 0.34% (0.28, 0.40). [2]				⨁⨁◯◯ LOW	IMPORTANT
Incidence of Rh D alloimmunisation (2 doses, estimated)												
10	observational studies	serious [c,d,e,f,l]	not serious [b]	not serious [h]	not serious	dose response gradient	In a meta-regression model, Turner 2012 estimated an OR of 0.31 (95%CI 0.09, 0.65) for two-doses of RAADP based on the relative effectiveness observed in published studies adjusted for bias and expert opinion. [1] Using only studies relevant to the UK health system, Pilgrim 2009 estimated the risk of sensitisation using two-doses to be 0.30% (95% CI 0.22, 0.38). [2]				⨁⨁◯◯ LOW	IMPORTANT

CI: Confidence interval; **RR:** Risk ratio; **OR:** Odds ratio

Explanations

a. Study is reported in a conference abstract and it is difficult to judge internal bias. Not all outcomes reported.

b. One study only. Heterogeneity not assessed.

c. One or more randomised studies with plausible bias that raises some doubts about the results

d. Missing data and exclusion of women may over-estimate the clinical effectiveness of RAADP

e. One or more comparative observational studies with some important problems that seriously weaken the confidence in the results

f. Studies include historical and/or geographic controls and it is not clear if intervention and control groups are comparable at baseline.

g. Significant heterogeneity with substantial variability in effect estimates (I^2 statistic greater than 50%). Reduces confidence in the results to inform decision making.

h. 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.

i. Includes one RCT and one quasi-RCT

j. No heterogeneity. I^2 statistic equals 0%. Does not reduce confidence in results to inform decision making.
k. Low event rate and/or wide confidence intervals that cross the line of no effect. Confidence in the results is weak.
l. Authors elicited expert opinion to estimate association between the relative and observed effectiveness for different dosing regimens.

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Question 2 – Universal sensitising event prophylaxis in the first trimester

Question: 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/without a curette),* does universal first trimester sensitising event prophylaxis with Rh D immunoglobulin prevent Rh D alloimmunisation?

Setting: Obstetrics and maternity, primary setting.

Bibliography: [1,2,3,4,5]

Certainty assessment							№ of women		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	sensitising event prophylaxis	placebo or no sensitising event prophylaxis	Relative (95% CI)	Absolute (95% CI)		
Incidence of Rh D alloimmunisation (4-6 months after spontaneous miscarriage and/or therapeutic evacuation) (assessed with: Enzyme-Coombs screening)												
1 [4]	randomised trials	very serious [a,b]	not serious [c]	serious [d,e]	serious [f]	publication bias strongly suspected [g]	0/19 (0.0%)	0/29 (0.0%)	not estimable		⊕◯◯◯ VERY LOW	CRITICAL
Incidence of Rh D alloimmunisation (4-6 months after incomplete miscarriage or medical termination of pregnancy) (assessed with: Indirect Coombs)												
1 [1]	observational studies	very serious [h,i]	not serious [c]	serious [d,j]	serious [k]	publication bias strongly suspected [g]	0/21 (0.0%)	2/36 (5.6%)	**RR 0.34** (0.02 to 6.69)	**37 fewer per 1,000** (from 54 fewer to 316 more)	⊕◯◯◯ VERY LOW	CRITICAL
Incidence of Rh D alloimmunisation (at subsequent pregnancy after spontaneous miscarriage and/or therapeutic evacuation) (assessed with: Enzyme-Coombs screening)												
1 [4]	randomised trials	very serious [a,b]	not serious [c]	serious [d,e]	serious [f]	publication bias strongly suspected [g]	0/3 (0.0%)	0/6 (0.0%)	not estimable		⊕◯◯◯ VERY LOW	IMPORTANT
Incidence of Rh D alloimmunisation (at subsequent pregnancy after inducted abortion) (assessed with: Papain-treated cells or Indirect Coombs)												
1 [5]	observational studies	very serious [h,i]	not serious [c]	not serious [l,m]	serious [k]	publication bias strongly suspected [g]	1/96 (1.0%)	2/145 (1.4%)	**RR 0.76** (0.07 to 8.21)	**3 fewer per 1,000** (from 13 fewer to 99 more)	⊕◯◯◯ VERY LOW	IMPORTANT

Certainty assessment							№ of women		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	sensitising event prophylaxis	placebo or no sensitising event prophylaxis	Relative (95% CI)	Absolute (95% CI)		
Incidence of Rh D alloimmunisation (after abdominal trauma, molar pregnancy, ectopic pregnancy) - not reported												
-	-	-	-	-	-	-	No comparative evidence found [2]				-	IMPORTANT
Incidence of a positive test for fetomaternal haemorrhage - not reported												
-	-	-	-	-	-	-	No comparative evidence found [3]				-	NOT IMPORTANT
Adverse neonatal events (e.g. jaundice) - not reported												
-	-	-	-	-	-	-	No comparative evidence found [3]				-	NOT IMPORTANT
Adverse maternal events attributed to anti-D - not reported												
-	-	-	-	-	-	-	No comparative evidence found [3]				-	NOT IMPORTANT

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. One randomised study with plausible bias that raises serious doubts about the results.

b. Method of randomisation not reported and unclear if treatment allocation concealed. Some concerns with reporting bias and missing data.

c. Single study. Heterogeneity not assessed.

d. The evidence is not directly applicable to the target population or the Australian healthcare context and it is difficult to judge if it could be sensibly applied. Obstetric practice and the baseline characteristics of the population may not be reflective of current practice.

e. The study was conducted in the United States among RhD negative women with complete miscarriage (n=9) or incomplete miscarriage with curettage (n=48). An unknown proportion of women had miscarriage outside the first trimester (after 12 weeks' gestation) and the intervention was administered at a dose higher than recommended in Australia (1500 IU vs 625 IU).

f. Small study not sufficiently powered to detect a statistically significant difference.

g. Single study. Publication bias likely.

h. Comparative study with some important problems that seriously weakens the confidence in the results.

i. Method of treatment allocation or blinding not reported. Some concerns with reporting bias and missing data.

j. The study was conducted in the United States among Rh D negative women who had medical termination of pregnancy (n=33) or were treated for incomplete miscarriage (n=24). Thirteen (22.8%) women were treated outside the first trimester (>13 weeks' gestation) and the dose of Rhogam was not stated.

k. Low event rate and/or wide confidence intervals that cross the line of no effect. Confidence in the results is weak.

l. The evidence is probably applicable to the Australian population and healthcare context with some caveats.

m. 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).

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1. Gavin, P.S.. Rhesus sensitization in abortion.. Obstetrics and Gynecology.; 1972.
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Question 3 – *Targeted* routine antenatal or sensitising event prophylaxis

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

Setting: Obstetrics and maternity, primary

Bibliography: [1,2,3,4]

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Incidence of Rh D alloimmunisation - not reported									
-	-	-	-	-	-	-	No studies directly assessed the effect of targeted routine antenatal or sensitising event prophylaxis on the incidence of Rh D alloimmunisation. One study (Saramago 2018) conducted a Monte Carlo simulation based on diagnostic accuracy of the test and expected management in women with positive and negative test results. The report estimated targeted RAADP increased the risk of Rh D alloimmunisation from 284 (base case scenario) or 309 (worst case scenario) per 100 000 pregnant women compared with 281 per 100 000 with universal RAADP. [1]	-	CRITICAL
Utilisation of anti-D - not reported									
-	-	-	-	-	-	-	No comparative studies directly assessed the effect of targeted routine antenatal or sensitising event prophylaxis on utilisation of Rh D immunoglobulin. One study (Saramago 2018) conducted a Monte Carlo simulation based on data from three noncomparative studies and estimated utilisation of Rh D immunoglobulin would decrease by approximately 33.1% to 36.9%. [1,2,3,4]	-	IMPORTANT
Incidence of a positive test for fetomaternal haemorrhage - not reported									
-	-	-	-	-	-	-	No studies directly assessed effect of targeted routine antenatal or sensitising event prophylaxis on the incidence of a positive test for fetomaternal haemorrhage.	-	NOT IMPORTANT
Adverse neonatal events - not reported									
-	-	-	-	-	-	-	No studies were identified that reported any data on adverse neonatal events relating to NIPT or antenatal Rh D immunoprophylaxis.	-	IMPORTANT

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Adverse maternal events attributed to Rh D immunoglobulin (e.g. allergic response, infection) - not reported									
-	-	-	-	-	-	-	No studies were identified that reported any data on adverse maternal events relating to NIPT or antenatal Rh D immunoprophylaxis.	-	IMPORTANT

CI: Confidence interval

References

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Subquestion 3 – Diagnostic accuracy of noninvasive prenatal testing for fetal Rh D status

Question: Should noninvasive prenatal testing be used to diagnose fetal Rh D status in Rh D negative pregnant women with no preformed anti-D (for routine or sensitising event prophylaxis)?

Sensitivity	0.93 to 1.00
Specificity	0.92 to 1.00

Included studies published after 2008 and with more than 100

Prevalences	55% Assumed lower estimate	62% Likely estimate for Australia	75% Maximum reported prevalence in identified studies

Outcome	№ of studies (№ of women)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 women tested			Test accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 55%	pre-test probability of 62%	pre-test probability of 75%	
True positives (women with fetal Rh D status)	48 studies 76 349 women	cohort & case-control type studies	not serious [a]	not serious [b,c]	not serious [d]	not serious [e]	none	510 to 550	575 to 620	696 to 750	⊕⊕⊕⊕ HIGH
False negatives (women incorrectly classified as not having fetal Rh D status)								0 to 40	0 to 45	0 to 54	
True negatives (women without fetal Rh D status)	48 studies 76 349 women	cohort & case-control type studies	not serious [a]	not serious [b,c]	not serious [d]	not serious [e]	none	412 to 450	348 to 380	229 to 250	⊕⊕⊕⊕ HIGH
False positives (women incorrectly classified as having fetal Rh D status)								0 to 38	0 to 32	0 to 21	

Explanations

a. 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, exclusion of sensitised women) or conduct of the index test (e.g., number of exons amplified, controls used) were small, and are not considered to substantially alter the test results. Cord blood serology was the reference standard in all studies and was usually conducted independent of the index test.

b. The evidence was considered applicable to the Australian healthcare context with some caveats. Much of the evidence is in Northern European countries with predominantly Caucasian majority. This was considered comparable to the Australian context in which the prevalence of RhD negative phenotype among donors is around 15%. The prevalence of RhD negative babies born to RhD negative women is estimated to be 38%; however, the prevalence of specific *RHD* genotypes is not known. The meta-analyses by Zhu 2014 and Geifman-Holtzman 2006 were not included as changes and improvements in conduct of the test have occurred. It is expected that the screening test would, at a minimum, include primers for 2 exons (either 4, 5, 7, or 10), involve RT-qPCR, and be conducted in duplicate.

c. Diagnostic performance may by overestimated if only high-throughput studies are considered (as reported in Saramago 2018), therefore the inclusion of Mackie (2016) and smaller studies was considered appropriate for the Australian context. Care should be taken when interpreting test results in women with multiple pregnancies as this subgroup was excluded from the meta-analysis by Mackie 2017 and other studies.

d. Almost all studies consistent, and inconsistencies could be explained. Samples taken prior to 12 weeks' gestation would reduce confidence in specificity of the test. Some studies did not report inconclusive results, which would favour the index test; however, this was not considered to substantially reduced the confidence in the overall quality of evidence.

e. Many studies included. Smaller confidence intervals observed in the large studies with central reference laboratories and those that used thresholds to maintain an acceptable level of sensitivity. Here, confidence in the evidence is high. In small, single centre studies, wider confidence interval would suggest a lower certainty of evidence.

Question 4 – Risk of failure of Rh D immunoprophylaxis due to increased BMI

Question: Does increasing BMI increase the risk of failure of anti-D administration in Rh D negative pregnant or postpartum women with no preformed anti-D?
Setting: Obstetrics and maternity, primary setting
Bibliography: [1,2,3,4]

Certainty assessment							№ of women	Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Increased dose of RAADP	Relative (95% CI)	Absolute (95% CI)		
Incidence of Rh D alloimmunisation (any timepoint)											
1 [1]	observational studies	not serious [a,b]	not serious [c]	not serious [d]	very serious [e]	none	No significant association between body mass index, mean body weight, weight >75 kg or weight >100 kg on the incidence of Rh alloimmunisation.			⊕◯◯◯ VERY LOW	IMPORTANT
Anti-D serum levels after administration of Rh D IgG (2 doses, 28 and 34 weeks gestation)											
1 [3]	observational studies	very serious [f,g]	serious [c]	not serious [h]	very serious [i]	none	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.			⊕◯◯◯ VERY LOW	NOT IMPORTANT
Anti-D serum levels after administration of Rh D IgG (single dose, 28 weeks gestation)											
1 [4]	randomised trials	extremely serious [j]	not serious [c]	serious [k]	very serious [i]	none	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); however, anti D IgG remained quantifiable in both women at last scheduled followup (Week 9 and 11).			⊕◯◯◯ VERY LOW	NOT IMPORTANT
Anti-D serum levels after delivery of an Rh D positive child											
1 [2]	observational studies	not serious [l]	not serious [c]	not serious [m]	very serious [i]	none	Based on the general linear model over time, the study authors found each kg/m^2 BMI higher than 27 kg/m^2 reduced the Rh D Ig G serum concentration by the calculated value.			⊕◯◯◯ VERY LOW	NOT IMPORTANT
Incidence of a positive test for fetomaternal haemorrhage - not reported											
-	-	-	-	-	-	-	No studies identified reported this outcome.			-	NOT IMPORTANT
Adverse neonatal events (e.g., jaundice) - not reported											

Certainty assessment							№ of women	Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Increased dose of RAADP	Relative (95% CI)	Absolute (95% CI)		
-	-	-	-	-	-	-	No studies identified reported this outcome.			-	IMPORTANT
Adverse maternal events											
1	randomised trials	extremely serious [j]	not serious [c]	not serious [d]	serious [n]	none	Seven adverse events reported among five women; none were considered related to study drug.			⊕◯◯◯ VERY LOW	IMPORTANT

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. One case-control study that appears to provide sound evidence for a nonrandomised study but cannot be considered comparable to a well-performed RCT.

b. There was an over-representation of women from the primary versus obstetric setting (3:1) in the control group compared with cases. This was considered to not seriously influence the overall certainty of effect. The authors corrected for this using weighted data in the analysis.

c. Single study. Heterogeneity not assessed. Certainty of evidence not downgraded.

d. 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 1000 IU of Rh D immunoglobulin at 30 weeks' gestation. This is different to the recommended dose of 625 IU at 28 and 34 weeks' gestation in Australia.

e. The study is not statistically powered to inform decision-making. A very small number of women with a high BMI included.

f. One study with some important problems that seriously weaken the confidence in the results.

g. Small cohort with some concerns with reporting bias and missing data.

h. Evidence is directly generalisable to the target population and applicable to the Australian healthcare system with some caveats. The study was conducted in the UK in Rh D negative pregnant women. Rh D immunoglobulin (500 IU) was administered at 28 and 34 weeks' gestation but the dose was lower than recommended in Australia (625 IU).

i. Small cohort with insufficient longer term data to provide meaningful information relating to BMI and incidence of Rh D alloimmunisation in a subsequent pregnancy.

j. The study is too problematic to provide any useful evidence on the outcome of interest.

k. Evidence is probably generalisable to the target population but difficult to judge if sensible to apply to the Australian healthcare 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 (625 IU at 28 and 34 weeks' gestation). The correlation between body weight and BMI is poor, with the BMI of subject 12 being 26.79 and the BMI of subject 9 being 32.29.

l. One observational study that appears to provide sound evidence for a nonrandomised study but cannot be considered comparable to a well-performed RCT.

m. 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 child. Rh D immunoglobulin was administered with 72 hours after birth, but at a dose higher than that recommended in Australia (1500 IU vs 625 IU).

n. Small study unlikely to be sufficiently powered to detect a statistically significant difference.

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