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https://doi.org/10.3310/hta22130

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HEALTH TECHNOLOGY ASSESSMENT

VOLUME 22 ISSUE 13 MARCH 2018
ISSN 1366-5278

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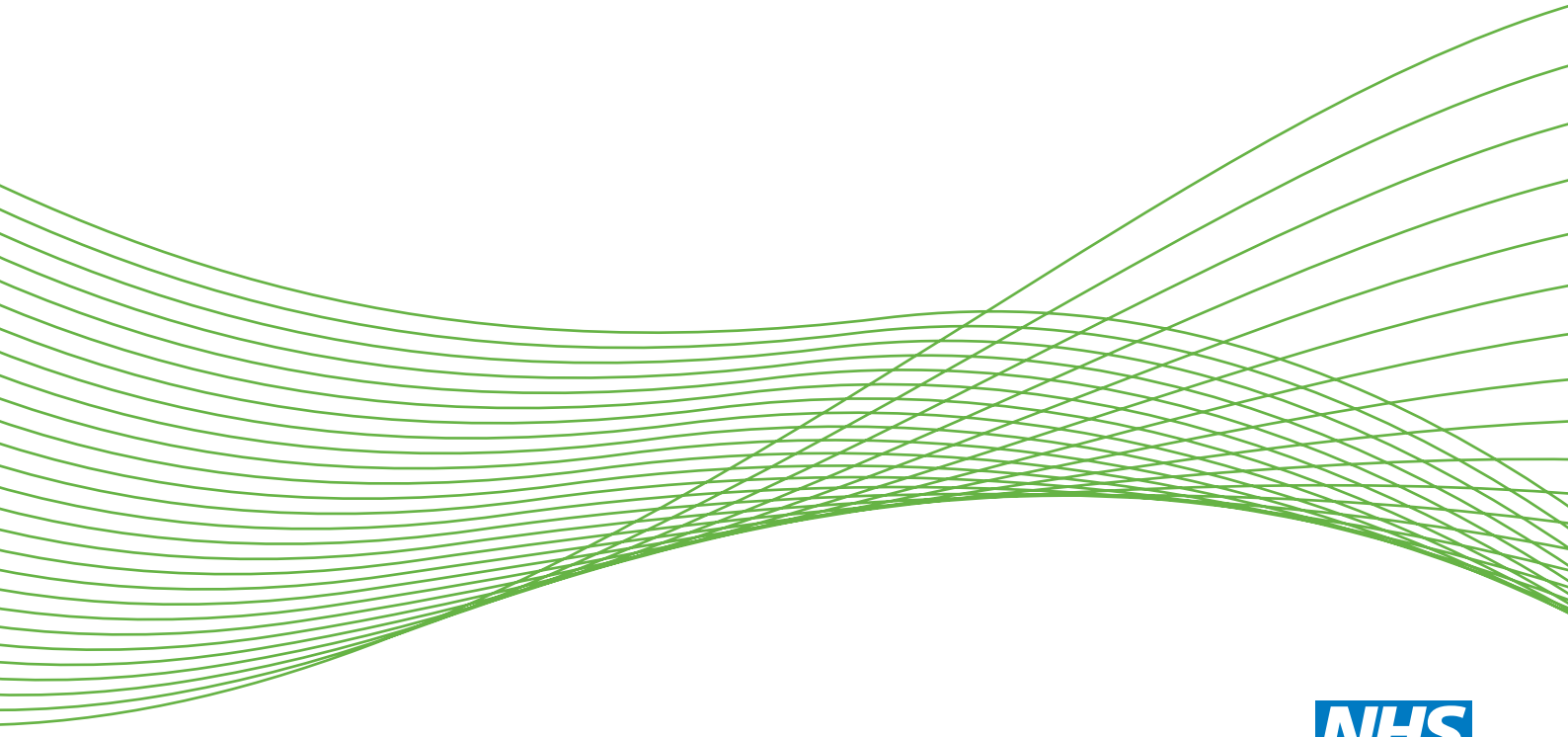

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National Institute for Health Research

DOI 10.3310/hta22130

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

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Declared competing interests of authors: none

Published March 2018
DOI: 10.3310/hta22130

This report should be referenced as follows:

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

Health Technology Assessment is indexed and abstracted in *Index Medicus*/MEDLINE, *Excerpta Medica*/EMBASE, *Science Citation Index Expanded* (SciSearch®) and *Current Contents®*/ Clinical Medicine.

Health Technology Assessment

NICE TAR and DAR

ISSN 1366-5278 (Print)

ISSN 2046-4924 (Online)

Impact factor: 4.236

Health Technology Assessment is indexed in MEDLINE, CINAHL, EMBASE, The Cochrane Library and the Clarivate Analytics Science Citation Index.

This journal is a member of and subscribes to the principles of the Committee on Publication Ethics (COPE) (www.publicationethics.org/).

Editorial contact: journals.library@nihr.ac.uk

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This report

The research reported in this issue of the journal was commissioned and funded by the HTA programme on behalf of NICE as project number 15/17/02. The protocol was agreed in January 2016. The assessment report began editorial review in June 2016 and was accepted for publication in February 2017. The authors have been wholly responsible for all data collection, analysis and interpretation, and for writing up their work. The HTA editors and publisher have tried to ensure the accuracy of the authors' report and would like to thank the reviewers for their constructive comments on the draft document. However, they do not accept liability for damages or losses arising from material published in this report.

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Abstract

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

Pedro Saramago,[1] Huiqin Yang,[2] Alexis Llewellyn,[3] Ruth Walker,[3] Melissa Harden,[3] Stephen Palmer,[1] Susan Griffin[1] and Mark Simmonds[3]*

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Background: High-throughput non-invasive prenatal testing (NIPT) for fetal rhesus (D antigen) (RhD) status could avoid unnecessary treatment with routine anti-D immunoglobulin for RhD-negative women carrying a RhD-negative fetus, although this may lead to an increased risk of RhD sensitisations.

Objectives: To systematically review the evidence on the diagnostic accuracy, clinical effectiveness and implementation of high-throughput NIPT and to develop a cost-effectiveness model.

Methods: We searched MEDLINE and other databases, from inception to February 2016, for studies of high-throughput NIPT free-cell fetal deoxyribonucleic acid (DNA) tests of maternal plasma to determine fetal RhD status in RhD-negative pregnant women who were not known to be sensitised to the RhD antigen. Study quality was assessed with the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) and A Cochrane Risk of Bias Assessment Tool: for Non-Randomised Studies of Interventions (ACROBAT-NRSI). Summary estimates of false-positive rates (FPRs) and false-negative rates (FNRs) were calculated using bivariate models. Clinical effectiveness evidence was used to conduct a simulation study. We 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 anti-D immunoglobulin antenatally and post partum. Sensitivity analyses (SAs) were conducted to address key uncertainties and model assumptions.

Results: Eight studies were included in the diagnostic accuracy review, seven studies were included in the clinical effectiveness review and 12 studies were included in the review of implementation. Meta-analyses included women mostly at or post 11 weeks' gestation. The pooled FNR (women at risk of sensitisation) was 0.34% [95% confidence interval (CI) 0.15% to 0.76%] and the pooled FPR (women needlessly receiving anti-D) was 3.86% (95% CI 2.54% to 5.82%). SAs did not materially alter the overall results. Data on clinical outcomes, including sensitisation rates, were limited. Our simulation suggests that NIPT could substantially reduce unnecessary use of antenatal anti-D with only a small increase in the risk of sensitisation. All large implementation studies suggested that large-scale implementation of high-throughput NIPT was feasible. Seven cost-effectiveness studies were included in the review, which found that the potential for the use of NIPT to produce cost savings was dependent on the cost of the test. Our de novo model suggested that high-throughput NIPT is likely to be cost saving compared with the current practice of providing routine antenatal anti-D prophylaxis to all women who are RhD negative. The extent of the cost saving appeared to

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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.

Limitations: There was very limited evidence relating to the clinical effectiveness of high-throughput NIPT, with no evidence on potential adverse effects. The generalisability of the findings to non-white women and multiple pregnancies is unclear.

Conclusions: 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 this evaluation.

Future work: Further research on the diagnostic accuracy of NIPT in non-white women is needed.

Study registration: This study is registered as PROSPERO CRD42015029497.

Funding: The National Institute for Health Research Health Technology Assessment programme.

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Glossary

Cost-effectiveness analysis An economic analysis that converts effects into health terms and describes the costs for additional health gain.

Decision modelling A theoretical construct that allows the comparison of the relationship between costs and outcomes of alternative health-care interventions.

False negative An incorrect negative test result – the number of diseased persons with a negative test result.

False positive An incorrect positive test result – the number of non-diseased persons with a positive test result.

Incremental cost-effectiveness ratio The difference in the mean costs of two interventions in the population of interest divided by the difference in the mean outcomes in the population of interest.

Index test The test for which performance is being evaluated.

Markov model An analytic method particularly suited to modelling repeated events or the progression of a chronic disease over time.

Meta-analysis Statistical techniques used to combine the results of two or more studies and obtain a combined estimate of effect.

Metaregression A statistical technique used to explore the relationship between study characteristics and study results.

Opportunity costs The cost of forgone outcomes that could have been achieved through alternative investments.

Receiver operating characteristic curve A graph that illustrates the trade-offs between sensitivity and specificity that result from varying the diagnostic threshold.

Reference standard The best currently available diagnostic test against which the index test is compared.

Sensitivity The proportion of people with the target disorder who have a positive test result.

Specificity The proportion of people without the target disorder who have a negative test result.

True negative A correct negative test result – the number of non-diseased persons with a negative test result.

True positive A correct positive test result – the number of diseased persons with a positive test result.

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List of abbreviations

ACROBAT-NRSI	A Cochrane Risk Of Bias Assessment Tool: for Non-Randomised Studies of Interventions
BNF	*British National Formulary*
CDSR	Cochrane Database of Systematic Reviews
CENTRAL	Cochrane Central Register of Controlled Trials
CI	confidence interval
CINAHL	Cumulative Index to Nursing and Allied Health Literature
DARE	Database of Abstracts of Reviews of Effects
DNA	deoxyribonucleic acid
FMH	fetal–maternal haemorrhage
FNR	false-negative rate
FPR	false-positive rate
HSROC	hierarchical summary receiver operating characteristic
HTA	Health Technology Assessment
ICER	incremental cost-effectiveness ratio
IU	international unit
MeSH	medical subject heading
NHB	net health benefit
NHS EED	NHS Economic Evaluations Database
NICE	National Institute for Health and Care Excellence
NIPT	non-invasive prenatal testing
PCR	polymerase chain reaction
PP1	postpartum scenario 1
PP2	postpartum scenario 2
PP3	postpartum scenario 3
PP4	postpartum scenario 4
QALY	quality-adjusted life-year
QUADAS-2	Quality Assessment of Diagnostic Accuracy Studies 2
RAADP	routine antenatal anti-D prophylaxis
RhD	rhesus blood group (D antigen)
ROC	receiver operating characteristic
RR	relative risk
SA	sensitivity analysis
TA	technology appraisal

Note

This monograph is based on the Technology Assessment Report produced for NICE. The full report contained a considerable number of data that were deemed confidential. The full report was used by the Appraisal Committee at NICE in their deliberations. The full report with each piece of confidential data removed and replaced by the statement 'confidential information (or data) removed' is available on the NICE website: www.nice.org.uk.

The present monograph presents as full a version of the report as is possible while retaining readability, but some sections, sentences, tables and figures have been removed. Readers should bear in mind that the discussion, conclusions and implications for practice and research are based on all the data considered in the original full NICE report.

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Plain English summary

About 3 in 20 women in the UK have a blood type called rhesus blood group (D antigen) (RhD) negative. If they become pregnant, around 6 in 10 of these women will have babies who have the opposite blood type (RhD positive) and the woman's immune system can react to the baby's blood (a process called 'sensitisation'). Following sensitisation, commonly in a subsequent pregnancy, the woman's immune system may attack the baby's blood, which potentially has severe consequences, such as a need for blood transfusions or even the death of the baby. The risk of sensitisation can be substantially reduced by injecting women with a blood-based product called anti-D immunoglobulin. Currently, all pregnant women with RhD-negative blood are offered this injection during later pregnancy and after birth. However, women carrying a RhD-negative baby do not need this injection. Non-invasive prenatal testing (NIPT) may determine the blood type of the baby during pregnancy and so the anti-D injection can be avoided in women who do not need it.

This report investigated whether or not using NIPT was a reliable, effective and safe way to manage RhD-negative pregnant women and whether or not it could reduce costs for the NHS. Based on eight studies, the test was found to be highly accurate, with an incorrect result in about 2% of women, which translates to between 3 and 27 additional sensitisations per 100,000 pregnancies compared with current practice, and a small risk of loss in health. However, the test is inconclusive in around 7% of women who could still be offered the anti-D injection, and there is an increased risk of adverse health outcomes for sensitised women. The evidence suggests that using NIPT would reduce the number of women receiving anti-D unnecessarily but would lead to a small increase in the number of additional sensitisations and that this may or may not be cost saving depending on the additional cost of NIPT.

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Scientific summary

Background

Approximately 17% of women giving birth in England and Wales are rhesus blood group (D antigen) (RhD) negative. Pregnant women who have RhD-negative blood type may carry a RhD-positive fetus. The entry of fetal RhD-positive cells into the maternal circulation can cause a mother who is RhD negative to produce anti-D antibodies against the RhD antigen. This process, called sensitisation, can happen at any time during pregnancy, although it is most common in the third trimester and during childbirth.

In a subsequent pregnancy with a RhD-positive fetus in women who have been sensitised, the woman's anti-D antibodies may respond to the presence of RhD-positive blood in the fetus, which may result in haemolytic disease of the fetus and newborn infant. Prophylaxis with anti-RhD immunoglobulin can substantially reduce the risk of sensitisation in RhD-negative women and the prevalence of haemolytic disease of the fetus and newborn infant.

High-throughput non-invasive prenatal testing (NIPT) for fetal RhD status may enable anti-D immunoglobulin to be withheld from RhD-negative women who are carrying a RhD-negative fetus. These women could avoid unnecessary treatment with routine anti-D immunoglobulin, as well as the potential risk associated with the administration of blood products, although this may also lead to an increased risk of RhD sensitisations. In addition, these women may not need the provision of anti-D immunoglobulin following potentially sensitising events and there may no longer be a need for serological cord testing at birth. However, the clinical effectiveness and cost-effectiveness of high-throughput NIPT for fetal rhesus D status in RhD-negative women not known to be sensitised to the RhD antigen for the NHS is uncertain.

Objectives

This assessment aims to evaluate both the clinical effectiveness and cost-effectiveness of using high-throughput NIPT to identify fetal rhesus D status in RhD-negative women not known to be sensitised to the RhD antigen and any consequent changes in treatment management.

Methods

Assessment of clinical effectiveness

Three systematic reviews were conducted. A range of bibliographic sources, including MEDLINE and EMBASE, were searched from inception to February 2016 for published and unpublished literature.

For diagnostic accuracy outcomes, we included prospective cohort studies reporting absolute numbers, which allowed for the calculation of diagnostic accuracy. For clinical effectiveness outcomes, we included any study in which high-throughput NIPT was used, in which anti-D prophylaxis was given as required and that reported relevant clinical outcomes. For implementation outcomes, we considered all publications reporting issues related to the implementation of, or practical advice relating to, high-throughput NIPT.

For all reviews, the eligible population were pregnant women who were RhD negative and not known to be sensitised to RhD antigen. The index test was high-throughput NIPT free-cell fetal deoxyribonucleic acid tests of maternal plasma used to determine fetal RhD status. The reference standard was serological cord blood testing at birth or any other suitable postnatal blood test of the infant.

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Two researchers independently screened the titles and abstracts of all reports identified by the search strategy and full-text papers were subsequently obtained for assessment. Data extraction and quality assessment were undertaken by one researcher and checked by a second. The risk of bias of diagnostic accuracy studies was assessed using a modified Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) checklist.

For diagnostic accuracy outcomes, bivariate models were fitted to calculate summary estimates of false-positive rates (FPRs) and false-negative rates (FNRs) with 95% confidence intervals (CIs).

For clinical effectiveness outcomes, data including sensitisation, NIPT uptake, anti-D prophylaxis uptake, reduction in anti-D use and adverse events were synthesised narratively. For the review of implementation studies, the following data were synthesised narratively: study findings, issues for implementation, practical guidance and recommendations for research. In addition, we performed a simulation study to simulate possible clinical outcomes of high-throughput NIPT in the UK based on results from the diagnostic accuracy review and existing reviews of antenatal anti-D prophylaxis.

Assessment of cost-effectiveness

A range of bibliographic databases were searched to identify relevant cost-effectiveness evidence. Citation searches were also undertaken. Only full economic evaluations were considered for review. Characteristics from the review findings were extracted and critically appraised using a published checklist. Studies were assessed with respect to the way in which NIPT was assumed to have an impact on the care pathway.

A de novo decision-analytic model using a decision tree cohort approach was developed to estimate, based on best available data, the costs and health outcomes. Four scenarios were designed to evaluate different impacts of NIPT on the existing postpartum care pathway. These evaluated how NIPT could impact on the use of cord serology, fetal–maternal haemorrhage (FMH) tests and anti-D immunoglobulin following delivery. First and subsequent pregnancies, together with the long-term consequences of sensitisations, in terms of costs and utilities, are evaluated within the model, with a yearly cycle and a lifetime horizon. The main outcomes of interest within the model were the total lifetime costs and total lifetime quality-adjusted life-years (QALYs) for each of the alternative pathways. The decision model was populated using the results from the systematic clinical review on the diagnostic accuracy of high-throughput NIPT. Various assumptions were based on the previous independent economic evaluation developed for NICE technology appraisal (TA) 156 on routine antenatal anti-D prophylaxis (RAADP). Primary model results are the total expected costs and expected QALYs for each alternative strategy. Population net health benefits are used to summarise the cost-effectiveness results in addition to the cost-effectiveness ratio. Uncertainty regarding the appropriate source of data, the appropriate assumptions or model structure and other scenarios are explored using one-way and two-way sensitivity analyses (SAs).

Results

Diagnostic accuracy

Eight studies were included in the diagnostic review of high-throughput NIPT, which were conducted in five European countries. There were three high-quality studies in which NIPT was performed by the NHS Blood and Transplant International Blood Group Reference Laboratory (Bristol, UK). The reference standard in all studies was cord blood serology at birth. The majority of included studies were judged as having a low risk of bias, but two studies were judged as having a high risk of bias.

Meta-analyses included women mostly at or post 11 weeks' gestation and showed very high diagnostic accuracy of high-throughput NIPT. In the primary analyses, in which women with inconclusive test results were treated as having tested positive, the pooled FNR (i.e. women at risk of sensitisation) was 0.34% (95% CI 0.15% to 0.76%) and the pooled FPR (i.e. women receiving anti-D unnecessarily) was 3.86% (95% CI 2.54% to 5.82%). SAs did not materially alter the overall result.

The diagnostic accuracy performance of high-throughput NIPT varied by gestational age. The data suggest that high-throughput NIPT was less accurate before around 11 weeks' gestation (i.e. in first trimester), but diagnostic accuracy was consistent at any time after 11 weeks' gestation. We were unable to conduct a subgroup analysis based on ethnicity because of a lack of relevant data from included studies.

Clinical effectiveness

Seven studies were included in the clinical effectiveness review. All studies were judged as having a high risk of bias. One large cohort study reported that implementation of NIPT for targeted antenatal anti-D prophylaxis was associated with a significant risk reduction in sensitisation (adjusted odds ratio 0.41, 95% CI 0.22 to 0.87) compared with historical controls.

Three non-comparative studies reported on the reduction in administration of anti-D. All suggested that anti-D administration was largely avoided in women with a RhD-negative fetus.

The compliance rate with antenatal anti-D prophylaxis ranged from 86% to 96.1% (four studies) and compliance rates with postpartum anti-D ranged from 92% to 99.7% (three studies) in women who undertook NIPT and received a positive result. High-throughput NIPT uptake rates ranged from 70% to > 95% (seven studies). None of the included studies reported data on adverse events associated with NIPT.

The results from the simulation study suggested that use of NIPT to determine antenatal anti-D use would substantially reduce the number of women receiving anti-D unnecessarily, from 38.9% to 5.7%, consistent with evidence identified by the review. The use of NIPT would cause an extra three sensitisations per 100,000 women if cord blood testing is continued (at least in women with a negative NIPT result) as the basis for administering postpartum anti-D. If cord blood testing is withdrawn (except for women who did not receive NIPT or who had an inconclusive test result) and NIPT is used to decide on postpartum anti-D administration, then there would be an extra 13 sensitisations per 100,000 women. These additional sensitisations are few compared with the underlying rate of sensitisation with antenatal anti-D (280 per 100,000 women). These results suggest that cord blood testing could potentially be withdrawn and NIPT results (if available and conclusive) may be used to prescribe postpartum anti-D. This conclusion will depend partly on whether or not the 10 extra sensitisations per 100,000 RhD-negative women caused by withdrawing cord blood testing can be considered an ethically acceptable increase.

Evidence on implementation

Twelve studies were included in the review of implementation. Most of the included studies were large cohort studies reporting implementation data alongside diagnostic accuracy data, although one study was a survey based in the UK (London). All the cohort studies suggested that high-throughput RhD genotyping of fetuses in all RhD-negative women was feasible. Key issues of implementation included ensuring anti-D prophylaxis compliance, the effective management of transporting samples and greater knowledge of NIPT among physicians, midwives and pregnant women.

Cost-effectiveness

The de novo health economic model suggested that high-throughput NIPT appears cost saving but also less effective than current practice, irrespective of the postpartum scenario evaluated. However, the magnitude of the potential cost savings appeared sufficient to outweigh the small increase in sensitisations and the associated small QALY loss when using NIPT compared with current practice. Based on a cross-section of 100,000 pregnancies, the probable magnitude of cost savings ranged between £485,000 and £671,000 across the separate postpartum strategies. In the base-case analysis, the strategy in which the NIPT result is used to guide RAADP only (i.e. all women continue to receive cord serology with FMH and postpartum anti-D immunoglobulin) had the highest probability of being cost-effective.

The magnitude of the cost saving appeared highly sensitive to the cost of NIPT itself to the NHS, which comprises the base unit cost per test, the level of any royalty fee and any increase in antenatal care costs

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required to accommodate an additional test. A small increase in the cost assumed of (confidential information has been removed) or more per test would alter these conclusions.

Our findings indicate that the timing of the test does not appear influential in determining the cost-effectiveness results, either in terms of diagnostic accuracy or in terms of the extent of management costs for potentially sensitising events that can be avoided. Another important consideration is the rate of high-throughput NIPT inconclusive results. Our findings demonstrate that even with a high-throughput NIPT inconclusive result rate of close to 15%, the introduction of NIPT appears to compare favourably with current practice.

Discussion

Limitations and uncertainties

Few studies reporting clinical effectiveness data of using high-throughput NIPT to detect fetal RhD status in RhD-negative women were identified. Results of the simulation study are sensitive to the parameters used and should be considered speculative.

Owing to the limited evidence, the potential clinical impact of high-throughput NIPT on the care pathway remains unclear. No studies compared NIPT with universal administration of RAADP. No studies were identified reporting comparative data relating to patient-related outcomes, such as quality of life or anxiety. Whether or not the diagnostic performance of high-throughput NIPT differs between different ethnic groups remains unclear.

There remains uncertainty regarding the cost of introducing the high-throughput NIPT, as the unit cost will potentially vary with throughput and may be subject to an additional royalty fee.

Generalisability of the findings

Diagnostic data from three UK (Bristol) studies are mostly generalisable to the UK setting. Differences in high-throughput NIPT devices and in antenatal care within different countries mean that the generalisability of the findings from those non-UK studies to the UK setting is likely to be limited, particularly for the reviews of clinical effectiveness and implementation studies. Owing to a lack of UK-based evidence, the generalisability of studies reporting compliance rates to antenatal anti-D treatment to the UK setting remains uncertain. As most participants in included studies were white Europeans, the generalisability of these findings to a non-white population also remains uncertain.

Conclusions

Implications for service provision

High-throughput NIPT is highly accurate for the detection of fetal rhesus D status in RhD-negative women, if performed after 11 weeks' gestation. Only 1% of women will have an incorrect test result (nearly all false positives) and around 7% will have an inconclusive result.

The use of NIPT can largely remove unnecessary exposure to prophylactic anti-D treatment, without substantially altering the rate of sensitisations. However, there will be a small number of women (about 0.1%) with a false-negative test result who are put at increased risk of sensitisation because they do not receive antenatal anti-D prophylaxis. This risk is unlikely to be substantially increased if postnatal cord blood testing is withdrawn. The test could be administered at any time after the first trimester without adversely affecting accuracy. Achieving high compliance rates may be important for the success of using NIPT, particularly through ensuring high compliance with NIPT and continuing to offer antenatal anti-D to women who refuse, or miss, NIPT.

Cost-effectiveness

Targeted provision of anti-D immunoglobulin prophylaxis through the use of high-throughput NIPT prophylaxis is estimated to be cost saving compared with the current practice of providing prophylactic prenatal anti-D immunoglobulin to all women who are RhD negative. A postpartum strategy that distinguishes between inconclusive results and positive results offers the greatest cost savings. The potential savings appear highly sensitive to the cost of NIPT.

Suggested research priorities

Evidence on the diagnostic accuracy of NIPT in women of non-white ethnicity is needed, for which large prospective cohort studies collecting diagnostic accuracy data will be required. This is of particular concern, as non-white women may be more likely to have inconclusive test results.

Further evidence on the clinical impact of NIPT is needed. If it is implemented, appropriate auditing of NIPT and anti-D administration processes should be considered, recording clinical outcomes, such as sensitisation rates, NIPT and anti-D compliance, and quality of life.

Further clarifications over the potential additional costs for blood drawing, the transportation of samples and antenatal care visits to administer the test and deliver counselling and results are needed.

Further research to comprehensively appreciate the full impact of sensitisations on mothers and children is warranted.

Study registration

This study is registered as PROSPERO CRD42015029497.

Funding

Funding for this study was provided by the Health Technology Assessment programme of the National Institute for Health Research.

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Chapter 1 Background

Description of the health problem

Pregnant women who have a rhesus blood group (D antigen) (RhD)-negative blood type may carry a RhD-positive fetus. The presence of fetal RhD-positive cells in the maternal circulation can cause a mother who is RhD negative to produce anti-D antibodies against the RhD antigen. This process, called sensitisation, can happen at any time during pregnancy, although it is most common in the third trimester and during childbirth. Sensitisation can follow events in pregnancy known to be associated with fetal–maternal haemorrhage (FMH). Potentially sensitising events include some medical interventions (e.g. chorionic villus sampling, amniocentesis or external cephalic version), terminations, late miscarriages, antepartum haemorrhage and abdominal trauma.

The process of sensitisation itself has no adverse effects to the mother and does not usually affect the pregnancy during which it occurs. However, in a subsequent pregnancy with a RhD-positive fetus in women who have been sensitised to the RhD antigen, the woman's anti-D antibodies may respond to the presence of RhD-positive blood in the fetus, resulting in haemolytic disease of the fetus and newborn infant. This can cause severe fetal anaemia, which may lead to fetal heart failure, fluid retention and swelling (hydrops) and intrauterine death.

Prophylaxis with anti-RhD immunoglobulin can substantially reduce the risk of sensitisation in RhD-negative women and the prevalence of haemolytic disease of the fetus and newborn infant.[1] Before anti-D immunoglobulin was available, the incidence of RhD sensitisation in RhD-negative women following the birth of two RhD-positive babies was approximately 16%. Haemolytic disease of the fetus and newborn infant was a significant cause of morbidity and mortality, which occurred in approximately 1% of all births. Since the introduction of routine postnatal administration of anti-D immunoglobulin, the incidence of RhD sensitisation dropped to approximately 2%. The introduction of routine antenatal prophylaxis during the third trimester of pregnancy has led to a further reduction in the sensitisation rate to between 0.17% and 0.28%. This has led to a decrease in mortality associated with haemolytic disease of the fetus and newborn infant, from 46 in 100,000 births before 1969 to 1.6 in 100,000 births by 1991.[2]

In England, there were 646,904 births from April 2013 to March 2014, of which approximately 15% (97,036 births) were to RhD-negative women.[3] Approximately 40% of these women will carry a RhD-negative fetus (around 39,000 per year) and therefore do not need administration of anti-D immunoglobulin. White populations of European descent have an approximately 15% incidence of RhD negativity; however, this is 3–5% in populations of African American ethnicity and is very rare in those of Eastern Asian origin.[4] Despite the mixing of genes, the majority of RhD-negative white people are RhD negative a result of gene deletion, and *RHD* gene variants are relatively rare in white people, who account for < 1% of all RhD-negative people. However, in people with black African ethnicity, an inactive *RHD* gene (known as the *RHD* pseudogene *RHDψ*), which is mostly the result of genes that contain RhD sequences but do not produce the D antigen, is present in 66% of RhD-negative people. The distribution of this gene varies between people with black African ethnicity and people with other African origins,[5] with 24% of people with African American ethnicity and 17% of people with black South African ethnicity having the gene.[6]

Current service provision and care pathway

The National Institute for Health and Care Excellence (NICE) guideline on antenatal care (2008)[7] recommends that women should be offered testing for blood group and rhesus D status in early pregnancy. All women identified as RhD negative will be tested for the presence of RhD antibodies, regardless of whether or not

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they are known to be sensitised. In those identified as RhD negative, administration of anti-D immunoglobulin is recommended both as prophylaxis and following potential sensitising events to prevent sensitisation. Routine antenatal prophylaxis with anti-D immunoglobulin can be given as two doses at weeks 28 and 34 of pregnancy or as a single dose between 28 and 30 weeks.[7] Following potentially sensitising events, anti-D immunoglobulin should be administered within 72 hours of the event.[2]

Anti-D immunoglobulin is produced from pooled plasma from large numbers of RhD-negative donors who have been transfused with RhD-positive red cells to stimulate the production of RhD antibodies. Thus, it carries a risk of transmission of human blood-borne viral and prion diseases. Despite this risk, the National Comparative Audit of Blood Transfusion from 2013[8] reports that of the women eligible for anti-D immunoglobulin, 99.0% received anti-D immunoglobulin.

For pregnant women who are RhD negative and are sensitised to RhD antigen, the Royal College of Obstetricians and Gynaecologists has published guidance on the management of women with red cell antibodies during pregnancy.[9] This guideline recommends that all RhD-negative women who are sensitised to RhD antigen should attend pre-pregnancy counselling with a clinician who has knowledge and expertise of this condition, have their blood group and antibody status determined at the booking appointment (ideally by 10 weeks of gestation) and at 28 weeks of gestation and be offered non-invasive fetal RhD genotyping using maternal blood if maternal RhD antibodies are present. Once a RhD-positive fetus is identified, additional monitoring and treatment are required during the pregnancy.

Description of the technology under assessment

Summary of technologies (index tests)

The technology under assessment is high-throughput non-invasive prenatal testing (NIPT) for fetal rhesus D status (International Blood Group Reference Laboratory, NHS Blood and Transplant, Bristol, UK).

High-throughput NIPT of fetal RhD status uses a real-time quantitative polymerase chain reaction (PCR) method for predicting the fetal RhD genotype from fetal deoxyribonucleic acid (DNA) in the plasma of RhD-negative women. The test principle is based on the analysis of cell-free fetal DNA, that is, small fragments of fetal extracellular DNA shed from the placenta and circulating freely in the maternal plasma. The level of cell-free fetal DNA in maternal blood increases throughout the pregnancy. A woman who is RhD negative does not have a copy of the *RHD* gene; therefore, the presence of a *RHD* gene in a RhD-negative pregnant woman suggests a RhD-positive fetus.

High-throughput NIPT is performed using samples of maternal anticoagulated blood. DNA extraction is performed using an automated robotic platform, which can rapidly process samples. The robotic platform is used as a liquid handler to dispense samples and reagents. In the UK, primers and probes for specific exons of the *RHD* gene are used, with a number of controls being tested (such as RhD-positive DNA, RhD-negative DNA, *RHD* pseudogene positive DNA and no DNA). An algorithm is employed to determine the fetal RhD status. The samples can be tested in batches of between 32 and 88 samples. The time to complete the test from sample receipt to report generation is 5–6 hours.

High-throughput NIPT for fetal RhD status may enable anti-D immunoglobulin to be withheld from RhD-negative women who are carrying a RhD-negative fetus. These women could avoid unnecessary treatment with routine anti-D immunoglobulin, along with the potential risk associated with administration of blood products. In addition, these women may not need the provision of anti-D immunoglobulin following potentially sensitising events and there may no longer be a need for serological cord testing at birth.

Identification of important subgroups

There are potential challenges for the detection of fetal rhesus D status when performing NIPT in pregnant women. Dealing with the presence of *RHD* pseudogene poses a challenge. The majority of RhD-negative

individuals with white European ethnicity have the pseudogene as a result of gene deletion; however, in people with African ethnicity the Rh-negative phenotype is mainly the result of genes that contain RhD sequences but do not produce D antigen (*RHD* pseudogene).[5] In the presence of the *RHD* pseudogene, prenatal determination of fetal Rh type from maternal blood would reveal a RhD-positive type, but this would be confirmed as RhD negative by serology because of the abundant maternal D gene sequences that are not expressed but are amplified. This may, therefore, lead to higher rates of false-positive results when performing NIPT in this population.

There is a diverse array of Rh variant genes and it is generally accepted that at least two exons of *RHD* should be targeted for accurate RhD status prediction. For instance, targeting only exon 7 (or exon 10) would not detect the presence of the *RHD* pseudogene and other variants and targeting only exon 10 would not detect the presence of the *RHD* pseudogene or the hybrid *RHD-CE-D*$^{(s)}$ gene, which are commonly present in people with African ethnicity.

Evidence suggests that the diagnostic accuracy of NIPT may vary according to different gestational ages at the time of sampling. Two meta-analyses found that the diagnostic accuracy of NIPT was higher in the first trimester than in the second and third trimester.[10,11] However, a recent UK cohort study found that fetal RhD genotyping was more accurate for the prediction of RhD status if it was performed after, rather than before, 11 weeks' gestation.[12]

In this assessment we aim to investigate findings of high-throughput NIPT from a number of subgroups, such as those based on different gestational ages and different ethnicities as well as on the usage of different exons of *RHD*, if data are available.

Current usage in the NHS

Currently, all high-throughput NIPT for fetal RhD status determination in the UK is performed by the NHS Blood and Transplant International Blood Group Reference Laboratory in Bristol. If all pregnant RhD-negative women in England were to be tested, approximately 100,000 samples would be tested each year. An increased capacity would be required for the International Blood Group Reference Laboratory to be able to cope with this demand by employing additional staff and acquiring more analytical platforms. Beyond this, extending the testing service to other laboratories is an alternative option. Blood samples would need to be transported from local hospital laboratories to the International Blood Group Reference Laboratory in Bristol or other laboratories. The established NHS Blood and Transplant transport system would be used to deliver blood samples across the country. This would need to be achieved in reasonable time, although there is evidence to suggest that cell-free fetal DNA is very stable.[13] There would also need to be reporting systems in place to ensure the accurate transmission of test results back to the women and their physicians and midwives.

Expected costs associated with technology

The potential costs associated with high-throughput NIPT to the NHS comprise two components. First, there is the unit cost of the diagnostic test itself, which varies with the level of throughput and to which a royalty fee may be added. An estimated unit cost for high-throughput NIPT of (confidential information has been removed) and a royalty payment of (confidential information has been removed) were considered. It should be noted that these estimates were provided in confidence by the company with the underlying assumption that the International Blood Group Reference Laboratory in Bristol will be the sole provider of the test nationally. Second, the potential costs of incorporating the test into routine antenatal care must be considered, which may bring additional costs relating to the time for antenatal care appointments to provide information about the test, counselling and delivering test results and also relating to blood drawing and blood sample transportation.

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Chapter 2 Definition of the decision problem

Decision problem

The clinical effectiveness and cost-effectiveness of high-throughput NIPT for assessing fetal rhesus D status in RhD-negative women not known to be sensitised to the RhD antigen for the NHS is uncertain. High-throughput NIPT for fetal RhD status may enable anti-D immunoglobulin to be withheld from RhD-negative women who are carrying a RhD-negative fetus. This subgroup of women could therefore avoid unnecessary prophylaxis with anti-D immunoglobulin during pregnancy, as well as the risk associated with exposure to blood products, which may have important resource implications for the NHS.

However, relying on NIPT to determine anti-D immunoglobulin use could lead to more women becoming sensitised, because women who incorrectly test negative on NIPT will not receive anti-D and so are at increased risk of sensitisation. This risk will be increased if cord blood testing is also withdrawn and postpartum anti-D given on the basis of the NIPT results. It is also unclear whether or not the cost of instituting NIPT screening will outweigh the savings from the reduced use of anti-D treatment.

This report, undertaken for the NICE Diagnostics Assessment Programme, examines the clinical effectiveness and cost-effectiveness of high-throughput NIPT. It considers the value of NIPT as a diagnostic test for RhD status, the clinical impact of using NIPT to determine anti-D immunotherapy use and the cost implications of implementing a NIPT screening programme. The report will allow NICE to make recommendations about how well the high-throughput NIPT works and whether or not the benefits are worth the cost of the tests for use in the NHS.

This report contains reference to confidential information provided as part of the NICE appraisal process. This information has been removed from the report and the results, discussions and conclusions of the report do not include the confidential information. These sections are clearly marked in the report.

Overall aims and objectives of the assessment

The purpose of this project was to assess the clinical effectiveness and cost-effectiveness of using high-throughput NIPT to identify fetal RhD status with any consequent changes in treatment management. In this assessment we addressed the following key objectives:

(a) to perform a systematic review and meta-analysis of the diagnostic accuracy of high-throughput NIPT for fetal RhD status
(b) to perform a systematic review of the clinical impacts of high-throughput NIPT, including incidence of sensitisation events, and adverse effects to the mother and fetus
(c) to systematically review the cost-effectiveness evidence on high-throughput NIPT and its impact on the management of pregnant women
(d) to produce a de novo cost-effectiveness model assessing the cost-effectiveness of high-throughput NIPT to identify fetal RhD status in RhD-negative women not known to be sensitised to the RhD antigen
(e) to assess the impact of alternative scenarios related to the timing of the test and the impact of the test on the use of antenatal anti-D prophylaxis for sensitising events and postdelivery testing.

This report is divided into two sections: clinical effectiveness (covering objectives a and b) is discussed in *Chapter 3*; and cost-effectiveness (covering objectives c–e) is discussed in *Chapter 4*.

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Chapter 3 Assessment of clinical effectiveness

The review of clinical effectiveness of high-throughput NIPT was broken down into the following three systematic reviews:

1. A review of the diagnostic accuracy of high-throughput NIPT for detecting RhD-positive fetuses.
2. A review of the clinical effectiveness of high-throughput NIPT, including numbers of sensitisations, test compliance and incidence of adverse events.
3. A review of the implementation of high-throughput NIPT in countries or regions in which it has been used, examining feasibility, guidance or recommendations for practice and need for further research.

In addition to these three reviews, we searched for existing systematic reviews of antenatal anti-D prophylaxis, identifying numbers of sensitisations, compliance and incidence of adverse events. Data from these existing reviews then facilitated the modelling of the probable clinical impact of high-throughput NIPT and supported the subsequent cost-effectiveness analyses.

The methodology of these reviews is described in the following sections.

Methodology of the clinical effectiveness reviews

The methods for systematic reviews of the diagnostic accuracy and clinical impacts of high-throughput NIPT for fetal RhD status are provided in the following sections.

Searches

The literature search aimed to systematically identify studies relating to the clinical effectiveness and cost-effectiveness of high-throughput, non-invasive, prenatal blood testing to determine fetal rhesus D status.

The search strategy was developed in MEDLINE (via Ovid) and then adapted for use in the other resources searched. The strategy included terms for rhesus D status combined, using the Boolean operator AND, with terms for the test. No language, date or geographical limits were applied and study design search filters were not used. EndNote X7 software (Thomson Reuters, CA, USA) was used to manage the references for the project.

Search strategies were developed by an information specialist with input from the project team. The search strategy was checked by a second information specialist.

The following databases were searched for relevant clinical effectiveness or cost-effectiveness studies from inception to November 2015: MEDLINE, MEDLINE In-Process & Other Non-Indexed Citations, Cumulative Index to Nursing and Allied Health Literature (CINAHL), Cochrane Central Register of Controlled Trials (CENTRAL), Cochrane Database of Systematic Reviews (CDSR), Database of Abstracts of Reviews of Effects (DARE), EMBASE, Health Technology Assessment (HTA) database, Maternity and Infant Care, NHS Economic Evaluations Database (NHS EED), PubMed and the Science Citation Index.

In addition, the following resources were searched for ongoing, unpublished or grey literature: ClinicalTrials.gov, Conference Proceedings Citation Index: Science, EU Clinical Trials Register, PROSPERO and the World Health Organization's International Clinical Trials Registry Platform portal.

The following websites were searched to identify any relevant guidelines: National Guidelines Clearinghouse, NICE, NHS Evidence, the Royal College of Obstetricians and Gynaecologists, the Turning Research into Practice database and the UK National Screening Committee. Reference lists of relevant

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reviews and included studies were checked to identify additional potentially relevant reports. The searches were updated in February 2016. A full search strategy can be found in *Appendix 1*.

Selection criteria

Types of studies

Diagnostic accuracy

Prospective cohort studies in which the index test (high-throughput NIPT) and reference standard test (cord blood sampling) were done independently in the same group of women to assess fetal RhD status were included. Included studies also had to report sufficient data to construct a 2 × 2 contingency table such that the cells in the table can be labelled as true positive, false positive, true negative and false negative.

Clinical effectiveness outcomes

Any experimental or observational study (controlled or non-controlled) was included provided that high-throughput NIPT was used to determine fetal RhD status and anti-D prophylaxis was given as required. Studies also had to report relevant clinical outcomes as listed in the following sections.

Implementation

Any publications discussing existing or experimental high-throughput NIPT screening programmes were included. Papers had to report issues related to the implementation of, or practical advice relating to, high-throughput NIPT as a screening tool to guide use of anti-D prophylaxis. This included publications that contained no numerical data but discussed practical issues of implementation, presented useful guidance or informed research recommendations.

Antenatal anti-D prophylaxis

Any systematic review reporting any aspect of the process of using routine antenatal anti-D to prevent sensitisation was included.

The following types of report were excluded: editorials and opinions, case reports and reports focusing only on technical aspects of the NIPT technology (such as technical descriptions of the testing process or specifications of machinery). Studies with a sample size of ≤ 10 were excluded. In the case of multiple reports for a given study or when the possibility of overlapping populations could not be excluded, the most recent or most complete reports were selected.

Population

For all reviews, the eligible population was pregnant women who were RhD negative and not known to be sensitised to RhD antigen.

Intervention

For all studies, high-throughput NIPT free-cell fetal DNA tests of maternal plasma used to determine fetal RhD status were eligible for inclusion. 'High-throughput' is a subjective concept and there is no clear consensus on its definition. For pragmatic reasons, we considered as high-throughput any NIPT that was conducted using an automated robotic platform (including automated DNA extraction and liquid handling) and that was able to process large numbers of samples rapidly for large-scale screening purposes. Studies in which this test was used for diagnosis (rather than screening) of sensitised women were excluded.

For clinical effectiveness studies, high-throughput NIPT had to be used to enable targeted anti-D prophylaxis.

Reference standard

For diagnostic accuracy studies, the reference standard considered was serological cord blood testing at birth or any other suitable postnatal blood test of the infant.

Outcomes

The following outcomes were included:

- test accuracy, including sensitivity and specificity
- number of inconclusive results, with reasons (e.g. no DNA detected)
- number of pregnant women who accept the test
- number of doses of anti-D immunoglobulin given (routine antenatal, following potentially sensitising events and postnatal)
- uptake of anti-D (antenatal and postnatal) immunoglobulin
- number of infections from anti-D immunoglobulin
- number of sensitisations
- number of cases of haemolytic disease of the fetus and newborn infant in subsequent pregnancies
- adverse effects of testing
- health-related quality of life.

At least two reviewers independently screened the titles and abstracts (if available) of all reports identified by the search strategy. Full-text copies of all studies deemed to be potentially relevant were obtained and two reviewers independently assessed them for inclusion. Any disagreements were resolved by consensus or by a third reviewer.

Data extraction

We selected the most recent or most complete report in cases of multiple reports for a given study or when we could not exclude the possibility of overlapping populations.

The data extraction forms were developed and piloted. One reviewer independently extracted details from full-text studies of study design, participants, index, comparator and reference standard tests and outcome data. The data extraction was checked by another reviewer. Any disagreements were resolved by consensus or by recourse to a third reviewer.

For studies reporting diagnostic data, we extracted the number of true positives, true negatives, false positives and false negatives for each index test evaluated in each study to construct 2 × 2 tables. If such data were not provided by the study authors, we attempted to contact them to construct the 2 × 2 table for the study population or the prespecified subgroups. Otherwise, we calculated the number of true positives, true negatives, false positives and false negatives from the summary estimates of sensitivity and specificity of the index test, if available. If reported, we extracted data on the number of undetermined or uninterpretable results. For studies in which only a subgroup of patients was included in the review, we extracted, analysed and presented data for this subgroup only. If some data were unclear or missing, we attempted to contact study authors to obtain additional data.

For studies reporting clinical outcomes, we extracted data as the numbers of women or fetuses experiencing the specified outcome. Mean differences, relative risks (RRs) or odds ratios [with 95% confidence intervals (CIs)] were extracted from comparative studies, when reported as unadjusted data.

For the implementation review, we summarised the findings and conclusions of the included publications using the following broad categories: study results and findings, issues for implementation, practical guidance and recommendations for research.

For the review of anti-D prophylaxis, we extracted summary results from syntheses or meta-analyses of studies on each clinical outcome reported. Mean differences, RRs or odds ratios with 95% CIs were extracted, when reported.

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Critical appraisal

One reviewer independently assessed the quality of all included studies in terms of risk of bias. Risk of bias from diagnostic accuracy studies was assessed using a modified version of the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) checklist.[14] The QUADAS-2 tool was adapted to ensure that it is applicable to assessing the quality of studies of non-invasive prenatal tests for detecting rhesus D status. The QUADAS-2 tool consists of four key domains: (1) patient selection, (2) index test, (3) reference standard and (4) flow of patients through the study and timing of the index test(s) and reference standard. Each domain was assessed in terms of the risk of bias. The first three domains were also assessed for concerns regarding their applicability in terms of whether or not the participants and setting; the index test, its conduct or interpretation; and the target condition, as defined by the reference standard, were applicable to nationwide screening in the UK.

A Cochrane Risk Of Bias Assessment Tool: for Non-Randomised Studies of Interventions (ACROBAT-NRSI) was used to assess risk of bias for each outcome of all comparative studies reporting other eligible clinical outcomes. The quality assessment was checked by another reviewer. Any disagreements were resolved by consensus or by recourse to a third party.

The quality of the studies in the implementation review was not assessed, as there is no validated tool for assessing the quality of studies on the implementation of health interventions.

Methods of data synthesis

Using extracted diagnostic accuracy data from the 2 × 2 tables, estimates of sensitivity, specificity, false-positive rates (FPRs) and false-negative rates (FNRs) were calculated and presented on forest plots and in receiver operating characteristic (ROC) space to examine the variability in diagnostic test accuracy within and between studies. In the primary analysis, undetermined or uninterpretable results were counted as being test positive, in accordance with current practice.

The hierarchical bivariate model described by Reitsma *et al.*[15] was fitted, which calculates summary estimates of sensitivity, specificity, FPRs, FNRs and the associated 95% CIs. The hierarchical summary receiver operating characteristic (HSROC) model[16] was fitted to produce summary ROC curves. Results of both models were presented in ROC plots.

Other eligible clinical outcomes were pooled if at least two studies reported on the same outcome and if data were reported consistently enough for analysis to be feasible. Otherwise, results were synthesised narratively. When meta-analyses were performed, data were pooled using standard random-effects DerSimonian and Laird meta-analyses. Analyses were conducted in R version 3 (The R Foundation for Statistical Computing, Vienna, Austria) and/or Stata® version 14 (StataCorp LP, College Station, TX, USA) software, as appropriate.

Investigation of heterogeneity

For diagnostic accuracy data, forest plots and ROC space were inspected to check for heterogeneity between study results. Subgroup analyses were conducted, when feasible, by performing separate bivariate and HSROC models in defined subgroups of studies.

If sufficient studies were available, we considered the following factors as potential sources of heterogeneity:

- gestational age at time of NIPT
- type of NIPT (e.g. test as used in Bristol vs. other)
- ethnicity (e.g. European vs. African).

For other clinical outcomes, when possible, heterogeneity was assessed using the I^2-statistic value and visual inspection of forest plots. Subgroup analyses and metaregression were used when feasible. Possible sources of heterogeneity were discussed and accounted for in the interpretation of the results.

Sensitivity analyses

We conducted sensitivity analyses (SAs) to explore:

- the impact of including and excluding undetermined or uninterpretable NIPT results on the pooled test accuracy estimates
- test accuracy in UK (Bristol)-based studies[12,17,18] only.

When participants from several studies were recruited from the same cohorts and significant overlap was suspected, data from only one study, with the most reliable reporting, were included in the main analyses.

Narrative synthesis

When quantitative synthesis and meta-analysis were not feasible, results for each study or systematic review were tabulated, categorised by outcome. For the review of implementation, we performed a narrative review of the findings of each included study, summarising their conclusions in terms of study findings, issues for implementation, practical guidance and recommendations for research.

Simulation study of clinical effectiveness

During the course of this report we found very little evidence on the probable clinical effectiveness of high-throughput NIPT and its impact on future sensitisation rates and adverse events. In order to investigate these issues, we opted to perform a simulation study to simulate possible outcomes of high-throughput NIPT in the UK, based on results from the diagnostic accuracy review and the results of published systematic reviews of antenatal anti-D prophylaxis and relevant audit data identified through additional literature searches.

The simulation sought to estimate the following in the UK population:

- rates of women with a RhD-positive fetus
- rates of women with positive/negative/inconclusive NIPT results
- rates of women who receive NIPT and/or antenatal anti-D prophylaxis
- number of sensitisations
- number of adverse effects on fetuses in subsequent pregnancies.

Data were extracted from the diagnostic accuracy review, existing systematic reviews of antenatal anti-D prophylaxis and other primary sources, when necessary.

We considered the following clinical scenarios:

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

Scenario 3 is equivalent (in clinical outcomes) to performing cord blood testing on women with negative NIPT but offering postpartum anti-D to all test-positive women without cord blood testing. Scenario 4 is equivalent (in clinical outcomes) to withdrawing cord blood testing and postpartum anti-D for women with negative NIPT but offering cord blood testing and postpartum anti-D (if needed) to all test-positive women.

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A Monte Carlo simulation of 10 million women was performed in R. Monte Carlo analysis is a modelling method that uses random number generation to simulate the running of multiple scenarios to define all potential outcomes of an event. We compared the amount of antenatal anti-D prescribed, the level of unnecessary anti-D use and the relative numbers of sensitisations and other adverse outcomes for each scenario.

Clinical effectiveness results

This chapter is structured as follows. The next section provides information on the quantity of research available, including characteristics and risk of bias of the included studies. This is then followed by the results sections with diagnostic accuracy, clinical effectiveness and implementation of high-throughput NIPT presented separately.

Quantity and quality of research available

Number of studies included

The literature searches of bibliographic databases identified 3921 references. After initial screening of titles and abstracts, 227 were considered to be potentially relevant and were ordered for full-text paper screening. In total, eight studies[12,17–23] were included in the diagnostic review of high-throughput NIPT, seven studies[18,20,22,24–27] were included in the clinical effectiveness review and 12 studies[13,17,18,20–28] were included in the review of implementation of high-throughput NIPT (with some overlap between studies). *Figure 1* shows a flow diagram outlining the screening process with reasons for exclusion of full-text papers.

All studies except two[8,28] were cohort studies. Most cohorts were reported in several papers and abstracts, with considerable overlaps in data and reporting. For each cohort and each review we selected the paper with the most up-to-date and complete data. Consequently, some papers were included in more than one review and some papers (mostly conference abstracts with limited or outdated data) were not

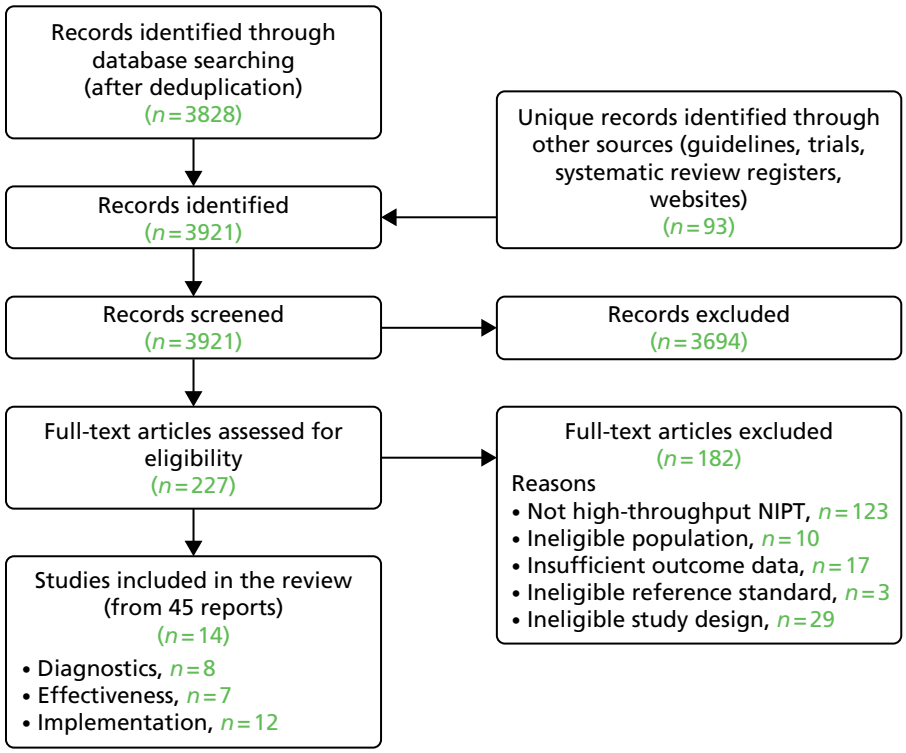

FIGURE 1 Flow diagram: study selection process.

included in any analysis. *Table 1* presents an overview of these cohort studies, the publications associated with each cohort and in which review the publications were included. *Appendix 2* presents a list of all included references.

Excluded studies

A list of full-text papers that were excluded, along with the reasons for their exclusion, is given in *Appendix 3*. These papers were excluded because they failed to meet one or more of the inclusion criteria in terms of the type of study, participants, test, reference standard or outcomes reported.

Results: assessment of diagnostic accuracy

Characteristics of the included studies

Table 2 presents the summary information of characteristics of the included diagnostic accuracy studies. There were eight studies[12,17–23] for the diagnostic review. All the studies were prospective studies and were conducted in European countries. Four studies were conducted in England,[12,17–19] three of which were based in Bristol.[12,17,18]

The sample size (number of patients/samples analysed) of studies ranged from 282 to 18,383. Most studies recruited pregnant women with a median gestational age of 10–28 weeks. Most participants were of white European ethnicity. All studies used maternal plasma as their sample source. A robotic DNA extraction instrument was employed in all studies. The studies used a number of robotic platforms such as MDx BioRobot (Qiagen, Crawley, UK), MagNa Pure 96 (Roche Ltd, Rotkreuz, Switzerland), MagNA Pure LC (Roche Ltd, Rotkreuz, Switzerland) and COBAS® AmpliPrep (Roche Ltd, Rotkreuz, Switzerland). For PCR, all studies targeted at least two exons (generally exons 5 and 7) and used at least two controls for *RHD* assay (RhD-positive DNA and RhD-negative DNA) except for the study by Wikman *et al.*,[23] which targeted only exon 4 and used glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) DNA as a control. The reference standard used in all studies was cord blood serology, except for Akolekar *et al.*,[19] which did not describe the reference standard. Inconclusive results were reported in all but two studies.[21,22] *Appendix 4* presents further details of included studies.

Risk of bias of the included studies

Each of the eight full-text papers was assessed for risk of bias using a modified version of the QUADAS-2 tool containing 14 items. *Table 3* presents a summary of the results for the risk of bias across all studies in the four main domains: patient selection, index test, reference standard, and flow and timing. *Appendix 5* presents results of quality assessment for the individual studies. Despite some gaps in reporting, most studies were considered to have a low risk of bias for these four domains. NIPT as an automated procedure was deemed to have a limited risk of human error, and multiple controls were used for *RHD* assays in all studies except one.[23] Cord blood serology was the reference standard in all studies. The index test of NIPT was conducted independently of the reference standard and the results of one were considered unlikely to influence the results of the other, so the risk of incorporation bias was considered low.

It appears that most studies prospectively recruited consecutive samples from clinical practice. Only three studies stated that multiple pregnancies were included.[17,22,23]

Multiple pregnancies can pose specific challenges for NIPT (e.g. twin fetuses may have discordant RhD status). Excluding them from the analyses may have introduced patient selection bias, although it was deemed unlikely that this bias would substantially affect diagnostic accuracy estimates. Only three studies stated that their diagnostic threshold was prespecified during the conduct of the screening programme.[12,17,20]

None of the studies reported whether or not there were any adverse events from the index test or reference standard.

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TABLE 1 Overview of included cohorts and studies

Cohort (country)	Number of full-text papers	Number of conference abstracts	Papers included in review			
			Diagnostic accuracy (full-text paper)	Clinical effectiveness (full-text paper)	Implementation (full-text paper)	Linked conference abstracts
UK (Bristol)	3	6	Chitty *et al.*, 2014;[12] Finning *et al.*, 2008;[17] and Soothill *et al.*, 2015[18]	Soothill *et al.*, 2015[18]	Finning *et al.*, 2008;[17] and Soothill *et al.*, 2015[18]	Chitty *et al.*, 2011;[29] Chitty *et al.*, 2012;[30] Daniels *et al.*, 2012;[31] Finning *et al.*, 2015;[32] Finning *et al.*, 2014;[33] and Ford and Soothill, 2016[34]
UK (London)	2	0	Akolekar *et al.*, 2011[19]	None	Oxenford *et al.*, 2013[28]	None
Denmark	4	5	Banch Clausen *et al.*, 2014[20]	Banch Clausen *et al.*, 2014;[20] Banch Clausen *et al.*, 2012;[24] and Damkjaer *et al.*, 2012[27]	Banch Clausen *et al.*, 2014;[20] Banch Clausen *et al.*, 2012;[24] Clausen *et al.*, 2013;[13] and Damkjaer *et al.*, 2012[27]	Banch Clausen 2012;[35,36] Dziegiel 2012;[37] Banch Clausen *et al.*, 2011;[38] and Steffensen *et al.*, 2012[39]
The Netherlands	2	10	Thurik *et al.*, 2015[21]	de Haas *et al.*, 2012[25]	de Haas *et al.*, 2012;[25] and Thurik *et al.*, 2015[21]	Veldhuisen *et al.*, 2014;[40] Veldhuisen *et al.*, 2013;[41] Thurik *et al.*, 2014;[42,43] Scheffer *et al.*, 2013;[44] van der Schoot *et al.*, 2005;[45] de Haas *et al.*, 2012;[46] de Haas *et al.*, 2013;[47] Grootkerk-Tax *et al.*, 2006;[48] and van der Ploeg *et al.*, 2015[49]
Spain	1	0	Grande *et al.*, 2013[22]	Grande *et al.*, 2013[22]	Grande *et al.*, 2013[22]	None
Sweden	2	10	Wikman *et al.*, 2012[23]	Tiblad *et al.*, 2013[26]	Wikman *et al.*, 2012;[23] and Tiblad *et al.*, 2013[26]	Wikman *et al.*, 2012;[50] Wikman *et al.*, 2011;[51] Wikman 2013;[52] Wikman *et al.*, 2010;[53] Tiblad *et al.*, 2010;[54] Tiblad *et al.*, 2012;[55] Neovius *et al.*, 2014;[56] Tiblad 2012;[57] and Neovius *et al.*, 2016[58]
Total			8	7	12	31

TABLE 2 Characteristics of the diagnostic accuracy studies

Study	Location	DNA extraction tool	Gestational age (weeks) at time of NIPT, median (range)	Sample size[a]	RhD-positive fetuses	RhD-negative fetuses	Inconclusive test results
Akolekar *et al.*, 2011[19]	UK (London)	MDx BioRobot (Qiagen, Crawley, UK)	12.4 (11–14)	586	410	176	84
Banch Clausen *et al.*, 2014[20]	Denmark	QIAsymphony SP (Qiagen, Hilden, Germany); MagNA Pure LC (Roche Ltd, Rotkreuz, Switzerland); MagNA Pure Compact Instrument (Roche Ltd, Rotkreuz, Switzerland)	25 (23–28)	12,668	7830	4838	274
Chitty *et al.*, 2014[12]	UK (Bristol)	MDx BioRobot (Qiagen, Crawley, UK)	19 (5–35)	4913	2890	2023	393
Finning *et al.*, 2008[17]	UK (Bristol)	MDx BioRobot (Qiagen, Crawley, UK)	28 (8–38)	1869	1156	713	64
Grande *et al.*, 2013[22]	Spain	COBAS® AmpliPrep (Roche Ltd, Rotkreuz, Switzerland)	24–26	282	186	96	NR
Soothill *et al.*, 2015[18]	UK (Bristol)	MDx BioRobot (Qiagen, Crawley, UK)	15–17 (mostly)	499[b]	315	184	61
Thurik *et al.*, 2015[21]	The Netherlands	MagNa Pure 96 (Roche Ltd, Rotkreuz, Switzerland)	26	18,383[b]	11,283	7100	NR
Wikman *et al.*, 2012[23]	Sweden	MagNA Pure LC (Roche Ltd, Rotkreuz, Switzerland)	8–40	3291[c]	2073	1218	13

NR, not reported.
a Number of blood samples unless otherwise specified.
b Number of participants.
c Excludes pre 8 weeks' gestation pregnancies.

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TABLE 3 Risk of bias and applicability of findings of included studies

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Akolekar *et al.*, 2011[19]	High	High	Unclear	Unclear	High	Low	Unclear
Banch Clausen *et al.*, 2014[20]	Low	Low	Low	Low	Unclear	Low	Low
Chitty *et al.*, 2014[12]	Low	Low	Low	Low	Low	Low	Low
Finning *et al.*, 2008[17]	Low	Low	Low	Low	Low	Low	Low
Grande *et al.*, 2013[22]	Low	Low	Low	Low	Low	Low	Low
Soothill *et al.*, 2015[18]	Low	Unclear	Low	Low	Low	Low	Low
Thurik *et al.*, 2015[21]	Low	High	Low	High	Low	Low	Low
Wikman *et al.*, 2012[23]	Low	Low	Low	Low	Unclear	High	Low

Two studies[19,21] were judged as having a high risk of bias. Akolekar *et al.*[19] stated that the targeted RhD-negative women were selected from a database; however, it was unclear whether or not this selection was performed on a random basis. The study recruited a large proportion of people with African ethnicity (19.3%), and so it may not be representative of the general population of pregnant women in the UK. This, combined with the fact that *RHD* variant analyses were not performed, may have contributed to the larger than average proportion of inconclusive results (15%). Akolekar *et al.*[19] excluded inconclusive results from their analyses, thereby potentially inflating their diagnostic accuracy estimates. Characteristics of the reference standard were also poorly reported.

Thurik *et al.*[21] excluded multiple pregnancies from their analysis and only 80% of participants received a reference standard. Reasons why cord blood serology was not performed in a significant proportion of the study population were not reported. The study also stated that their prediction algorithm was judged daily and adjusted as needed, and it was likely that this introduced bias in the diagnostic accuracy estimates (the authors reported the estimated impact of these changes on their diagnostic accuracy results).

The results of the studies were considered broadly applicable to the use of high-throughput NIPT for nationwide screening purposes in the UK, except for two studies.[19,23] The test used by Wikman *et al.*[23] targeted only exon 4, unlike all other included studies, which targeted at least two exons (5, 7 and/or 10). It is generally advocated that a combination of exons 5 and 7 is targeted to discriminate the pseudogene *RHDφ*, which is particularly present in individuals of African origin.[6,59] In addition, most participants in Wikman *et al.*[23] received NIPT in the first trimester of pregnancy. There is evidence to suggest that NIPT is less accurate before around 11 weeks' gestation. These potential issues may have negatively affected the diagnostic accuracy of the test. Although it was a UK study, Akolekar *et al.*[19] recruited a significantly higher proportion of patients with African ethnicity (19.3%) than the population of pregnant women in the UK (3%).[60] As patients with black African ethnicity may be harder to diagnose, because of the high prevalence of *RHDφ* in this population, this may limit the applicability of the study's findings to the UK population of pregnant women.

Overall, the majority of included studies were judged as having a low risk of bias, but two studies, Akolekar *et al.*[19] and Thurik *et al.*,[21] were judged as having a high risk of bias.

Meta-analyses of diagnostic accuracy

This section presents the results of the meta-analyses of the diagnostic accuracy studies. One key issue when considering the diagnostic accuracy of NIPT is how women with inconclusive test results are handled. It is expected that, in the UK, such women will be treated as having a positive test with no

further testing. Although this was the policy in the three high-quality studies performed in Bristol, data on inconclusive tests were not reported in two studies.[21,22]

Given these differences we considered four approaches to the diagnostic analysis:

1. women with inconclusive tests treated as test positive (including Thurik *et al.*[21] and Grande *et al.*[22] studies)
2. women with inconclusive tests treated as test positive (excluding Thurik *et al.*[21] and Grande *et al.*[22] studies)
3. excluding all women with inconclusive test results
4. studies conducted in Bristol only.[12,17,18]

This last analysis is likely to represent the most plausible results for UK practice, assuming that the methods used in Bristol are retained nationwide.

In all analyses, women whose NIPT was conducted at or before 11 weeks' gestation were excluded when possible because of concerns that the diagnostic accuracy is poorer before 11 weeks and that the test should not be conducted before then (see *Subgroup analyses*). Some tests were performed between 8 and 11 weeks' gestation in two studies,[17,23] most women were tested between 8 and 12 weeks' gestation in Wikman *et al.*[23] and < 8% of tests were performed before 11 weeks in Finning *et al.*,[12] but it was not possible to remove those women from the analysis.

In diagnostic analyses it is conventional to report results in terms of sensitivity (women who correctly test positive) and specificity (women who correctly test negative). NIPT is highly accurate and the focus should be on women with an incorrect test result, so in these analyses results are presented in terms of the FPRs (women incorrectly testing positive and so offered unnecessary anti-D) and FNRs (women incorrectly testing negative and so at risk of sensitisation, as they do not receive anti-D treatment).

A summary of all the results of the bivariate meta-analyses of FPRs and FNRs is presented in *Table 4*.

It can be seen that results are broadly consistent across the four scenarios. NIPT is very accurate among women with a RhD-positive fetus: only 2–4 in 1000 of such women will have a negative test result and so be at risk of sensitisation as a result of not being offered anti-D. NIPT is slightly less accurate among women with a RhD-negative fetus: between 1.3% and 5.7% of such women will test positive (depending on the analysis performed) and so may be offered NIPT unnecessarily. If women with inconclusive test results are excluded from analyses, the FPR was 1.3%, rising to 3.9–4.4% if women with inconclusive test results are treated as having tested positive. This suggests that the main cause of test error is treating women with an inconclusive NIPT result as if they had tested positive.

Assuming that 60% of RhD-negative women have a RhD-positive fetus, about 0.5% of women have a conclusive, but incorrect, positive test result. About 0.1–0.2% of women have a false-negative test result.

We consider the results of each analysis in more detail in the following sections.

TABLE 4 Bivariate meta-analyses of FPRs and FNRs

Analysis case	Number of studies	FNR (at risk of sensitisation)		FPR (unnecessary anti-D)	
		Estimate (%)	95% CI	Estimate (%)	95% CI
Inconclusive tests treated as test positive (including Thurik *et al.*[21] and Grande *et al.*[22])	8	0.34	0.15 to 0.76	3.86	2.54 to 5.82
Inconclusive tests treated as test positive (excluding Thurik *et al.*[21] and Grande *et al.*[22])	6	0.38	0.15 to 0.94	4.37	2.79 to 6.78
Excluding all women with inconclusive test results	8	0.35	0.15 to 0.82	1.26	0.87 to 1.83
Studies conducted in Bristol only	3	0.21	0.09 to 0.48	5.73	4.58 to 7.16

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Considering inconclusive results as test positive

Figure 2 shows forest plots of FNRs and FPRs when counting an inconclusive test result as being test positive. The results of these figures are slightly different from those in *Table 4*, because the figure shows separate analyses of FPR and FNR, rather than a full bivariate analysis.

There was some evidence of inconsistency across studies. The I^2-statistic for heterogeneity was 75% for the FNR and 99% for the FPR. It should be noted that these high heterogeneities are, in part, a consequence of the high accuracy of the test and the large size of the studies (and consequent small within-study variance, because I^2 increases as the average within-study variance declines). They do not necessarily

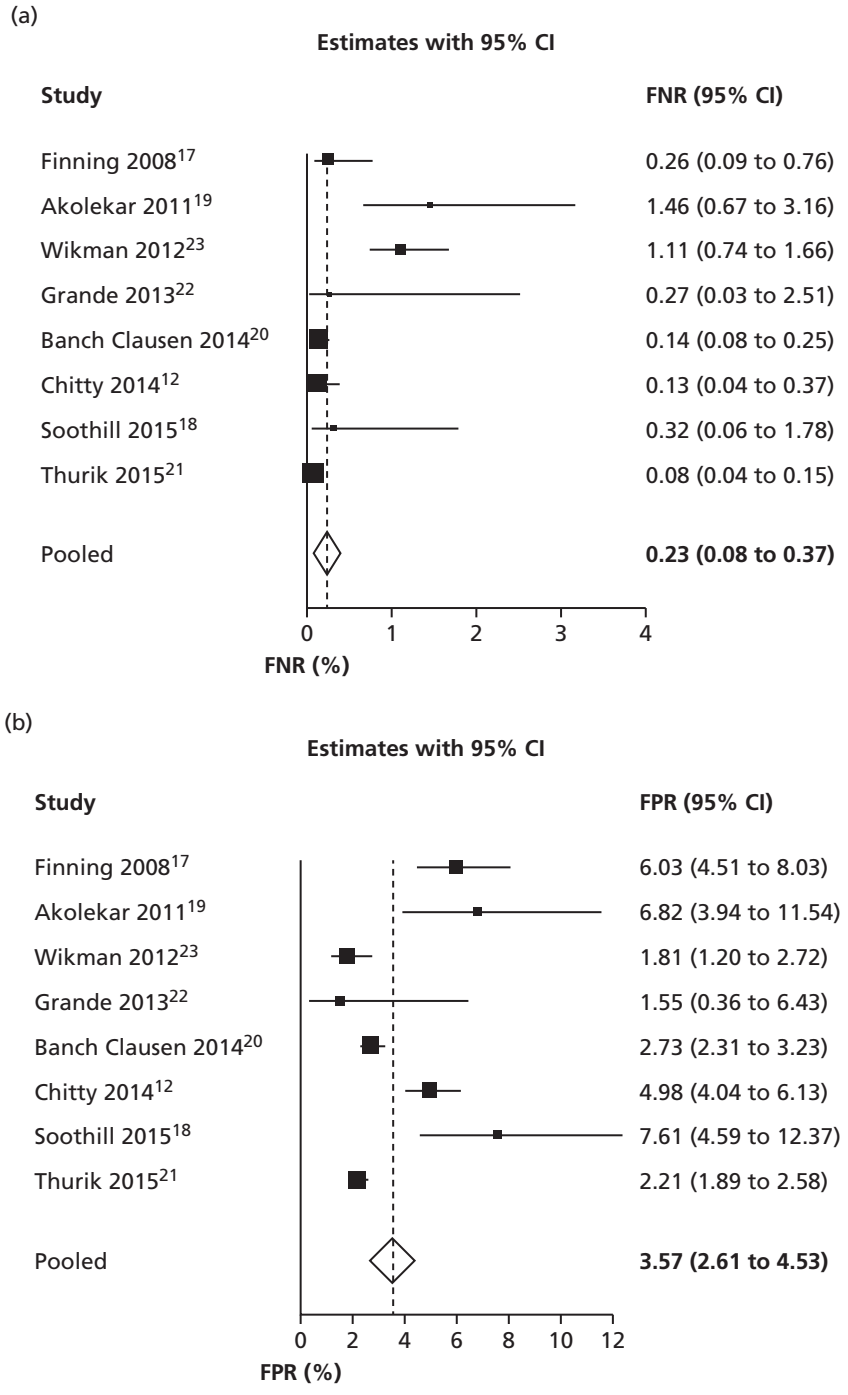

FIGURE 2 Forest plots of (a) FNR and (b) FPR when counting an inconclusive test result as being test positive.

indicate any clinically meaningful differences between studies. The heterogeneity in FPRs is likely to be a consequence of differing reporting and handling of inconclusive tests.

Figure 3 shows the results of each study, the results of the bivariate analysis (black circle) and the summary HSROC curve (black curve) for this analysis. As for other analyses, this is presented in terms of FPR and FNR rather than sensitivity and specificity. This plot shows the consistency of false-negative results, except for two outlying studies.[19,23] The Wikman *et al.*[23] study performed most NIPT in the first trimester, earlier than other studies. As discussed later (see *Subgroup analyses*), the timing of NIPT may have an impact on the FNR. The studies are less consistent in FPRs. This is most probably because the studies have different numbers of inconclusive test results and different methods of handling such results. As women with an inconclusive result are treated as RhD positive, women with an inconclusive result but a RhD-negative fetus will have a false-positive result. There may also be some heterogeneity because of differences in the threshold used and how different testing machines operated.

When excluding the two studies that did not report numbers of inconclusive tests,[21,22] the results were broadly similar, as seen in *Table 4*. The forest plots of FPR and FNR for this analysis are given in *Appendix 6*.

Excluding inconclusive results

We considered the diagnostic accuracy of NIPT, excluding all inconclusive test results, to identify the 'optimal' diagnostic accuracy in which a test result is obtained for every woman. This analysis excluded women who were difficult to diagnose, so it may overestimate diagnostic accuracy. Forest plots for FNR and FPR are shown in *Figure 4*.

Excluding women with inconclusive test results has no meaningful impact on false-negative results (as those women are always assumed to have a positive result). It does, however, considerably reduce the FPR. The FPR, at 1.2%, is low but still considerably higher than the FNR. This suggests that NIPT is more accurate in

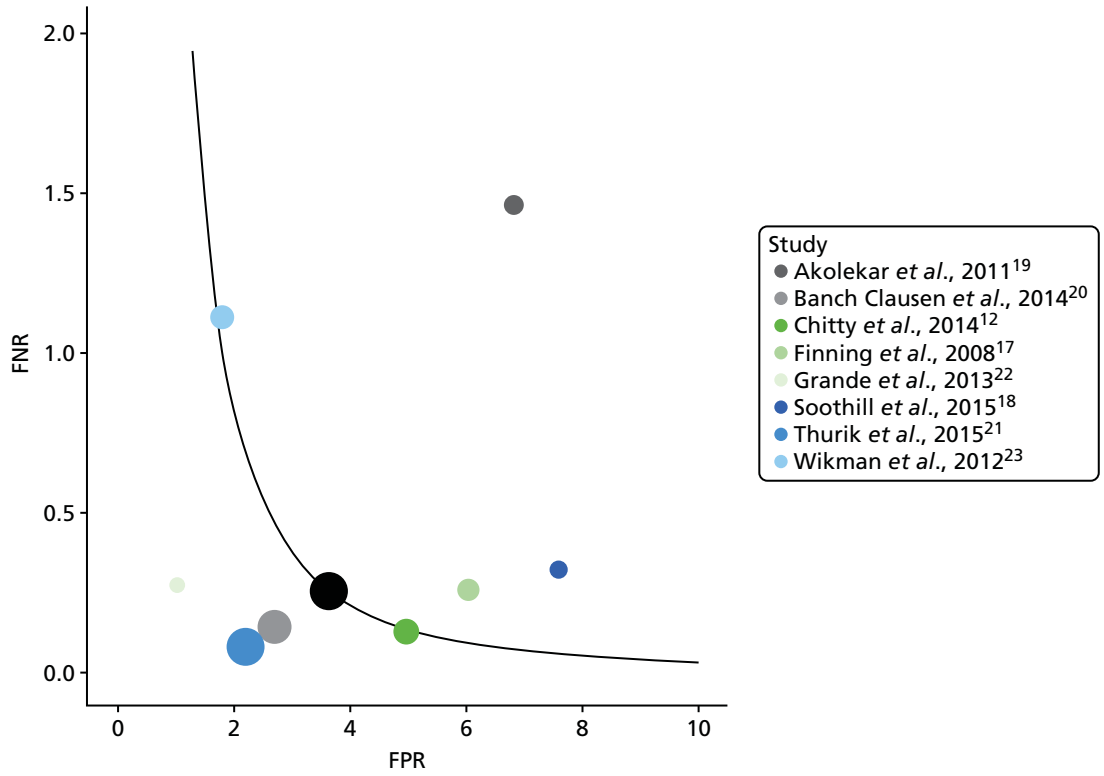

FIGURE 3 Hierarchical summary receiver operating characteristic and bivariate analysis when counting an inconclusive test result as being RhD positive.

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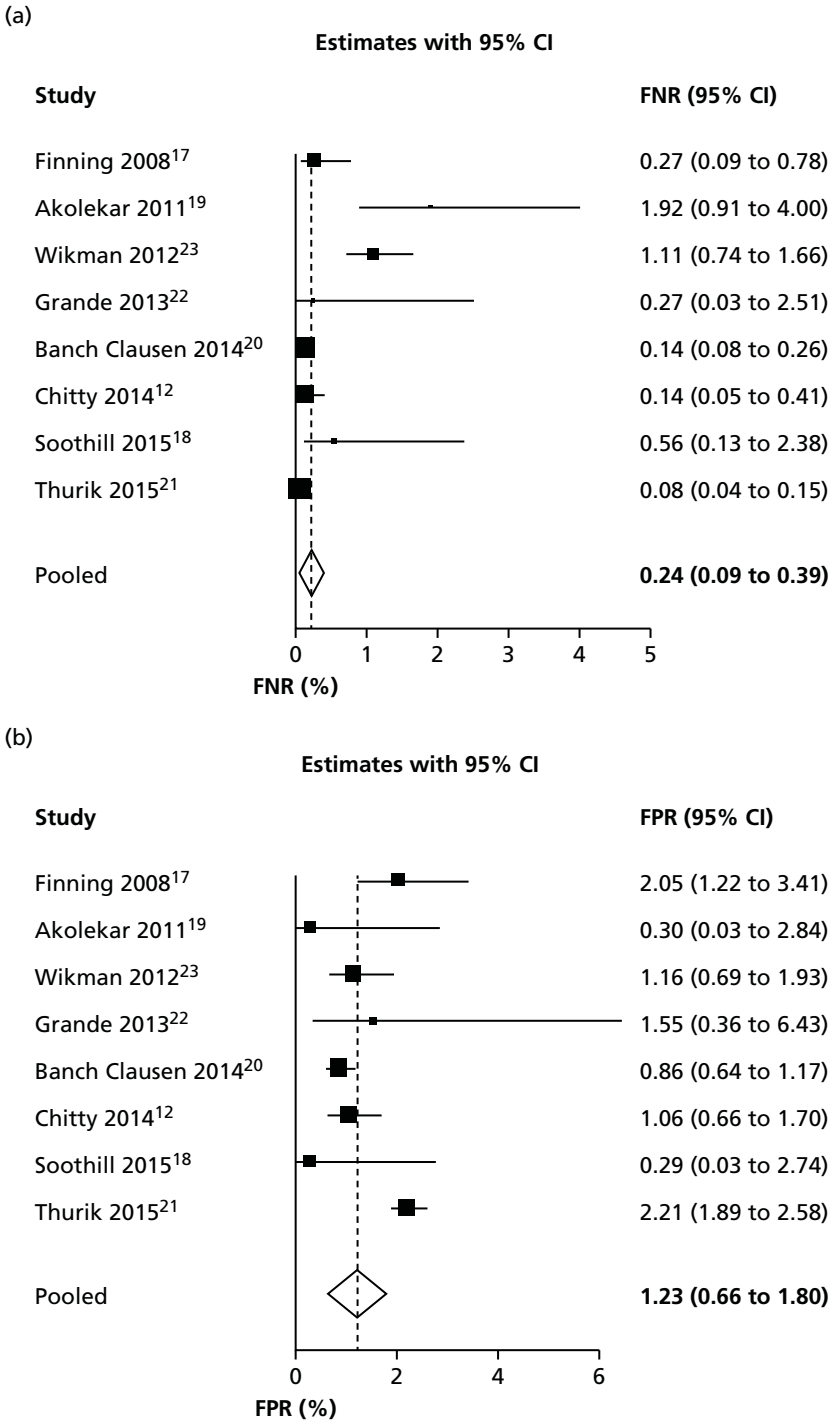

FIGURE 4 Forest plots of (a) FNR and (b) FPR excluding women with inconclusive test results.

women with a RhD-positive fetus than in those with a RhD-negative fetus. There was some evidence of heterogeneity across studies. The I^2-statistic for heterogeneity was 75% for the FNR and 99% for the FPR. The ROC plot with bivariate and HSROC analyses is given in *Appendix 6*.

Bristol studies

We performed a subgroup meta-analysis of only the high-quality studies based in Bristol[12,17,18] in order to assess the most likely performance of NIPT in the UK. We excluded the study by Akolekar *et al.*[19] (based in London but with NIPT run in Bristol) from this analysis on the grounds of it having a high risk of bias, as it was not primarily intended to assess NIPT screening and because of the limited applicability of recruited

participants. A higher proportion of people with African ethnicity (19.3%) in this study means that it may not be representative of the general population of pregnant women in the UK.

In this analysis, women with an inconclusive test result were treated as having a positive result, in line with the practice in the studies.

As observed in *Table 4* and *Figure 5*, the three Bristol studies have a slightly lower FNR and a higher FPR than other studies. This suggests that the Bristol high-throughput NIPT approach in which the MDx Bio Robot machine is used may be using a different test threshold from other countries, which further minimises false-negative findings, with a consequent increase in the FPR. This may explain some of the heterogeneity observed in previous analyses.

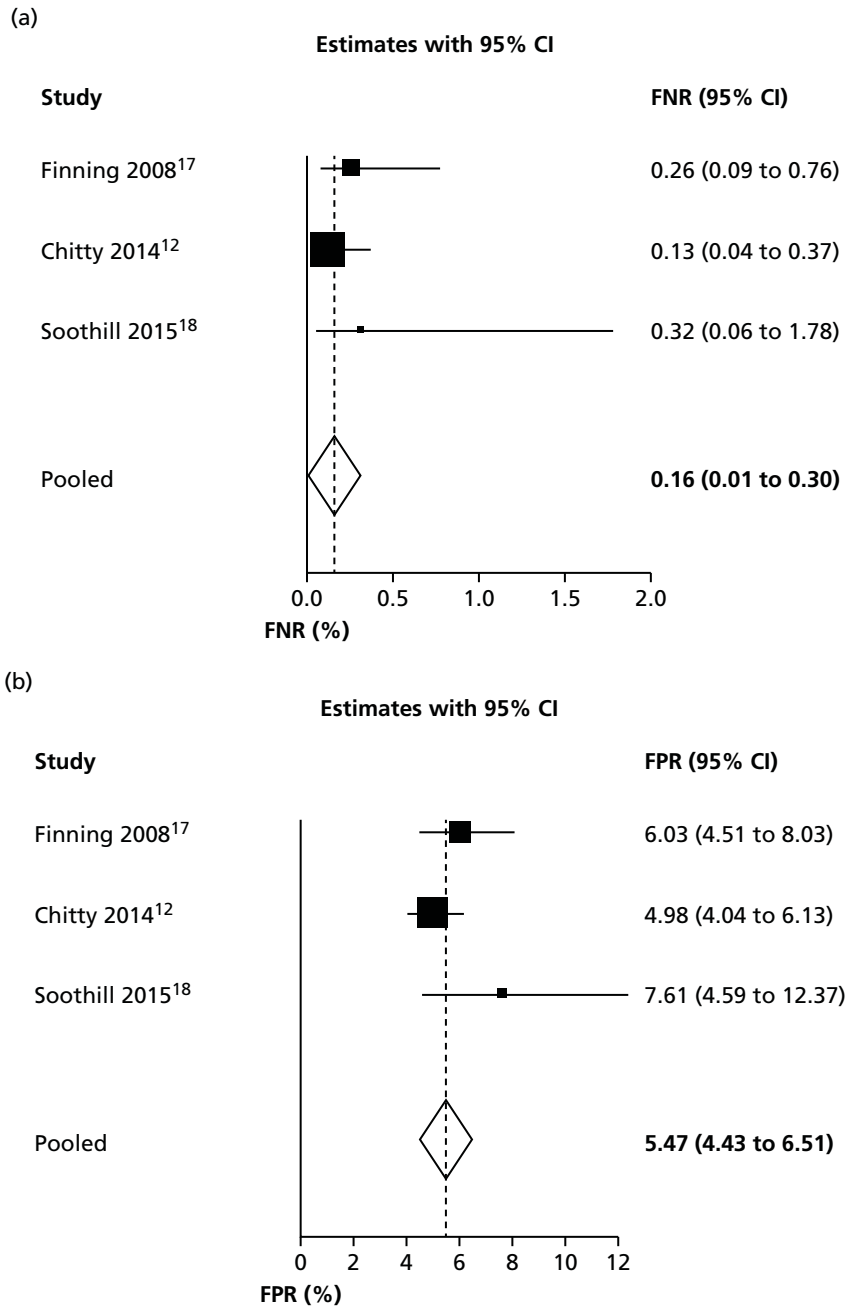

FIGURE 5 Forest plots of (a) FNR and (b) FPR for the Bristol studies.

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If inconclusive tests results were excluded from the Bristol studies, the summary FNR was 0.263% (95% CI 0.13% to 0.56%) and the FPR was 1.474% (95% CI 0.82% to 2.63%). This confirms that most false-positive results arise from treating women with an inconclusive test result as being test positive.

Inconclusive test results

As seen in *Table 4*, treating women with inconclusive test results as if they had a positive test has a substantial impact on diagnostic accuracy. Knowing the incidence of inconclusive test results is therefore important when determining diagnostic accuracy. *Table 5* summarises the rates of and reasons for inconclusive test results across included studies. When reported, the most common reasons for inconclusive results were the presence of a maternal/fetal *RHD* variant.

These results show that there is considerable variation in the rates of inconclusive tests across studies. The most likely cause for this variability is differences in how NIPT was conducted (e.g. different numbers and types of exons considered). However, even in the studies in which tests were conducted in Bristol using the same test, there is considerable unexplained variation. Differences in the characteristics of study populations (e.g. different proportions of people of black African ethnicity) may also explain some of this variation.

TABLE 5 Inconclusive test results in the included studies

Study	Location	RhD-positive fetuses (%)	Inconclusive test results (%)	RhD-positive fetuses in women with inconclusive test results (%)	Reported reasons for inconclusive results (number of cases)
Akolekar *et al.*, 2011[19]	UK (London)	70.0	14.3	85.7	Insufficient DNA ($n = 5$); *RHD* variant ($n = 44$); NR ($n = 40$)
Banch Clausen *et al.*, 2014[20]	Denmark	61.8	2.2	66.8	Maternal weak D ($n = 93$); maternal silent *RHD* variant ($n = 38$); high level of maternal background DNA ($n = 29$); technical problems ($n = 19$); maternal DVI ($n = 14$); weak PCR signal ($n = 13$); suspected maternal *RHD* positive ($n = 3$); no reported cause ($n = 65$)
Chitty *et al.*, 2014[12]	UK (Bristol)	58.8	7.0	76.6	NR
Finning *et al.*, 2008[17]	UK (Bristol)	61.9	3.4	54.7	Insufficient DNA ($n = 30$); suspected maternal *RHD* gene ($n = 25$); failure to extract DNA from plasma ($n = 1$)
Grande *et al.*, 2013[22]	Spain	66.0	NR	NR	NR
Soothill *et al.*, 2015[18]	UK (Bristol)	63.1	12.2	77.0	NR
Thurik *et al.*, 2015[21]	The Netherlands	61.4	NR	NR	Maternal *RHD* variant ($n = 55$); fetal variant ($n = 45$); weak PCR signals ($n = 70$); incorrect blood sample ($n = 11$)
Wikman *et al.*, 2012[23]	Sweden	63.0	0.4	38.5	*RHD* variant ($n = 14$); no second sample ($n = 18$, of which 13 were spontaneous abortions and miscarriages)

NR, not reported.

We performed a meta-analysis to estimate average rates of inconclusive test results. The results of this analysis are shown in *Table 6*. Based on these results, we would estimate that 6.7% of women in the UK would have an inconclusive test result, but this is subject to considerable uncertainty.

Table 5 also shows that, in general, most women with an inconclusive test result have a RhD-positive fetus (and it is more common than in the general population) and so treating all women with inconclusive test results is reasonable, if no further testing is possible. However, there are still many women with a RhD-negative fetus who would receive anti-D unnecessarily.

Subgroup analyses

We considered the effect of the timing of NIPT on its diagnostic accuracy. *Figure 6* shows the FNRs plotted by gestational age at time of high-throughput NIPT. It suggests that FNRs after 11 weeks' gestation were consistent, irrespective of timing, but that FNRs were higher before 11 weeks' gestation. *Figure 7* shows the FPRs plotted by gestational age at time of high-throughput NIPT. There was no obvious pattern from this figure. Only one study[12] examined test performance at multiple time points. *Figure 8* shows the FPRs and FNRs at different times for this study. It indicates that FNRs were higher before 11 weeks' gestation and were generally stable after 11 weeks' gestation. We did not perform any formal statistical analyses on the timing data (such as a metaregression) because the relationship appears to be a step change in accuracy, rather than a linear trend over time. These results together suggest that NIPT is insufficiently accurate before around 11 weeks' gestation (i.e. in first trimester) but is accurate at any time after the end of the first trimester.

TABLE 6 Meta-analyses of inconclusive results

Studies included	Estimated inconclusive rate (%)	95% CI (%)
All reporting inconclusive tests	4.0	1.5 to 10.3
Bristol studies[12,17,18] only	6.7	3.7 to 11.7

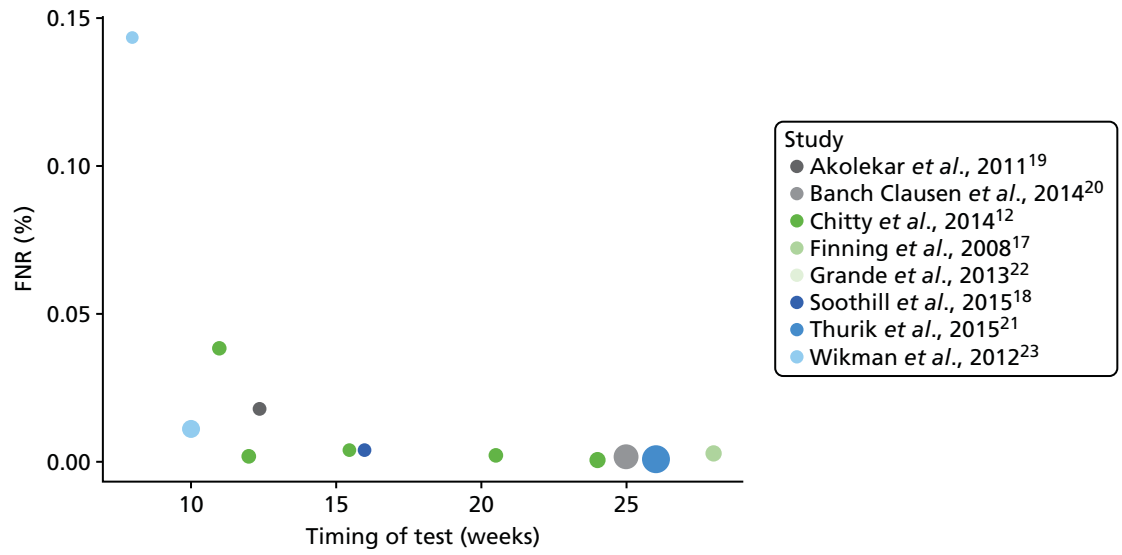

FIGURE 6 False-negative rate by gestational age at time of NIPT.

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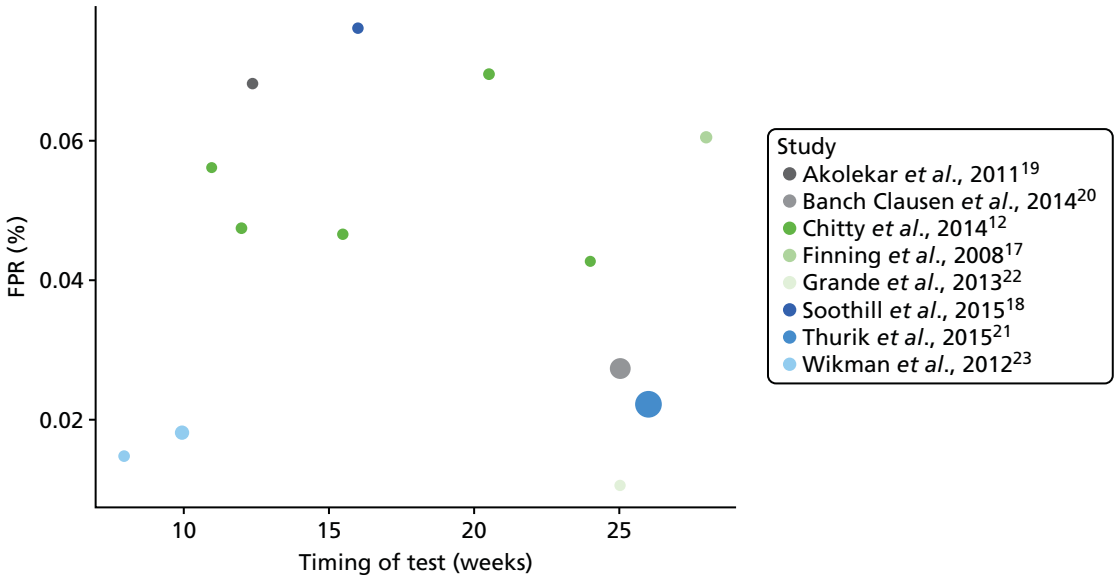

FIGURE 7 False-positive rate by gestational age at time of NIPT.

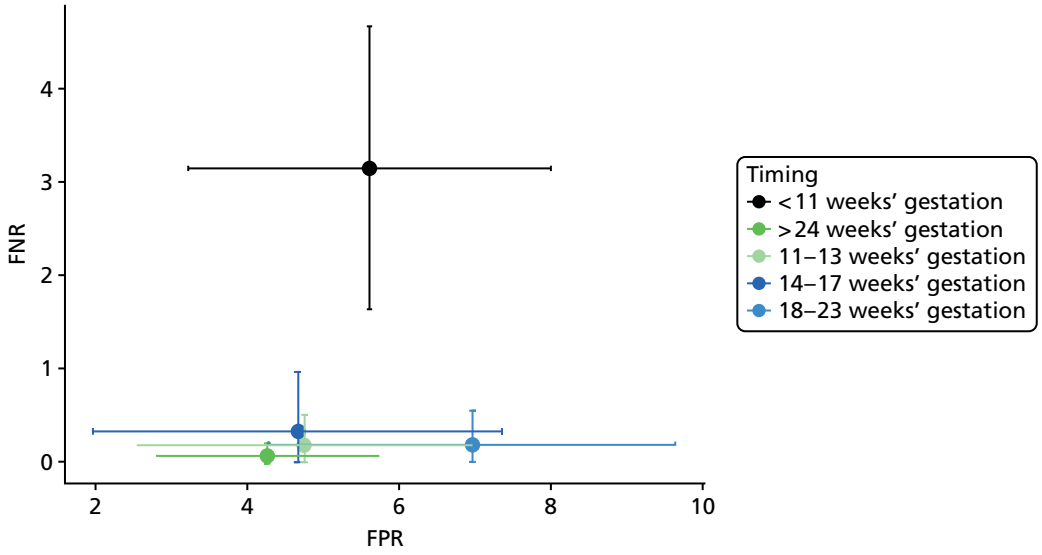

FIGURE 8 False-negative rate against FPR for Chitty *et al.*[12] study.

We also considered the impact of the timing of high-throughput NIPT on the number of inconclusive test results (*Figure 9*). Despite the data from Wikman *et al.*[23] being heterogeneous, there appears to be a trend that the percentage of inconclusive results for this test reduces as the gestational age increases from 11 weeks. This is most obvious in the Chitty *et al.*[12] study, which reported numbers of inconclusive tests at different times.

We were unable to conduct any subgroup analysis based on ethnicity, as the relevant data were not reported in any publication. As all studies were conducted in Europe, numbers of participants of non-white ethnicity were few. Any diagnostic analysis of non-white ethnicities may therefore not give reliable results.

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. We have considered a subgroup analysis including the Bristol-based studies only, as reported in *Meta-analyses of diagnostic accuracy*.

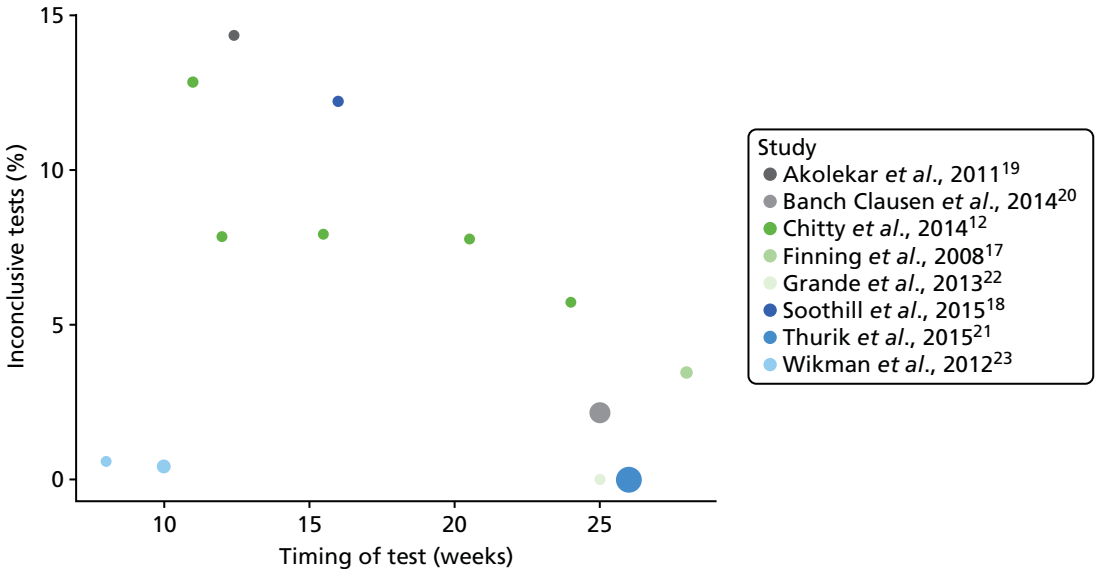

FIGURE 9 Inconclusive results by test timing.

Sensitivity analyses

We performed two post hoc SAs. The first excluded the two studies considered to have a risk of bias[19,21] and the second excluded the Wikman *et al.*[23] study, as this included a substantial number of women with NIPT performed before 11 weeks' gestation. Bivariate meta-analyses as in *Table 4* were performed excluding these studies. The results are presented in *Appendix 6*.

Excluding the two studies that were considered to have high risk of bias had limited impact on the FPRs and FNRs and does not alter any conclusions. Excluding the Wikman *et al.*[23] study marginally reduced the FNRs, which is consistent with the finding that the FNR is higher before 11 weeks' gestation. It also slightly increased the FPR when counting inconclusive test results as positive. This is because there were few inconclusive tests in the Wikman *et al.* study (see *Table 5*). None of the SAs meaningfully alters any of the conclusions of these meta-analyses.

Results: assessment of clinical effectiveness

Characteristics of the included studies

Table 7 presents a summary of the characteristics of the seven studies included in the review of clinical effectiveness studies. All studies were observational and conducted in European countries, including Denmark, the Netherlands, Spain, the UK and Sweden. The sample size of studies ranged from 284 to 15,126. All participants were RhD-negative pregnant women and most participants were white European. Most studies recruited women with a gestational age median of 10–26 weeks. Three studies reported using routine antenatal anti-D prophylaxis (RAADP) at between 28 and 30 weeks.

Only two studies compared women receiving NIPT to controls.[20,26] One study[26] compared patients undergoing NIPT with routine management with no NIPT and routine postnatal anti-D prophylaxis only (historical control). The other comparative study[20] reported data on anti-D compliance in a small subgroup of participants from one region in Denmark, comparing participants receiving NIPT with those receiving no NIPT.

Risk of bias of the included studies

The results of the quality assessment of the two comparative studies are given in *Appendix 7*. In summary, both studies had significant limitations. Tiblad *et al.*[26] was considered as having a serious risk of bias, primarily owing to concerns about patient selection, confounding and missing data. Banch Clausen *et al.*[20] was considered as having a critical risk of bias across all outcomes because of concerns about patient

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TABLE 7 Characteristics of effectiveness studies

Study	Location	Study dates	Sample size[a]	Gestational age at time of NIPT (weeks)	Routine antenatal anti-D prophylaxis	Comparator
Banch Clausen *et al.*, 2014[20]	Denmark: one region	January–June 2010	591	Median 25	250–300 µg at 29 weeks	Postnatal anti-D only ($n = 109$)
Banch Clausen *et al.*, 2012[24]	Denmark: nationwide	January–June 2010	2312	Median 25	250–300 µg at 29 weeks	None
Damkjaer *et al.*, 2012[27]	Denmark: one hospital	June–September 2010	239	Mean 27	250–300 µg at 29 weeks	None
de Haas *et al.*, 2012[25]	The Netherlands: nationwide	July 2011–January 2012	15,126[b]	Mean 26	250 µg at 30 weeks and after birth	None
Grande *et al.*, 2013[22]	Spain: Barcelona	February 2010–October 2011	284	Range 24–26	NR	None
Soothill *et al.*, 2015[18]	England: three NHS trusts in south-west England	April–September 2013	529	Range 15–26	500 or 1500 µg (timing NR)	None
Tiblad *et al.*, 2013[26]	Sweden: Stockholm area	September 2009–March 2012 (reference cohort: 2004–8)	8347[c]	Median 10 (range 3–40)	250–300 µg at 28–30 weeks	Postnatal anti-D only (historical control) ($n = 18,546$)

NR, not reported.
a Number of blood samples undergoing NIPT unless otherwise specified.
b Number of participants undergoing NIPT.
c Number of pregnancies undergoing NIPT.

selection and lack of adjustment for potential confounders. The generalisability of these two studies to the UK context was limited given that participants in the control group did not receive RAADP.

The remaining five studies reported non-comparative effectiveness data for women receiving NIPT only. We did not perform a formal quality assessment of these studies for clinical effectiveness, as we considered the evidence from non-controlled studies to be of poor quality.

Results of studies on clinical effectiveness

Studies reported various clinical effectiveness outcomes, including sensitisation rate, NIPT uptake, rates of women receiving antenatal and postpartum anti-D prophylaxis and number of women avoiding unnecessary anti-D immunoglobulin use. We performed a narrative synthesis owing to the considerable heterogeneity in outcomes and study designs.

Sensitisations

One study reported data on the incidence of sensitisation (defined as having developed anti-D antibodies after the first trimester) and haemolytic disease of the newborn infant. Tiblad *et al.*[26] compared targeted routine antenatal anti-D in the first trimester with routine care (postnatal anti-D only, historical control) in the Stockholm region, Sweden. The study reported that the incidence of RhD sensitisation in the cohort that underwent high-throughput NIPT was 0.26% (95% CI 0.15% to 0.36%, $n = 8347$), compared with 0.46% (95% CI 0.37% to 0.56%, $n = 18{,}546$) in the historical control cohort. The absolute risk difference in the incidence of sensitisation was 0.20%. The high-throughput NIPT for targeted antenatal anti-D was associated with a significant risk reduction in sensitisation (unadjusted RR 0.55, 95% CI 0.35 to 0.87) compared with historical controls. An updated analysis by Neovius *et al.*[58] found an adjusted odds ratio of 0.41 (95% CI 0.22 to 0.87). In addition, this study reported one case of severe haemolytic disease diagnosed soon after birth in a nulliparous mother who did not receive routine anti-D prophylaxis.

Non-invasive prenatal testing uptake

Rates of NIPT uptake are presented in *Table 8*. Seven studies reported on uptake rates of NIPT screening.[18,20,22,25–27] Uptake rates ranged from 70% to > 95% across the studies. In the pilot study conducted by Soothill *et al.*[18] in three maternity services in the south west of England, only 70% of eligible women joined the study in the initial 6 months. The larger English study conducted by Chitty *et al.*[12] reported that 88% of the 3069 participants consented to receive *RHD* genotyping. The only country that reported nationwide NIPT screening uptake data was the Netherlands, where > 95% of eligible women underwent fetal *RHD* genotyping. The studies generally noted that uptake is likely to increase over time if a nationwide screening programme is implemented.

TABLE 8 Uptake of NIPT

Study	Country	Rates of NIPT uptake, % (*n*/*N*)
Banch Clausen *et al.*, 2014[20]	Denmark	84.2 (581/690)
Chitty *et al.*, 2014[12]	England	88 (372/3069)
Damkjaer *et al.*, 2012[27]	Denmark	90 (215/239)
de Haas *et al.*, 2012[25]	The Netherlands	> 95 (15,126/approximately 15,750)
Grande *et al.*, 2013[22]	Spain	94 (284/302)
Soothill *et al.*, 2015[18]	England	70 (approximately) (numbers not reported)
Tiblad *et al.*, 2013[26]	Sweden	89 (8374/9380)

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Antenatal anti-D prophylaxis uptake

Rates of women receiving antenatal anti-D uptake according to NIPT uptake are presented in *Table 9*. Four studies reported uptake rates of RAADP in women who accepted NIPT and received a positive result, ranging from 86% to 96.1%.[20,26,27,49] One study reported nationwide data in women receiving RhD genotyping in the Netherlands, where 96.1% of approximately 18,383 women received antenatal prophylaxis anti-D.

TABLE 9 Uptake routine antenatal and postpartum anti-D prophylaxis according to NIPT uptake

RAADP	% (*n*/*N*)	Source	Country
1. Uptake of RAADP with no NIPT (current practice)	99 ($n = 5276$) receiving at least one injection; 87.5% ($n = 5276$) receiving the correct dose at the correct time; 90%[a] (NR/5276) receiving all injections at correct doses	[b]UK anti-D audit[8]	UK
	100 (10/10)	Soothill *et al.*, 2015[18]	England
2. Uptake of RAADP in those who refuse NIPT	0 (0/23)	Damkjaer *et al.*, 2012[27]	Denmark
	80 (4/5)	Soothill *et al.*, 2015[18]	England
3. Uptake of RAADP in those who accept NIPT and receive a positive result	93.2 (330/354)	Banch Clausen *et al.*, 2014[20]	Denmark
	86 (NR)	Damkjaer *et al.*, 2012[27]	Denmark
	90 (4590/5104)	Tiblad *et al.*, 2013[26]	Sweden
	96.1 (of approximately 18,383)	van der Ploeg *et al.*, 2015[49]	The Netherlands
4. Uptake of RAADP in those who accept NIPT and receive an inconclusive result	100 (5/5)	Soothill *et al.*, 2015[18]	England
5. Uptake of RAADP in those who accept NIPT and receive a negative result	6 (1/18)	Soothill *et al.*, 2015[18]	England
	5 (5/95)	Grande *et al.*, 2013[22]	Spain
Postnatal routine anti-D uptake			
6. Uptake of postnatal anti-D with no testing	98.4 (91.6% had the correct dose at the correct time) (NR/3392)	[b]UK anti-D audit[8]	UK
	95.7 (66/69)	Banch Clausen *et al.*, 2014[20]	Denmark
7. Uptake of postnatal anti-D in those who refuse NIPT	> 99 (NR)	Damkjaer *et al.*, 2012[27]	Denmark
8. Uptake of postnatal anti-D in those who accept NIPT and receive a positive result	99.7 (353/354)	Banch Clausen *et al.*, 2014[20]	Denmark
	99.3 (151/152)	Damkjaer *et al.*, 2012[27]	Denmark
	92 (of approximately 18,383)	van der Ploeg *et al.*, 2015[49]	The Netherlands
9. Uptake of postnatal anti-D in those who accept NIPT and receive an inconclusive result	No data	N/A	N/A
10. Uptake of postnatal anti-D in those who accept NIPT and receive a negative result	0 (0/227)	Banch Clausen *et al.*, 2014[20]	Denmark
	0 (0/85)	Damkjaer *et al.*, 2012[27]	Denmark
	0.087 (2/NR)	Banch Clausen *et al.*, 2012[24]	Denmark
	0 (NR)	Soothill *et al.*, 2015[18]	England

N/A, not applicable; NR, not reported.
a Full compliance (correct dose, correct time) with single-dose regime. A total of 99% received at least one dose.
b Although this study did not meet the selection criteria for this review (no NIPT), it is included here for informative purposes.

Tiblad *et al.*[6] reported a slightly lower rate, with 90% of 5104 women with a positive NIPT result receiving RAADP. Further data on uptake of RAADP in women who received a negative result (two studies),[18,22] those who received an inconclusive result (one study)[18] and those who refused NIPT (two studies)[18,27] were limited. None of the included studies reported whether or not all women who received antenatal anti-D prophylaxis received the intended dosage at the intended time, or what proportion of women received additional anti-D owing to a potentially sensitising event.

Postpartum anti-D prophylaxis uptake

Rates of women receiving postpartum anti-D uptake according to NIPT uptake are presented in *Table 9*. Three studies reported uptake of postnatal anti-D prophylaxis in women who accepted NIPT and received a positive result, ranging from 92% to 99.7%.[20,27,49] One study reported nationwide data in women receiving RhD genotyping in the Netherlands, where 92% of approximately 18,383 women received postnatal prophylaxis anti-D. A subgroup analysis by Banch Clausen *et al.*[20] (including a total of 690 pregnancies) found a slightly higher uptake of postnatal anti-D among women who received NIPT (99.7%, 353/354) than in those who did not undergo NIPT (95.7%, 66/69). Another Danish study reported a similar rate among women who received NIPT (99.3%, 151/152).[27] None of the included studies reported whether or not all women who received postpartum anti-D prophylaxis received the intended dosage at the intended time.

Reduction in anti-D use

Three non-comparative studies reported outcome measures relating to anti-D doses administered. Soothill *et al.*[18] reported a significant 6% reduction per month of anti-D administration (95% CI 4% to 8%, Poisson regression) within 6 months in the three maternity services in the south-west of England. The total use of anti-D doses fell by about 29%, corresponding to 35% of RhD-negative women not receiving anti-D in their pregnancy unnecessarily. Similar results were also observed in Banch Clausen *et al.* study,[20] which reported that, of 12,668 pregnant women, 4706 (37.1%) avoided unnecessary anti-D administration within 2 years of prenatal *RHD* screening programme. The study by Grande *et al.*[22] reported that, of 95 women carrying a RhD-negative fetus, five requested anti-D administration; unnecessary anti-D administration was therefore avoided in 95% of women carrying a RhD-negative fetus.

Adverse events

None of the studies reported any data on adverse events of either NIPT or antenatal anti-D administration. In particular, there were no data on adverse reactions (such as allergic reactions) to anti-D, on transmission of blood-borne diseases, or on social consequences of NIPT (such as revealing false paternity). No studies reported data on health-related quality of life and patients' anxiety associated with NIPT.

Simulation study of clinical effectiveness

As seen in the review of clinical effectiveness (see *Results: assessment of clinical effectiveness*), very limited comparative evidence on the clinical outcomes of NIPT has been reported. In order to better understand the probable consequences of implementing NIPT, and basing anti-D administration on its results, we performed a simulation study.

The parameters of this simulation study are drawn primarily from the systematic reviews of diagnostic accuracy and clinical effectiveness. Prevalence and diagnostic accuracy parameters are derived from the three high-quality Bristol-based studies[12,17,18] whenever possible to best represent the UK population. Data on compliance with NIPT and anti-D are drawn from a recent audit of antenatal anti-D administration in the UK, or papers in the clinical effectiveness review, favouring UK-based results whenever available. Some important parameters, such as incidence of sensitisation with and without anti-D, were not reported in any papers included in the diagnostic accuracy or clinical effectiveness reviews. To inform other parameter estimates for this simulation, we conducted an additional literature search to identify relevant systematic reviews of antenatal anti-D prophylaxis. Four relevant reviews[61–64] were identified. These reviews provided data on the probability estimates of the events used in the simulation study, including sensitisation and compliance rates. These reviews are summarised in *Appendix 8*.

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Table 10 summarises the parameter estimates used in the simulation and gives their source. All these parameter estimates assume the current practice of offering antenatal anti-D at around 28 weeks and offering postpartum anti-D on the basis of a cord blood test (assumed to be 100% accurate). We assume that there are no adverse consequences of administering anti-D. We note that this simulation considers only women who would be eligible for NIPT at the time it would be received. Women who might not receive NIPT, for example because the father is confirmed as RhD negative, are excluded.

The simulation study assumes that these input probabilities are accurate and does not account for any uncertainty in their estimation. Therefore, results of the simulation study should be considered illustrative of the probable consequences of the use of NIPT and not definitive estimates of effect.

The results of the simulation study are summarised in *Table 11*. These results are subject to a Monte Carlo error of approximately ± 0.002%.

These results show that using NIPT leads to a substantial reduction in antenatal anti-D prophylaxis use, from 99% of RhD-positive women (i.e. assuming 99% compliance) to 65.9%. This decline is similar in magnitude to that observed by Soothill *et al.*[18] This is a consequence of the substantial drop in unnecessary anti-D administration in women with RhD-negative fetuses, from 39% of women to 5.7%. Using the NIPT approach means that about 1.2% of women miss out on potentially beneficial prophylaxis, mainly because of non-compliance, compared with 0.6% with universal anti-D administration.

TABLE 10 Probability estimates, derived from published data, that were used in the simulation study

Probability	Estimate (%)	Source
RhD-positive fetus	60.7	Bristol-based diagnostic studies[12,17,18]
RhD-positive fetus (with inconclusive NIPT)	70.7	Bristol-based diagnostic studies[12,17,18]
False-negative NIPT	0.21	Diagnostic meta-analysis (of the Bristol studies)
Inconclusive NIPT	6.7	Bristol-based diagnostic studies[12,17,18]
False-positive test (if conclusive)	1.5	Diagnostic meta-analysis (of the Bristol studies)
Compliance with antenatal anti-D (without NIPT) (received at least one dose of anti-D)	99	UK NHS Blood and Transplant, *2013 Audit of Anti-D Immunoglobulin Prophylaxis*[8]
Uptake of NIPT	96	de Haas *et al.*, 2012[25] (clinical effectiveness review)
Compliance with postpartum anti-D	99	UK NHS Blood and Transplant, *2013 Audit of Anti-D Immunoglobulin Prophylaxis*[8]
Compliance with antenatal anti-D (if NIPT refused or missed)	80	Soothill *et al.*, 2015[18] (clinical effectiveness review)
Compliance with antenatal anti-D (if NIPT inconclusive)	99	Soothill *et al.*, 2015[18] (clinical effectiveness review)
Uptake of antenatal anti-D in women with negative NIPT	6	Soothill *et al.*, 2015[18] (clinical effectiveness review)
Compliance with postpartum anti-D after NIPT process	99	No data, assumed same as without NIPT
Sensitisation with antenatal anti-D and postpartum anti-D	0.35	Pilgrim *et al.*, 2009[62] (HTA report)
Sensitisation with only postpartum anti-D	0.95	Pilgrim *et al.*, 2009[62] (HTA report)
Sensitisation with no anti-D	10.7	Pilgrim *et al.*, 2009[62] (HTA report) and Crowther and Middleton[65]
Subsequent pregnancy in sensitised women	62	Used by Chitty *et al.*, 2014,[12] no source given
Death of RhD-negative fetus in sensitised women	5	Used by Chitty *et al.*, 2014,[12] no source given

TABLE 11 Results of the simulation study

Outcome	Treatment approach	Percentage of women
Antenatal anti-D given	Universal anti-D	99
	Based on NIPT	65.9
Unnecessary anti-D given (RhD-negative fetus)	Universal anti-D	38.9
	Based on NIPT	5.7
Anti-D not given (RhD-positive fetus)	Universal anti-D	0.6
	Based on NIPT	1.2
Sensitised during or after pregnancy	Postpartum/emergency anti-D only	0.641
	Universal anti-D	0.281
	Based on NIPT with postpartum anti-D	0.284
	Based on NIPT with no postpartum anti-D for test negatives	0.294
Deaths in subsequent pregnancies	Postpartum/emergency anti-D only	0.0198
	Universal anti-D	0.0086
	Based on NIPT with postpartum anti-D	0.0091
	Based on NIPT with no postpartum anti-D for test negatives	0.0091

Because sensitisation is rare, very few additional women will be sensitised if NIPT is used. Assuming that all women still receive a postnatal cord blood test and anti-D if required, NIPT will result in about three extra sensitisations per 100,000 women. If cord blood testing is not performed, then there will be approximately 13 extra sensitisations per 100,000 women. These increases are small compared with the total number of sensitisations attributable to failure of anti-D treatment (around 284 per 100,000 women) and compared with not using antenatal anti-D at all (around 641 per 100,000).

The use of NIPT is unlikely to have any meaningful impact on mortality in subsequent pregnancies. Even if postpartum anti-D is never given to women with a negative NIPT result, only approximately five extra deaths will occur per 1 million RhD-negative women.

This simulation assumes that women who do not receive NIPT, for whatever reason, would still be offered, and generally receive, antenatal anti-D. As a SA we consider the impact of a strategy of requiring NIPT as a prerequisite to antenatal anti-D, or, equivalently, of assuming that women who do not comply with NIPT would not comply with the whole antenatal anti-D immunisation process. These results are shown in *Table 12*.

These results show that anti-D administration rates will be further reduced (to 62.7%) if women who do not receive NIPT do not receive antenatal anti-D. The number of women who miss out on potentially beneficial anti-D will rise to 3.2%. This means that there will be more sensitisations: an extra 15 per 100,000 women if postpartum cord blood testing continues or 28 per 100,000 if it is withdrawn.

This simulation study suggests that the use of NIPT to determine antenatal anti-D use will substantially reduce the number of women receiving anti-D unnecessarily and so is likely to be beneficial, provided that the cost of the test does not outweigh this saving. The use of NIPT could also reduce the use of anti-D administration after potentially sensitising events during pregnancy, in women with a negative test result. The additional number of sensitisations compared with a universal offering of antenatal anti-D is very small, provided that care is taken to ensure that women who do not receive NIPT are still offered, and receive, anti-D.

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TABLE 12 Results of the simulation study assuming that women who do not receive NIPT are not offered anti-D

Outcome	Treatment approach	Percentage of women
Antenatal anti-D given	Universal anti-D	99
	Based on NIPT	62.7
Unnecessary anti-D given (RhD-negative fetus)	Universal anti-D	38.9
	Based on NIPT	4.5
Anti-D not given (RhD-positive fetus)	Universal anti-D	0.6
	Based on NIPT	3.2
Sensitised during or after pregnancy	Postpartum/emergency anti-D only	0.641
	Universal anti-D	0.281
	Based on NIPT with postpartum anti-D	0.296
	Based on NIPT with no postpartum anti-D for test negatives	0.309
Deaths in subsequent pregnancies	Postpartum/emergency anti-D only	0.0198
	Universal anti-D	0.0086
	Based on NIPT with postpartum anti-D	0.0096
	Based on NIPT with no postpartum anti-D for test negatives	0.0096

The results suggest that if a woman receives a conclusive NIPT, then test cord blood testing could potentially be withdrawn and postpartum prophylaxis offered on the basis of NIPT. This conclusion depends on whether or not the increase in sensitisations (approximately 13 per 100,000 RhD-negative women) is considered ethically acceptable and cost-effective.

Results: assessment of implementation

Characteristics of included studies

Table 13 presents a summary of the characteristics of the 12 studies[13,17,18,20–28] included in the review of implementation of high-throughput NIPT. Most of these were also included in the diagnostic accuracy and/or clinical effectiveness reviews. These studies were conducted in five countries: Denmark, the UK, Spain, the Netherlands and Sweden. Fetal RhD screening programmes were implemented nationally in the Netherlands and Denmark and regionally in England, Sweden and Spain. Most included studies were large cohort studies that reported implementation data as well as diagnostic accuracy data. One study was a UK-based survey (London). The number of included women ranged from 282 to 18,383.

Results of implementation studies

Table 14 presents a summary of implementation data for high-throughput NIPT. All the large cohort studies reported high diagnostic accuracy of high-throughput NIPT (see *Meta-analyses of diagnostic accuracy*) and suggested that high-throughput RhD genotyping of fetuses in all RhD-negative women was feasible. These studies reported high compliance with anti-D immunoglobulin administration and moderate to high compliance with NIPT (see details in *Results of studies on clinical effectiveness*).

One UK study[18] conducted in the south west of England stated that it is feasible to implement routine cell-free fetal DNA fetal blood grouping in RhD-negative women in the NHS. This study also stated that the requirements of patient information, patient consent, sample handling, sample transfer and implementation of the changed management were all successfully met.

TABLE 13 Study characteristics of implementation studies

Study	Location	Study dates	Sample size[a]	Gestational age (weeks) at time of NIPT, median (range)
Finning *et al.*, 2008[17]	England: Birmingham and Sheffield centre of the National Blood Service	NR	1869	28 (8–38)
Soothill *et al.*, 2015[18]	England: south west, three NHS trusts	April–September 2013	526	15–17 (mostly)
Oxenford *et al.*, 2013[28]	England: four hospitals (Birmingham, London, Newcastle, Sunderland)	NR	289 (270 survey respondents, 19 interviews/ focus groups)	> 12
Banch Clausen *et al.*, 2014;[20] Banch Clausen *et al.*, 2012;[24] Banch Clausen *et al.*, 2013;[13] and Damkjaer *et al.*, 2012[27]	Denmark: nationwide, five regions	2010–11	14,547	25 (73% between 23 and 28)
de Haas *et al.*, 2012;[25] Thurik *et al.*, 2015[21]	The Netherlands: nationwide	July 2011–January 2012	18,383[b]	26
Grande *et al.*, 2013[22]	Spain: Barcelona, six maternity care units	February 2010–October 2011	282	24–26
Wikman *et al.*, 2012;[23] and Tiblad *et al.*, 2013[26]	Sweden: Stockholm, 83 maternity care centres, six delivery units	September 2009–March 2012 (reference cohort: 2004–8)	8374[c]	8–40

NR, not reported.
a Number of blood samples undergoing NIPT unless otherwise specified.
b Number of participants undergoing NIPT.
c Number of pregnancies undergoing NIPT.

A number of studies reported issues related to the implementation of prenatal RhD screening programmes. For example, Banch Clausen *et al.*[20] stated that the challenges to the implementation of the prenatal RhD screening programme were related to programme anti-D prophylaxis compliance. Another study by Banch Clausen *et al.*[24] noted that there may be challenges in logistics concerning the transportation of samples from remote sites to testing laboratories and in getting results back to the correct general practitioner.

The UK-based survey[28] investigated 290 women's preferences and information needs for routine implementation of NIPT. A total of 92.1% women agreed that NIPT should be offered but only 75.9% stated that they would accept the test. Women preferred having the test when it was most accurate, even if later in pregnancy. The study revealed that women's current knowledge of rhesus blood groups and anti-D administration was limited, which could be a barrier to implementation. Although women may agree to extra appointments for NIPT, health professionals recruited from one London hospital thought that this may be impractical. The data from this survey showed that women hold positive views regarding the introduction of routine fetal RhD genotyping using cell-free fetal DNA. Given women's limited knowledge of rhesus blood groups and anti-D administration, the authors stated that developing information leaflets and health professional training will be critical for successful implementation. They stated that this work will be important for the development of policies and guidelines on the introduction of fetal RhD genotyping into routine care.

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TABLE 14 Summary of implementation studies

Screening programme (country)	Study	Details of screening programme	General results	Issues to implementation	Authors' practical advice	Authors' research recommendation
Denmark	Banch Clausen *et al.*, 2014[20]	National programme delivered in five regions in Denmark	Very good screening accuracy (see diagnostic review). False-negative results were mainly because of poor DNA yields or handling errors. False-positive results were a result of contamination and genetic variants. Inconclusive results were because of weak D genotypes. High compliance with anti-D/moderate compliance with NIPT (see effectiveness review)	The challenges to implement the prenatal *RHD* screening programme are related to programme anti-D prophylaxis compliance	Implement external quality assurance programmes as well as regular in-house testing to optimise effectiveness of the screening programme Postnatal prophylaxis should be based exclusively on the result from the prenatal *RHD* screening. An increased effort to improve anti-D prophylaxis compliance is important to further reduce the number of RhD immunisations Issuing focused statements to GPs may avoid sending samples from early pregnancy, which may help reduce false-negative results Increase information given directly to pregnant women, GPs, midwives and obstetricians and systems, such as a reminder system integrated into the GPs' software, which may help to increase women's compliance with the programme	None
	Banch Clausen *et al.*, 2012[24]	Earlier report on Danish screening programme	As above	There may be challenges in the logistics concerning the transportation of samples from remote sites to testing laboratories and in getting results back to the correct GP	Cord blood typing continues to ensure that postnatal anti-D is given if NIPT compliance is poor. RhD testing should be based on a single sample	Long-term follow-up is required to assess clinical effects of NIPT screening
	Clausen *et al.*, 2013[13]	Paper focused on issues around transportation of blood samples in the Danish screening programme	Total DNA declines over time from sampling. Fetal DNA was not generally affected over time from sampling	Not applicable. The paper did not consider implementation of the screening programme as a whole	The aim should be for a transportation time of up to 4 days and no more than 7 days	None

Screening programme (country)	Study	Details of screening programme	General results	Issues to implementation	Authors' practical advice	Authors' research recommendation
	Damkjaer *et al.*, 2012[27]	Earlier report on Danish screening programme, focused on compliance issues	Compliance with NIPT was around 90%, improving over time	No additional implementation issues reported	For GPs: 1. Higher level of physician information regarding antenatal *RHD* screening and targeted anti-D prophylaxis 2. Use of new maternity reports with separate text boxes for information on antenatal *RHD* screening and the injection of anti-D, which standardises the communication between departments For midwives: 1. Increased attention to documentation in the maternity report 2. Obligatory disclosure to the patient of the information letter from the Danish National Board of Health at the first meeting with the midwife 3. For patients: Encouragement to make an appointment with their GP at 25 weeks' gestation for blood sample collection for antenatal RhD screening For obstetricians: 1. To give extra antenatal prophylaxis in case of potentially sensitising events and to register whether or not extra doses are given	None

continued

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TABLE 14 Summary of implementation studies (*continued*)

Screening programme (country)	Study	Details of screening programme	General results	Issues to implementation	Authors' practical advice	Authors' research recommendation
UK (Bristol)	Finning *et al.*, 2008[17]	Two regions in England (Birmingham and Sheffield), centres of the National Blood Service for routine ABO and RhD blood grouping and antibody screening	Very good diagnostic accuracy (see review). Inconclusive results were often a result of substantial maternal DNA, for example because samples were old	No issues to implementation were reported. The modest apparent increase in risk of sensitisation in false-negative women might be offset by an increased uptake of prophylaxis among mothers who have been correctly identified as carrying a RhD-positive fetus	If the policy on routine antenatal prophylaxis were changed to a single dose of anti-RhD immunoglobulin given at 30 weeks' gestation in RhD-negative women, then *RHD* genotyping testing at 28 weeks would be suitable Commencement of anti-D treatment at 30 weeks' gestation, rather than 28 weeks', has been considered an option in the UK. Anti-D could be avoided after sensitising event in test-negative women. Treating inconclusive results as positive seems to be the best approach Testing only samples that are < 7 days old would increase logistical issues of transport over large geographic areas but would reduce the risk of false-negative results	Feasibility trials on testing maternal blood samples obtained during the earlier stages of pregnancy are required
	Soothill *et al.*, 2015[18]	Three maternity services in the south west of England	29% drop in use of anti-D at a cost reduction of £60,000 per year	It is possible to implement routine cffDNA fetal blood grouping in RhD-negative women in the NHS The requirements of patient information, patient consent, sample handling, sample transfer and implementation of the changed management were all successfully met	This service should be extended to the whole of the UK, because it has led to a more targeted use of anti-D. The cost of the tests seems to be covered by the resulting savings in the use of anti-D immunoglobulin. Continued use of anti-D in women who can be shown to have RhD-negative fetuses may be unethical	Further research on high-throughput NIPT to improve the test accuracy and reduce the inconclusive rates is required

Screening programme (country)	Study	Details of screening programme	General results	Issues to implementation	Authors' practical advice	Authors' research recommendation
UK (London)	Oxenford *et al.*, 2013[28]	Survey conducted in one hospital in London, UK	This study investigated women's preferences and information needs for routine implementation of NIPT. Around 290 women included: 92.1% agreed that NIPT should be offered. Only 75.9% said they would accept the test. Women preferred having the test when most accurate, even if later in pregnancy	Women hold positive views regarding the introduction of routine fetal RhD genotyping using cffDNA but women's current knowledge of rhesus blood groups and anti-D administration was found to be limited Although women may agree to extra appointments for the test, health professionals ($n = 13$) all thought that this may be impractical	Developing information leaflets and health professional training will be critical for successful implementation	None
Spain	Grande *et al.*, 2013[22]	Six health centres of Barcelona-West health district in Spain	High diagnostic accuracy (see diagnostic review). False-negative results were mainly related to specific DNA extraction methods, prolonged storage time before sample processing and early gestational age	No issues to implementation were reported	High-throughput NIPT of exons 5, 6, 7 and 10, before 28 weeks' gestation in their mixed population should be considered for further clinical application	None
The Netherlands	Thurik *et al.*, 2015[21]	One region in the Netherlands	Discordant test results were mainly caused by RhD variant genes and weak PCR signals and the 'vanishing twin' phenomenon	No issues to implementation were reported	Discordant positive results due to co-twin demise would have greater clinical impact in other non-invasive prenatal tests. The authors therefore advised documenting a vanishing twin at any early pregnancy scan and counselling against NIPT. False-positive findings will have little impact in NIPT, as the test causes only unnecessary anti-D use	Prospective studies in pregnancies with a vanishing twin will be required to test whether or not discrepant NIPT results may be compatible with a vanishing co-twin as a source of a third genomic cell line
	de Hass *et al.*, 2012[25]	Earlier report on the Netherlands screening programme	Compliance with NIPT screening was around 95%. The FPR was 1.1%	It is possible to guide both antenatal and postnatal anti-D immunoprophylaxis by fetal *RHD* screening in maternal blood obtained at 27 weeks' gestation. No further issues relating to implementation were reported	None stated	A longer period of evaluation based on local analyses of cord blood testing is required

continued

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TABLE 14 Summary of implementation studies (*continued*)

Screening programme (country)	Study	Details of screening programme	General results	Issues to implementation	Authors' practical advice	Authors' research recommendation
Sweden	Wikman *et al.*, 2012[23]	83 maternity care centres in the Stockholm area, Sweden	NIPT had high diagnostic accuracy with > 99% sensitivity and specificity. Before 8 weeks' gestation, fetal RhD genotype could not be reliably determined (see diagnostic accuracy review)	Fetal *RHD* detection in early pregnancy in a routine clinical setting is feasible and accurate. No further issues relating to implementation were reported This screening programme can be included in the routine antenatal care management and will not require any extra appointment for maternal blood sampling	NIPT should not be performed before 8 weeks' gestation. Maternal DNA levels may be too large after 4 days' storage for reliable testing in first trimester	The cost-effectiveness of fetal *RHD* screening combined with targeted antenatal Rh prophylaxis will be an important area for further research
	Tiblad *et al.*, 2013[26]	See Wikman *et al.*, 2012[23]	RhD immunisation rate was 0.26% in the screening cohort and 0.46% in historical controls (see effectiveness review)	Using first-trimester screening significantly reduces the incidence of new RhD immunisation but test sensitivity is lower than for later screening	No further advice given	Cost-effectiveness of first-trimester screening should be evaluated

cffDNA, cell-free fetal DNA; GP, general practitioner.

Several studies offered practical advice for implementing high-throughput NIPT. For example, Finning *et al.*[17] stated that if the policy on routine antenatal prophylaxis were changed to a single dose of anti-RhD immunoglobulin given at 30 weeks' gestation in RhD-negative women, then RhD genotyping testing at 28 weeks would be suitable. This study also suggested that treating inconclusive results as positive seems to be the best approach to minimise the risk of not treating women with a RhD-positive fetus. Another recent UK (Bristol) study[18] stated that this service should be extended to the whole of the UK, because it has allowed the use of anti-D in a more targeted way and the cost of the tests seems to be offset by the resulting savings in the use of anti-D. This study also stated that continued use of anti-D in women who can be shown to have RhD-negative fetuses may be unethical. Banch Clausen *et al.*[24] recommended continuing cord blood typing in practice to ensure that postnatal anti-D is given if NIPT compliance is poor. Damkjaer *et al.*[27] suggested improvement in relevant knowledge on prenatal RhD screening among general practitioners and midwives in Denmark.

Clausen *et al.*[13] focused on issues around transportation of blood samples in the Danish screening programme and suggested that the aim should be for a transportation time of up to 4 days and no more than 7 days. Wikman *et al.*[23] noted that testing before 8 weeks may be inappropriate because of the instability of samples and consequent difficulties of transportation.

In summary, the findings from these studies suggest that high-throughput NIPT for fetal RhD screening in all RhD-negative women is feasible. They also suggest that effective education, particularly for pregnant women but also for general practitioners and midwives, on the role of NIPT and the importance of anti-D immunisation is important. Any nationwide NIPT screening programme will require careful logistical management to ensure that blood samples are transported to laboratories and tested quickly and that results are reliably returned to general practitioners and midwives. NIPT could be carried out at any time between 25 and 28 weeks, preferably as part of an existing antenatal appointment. Anti-D, if required, should be administered as a single dose at around 30 weeks.

Clinical effectiveness summary and conclusions

Diagnostic accuracy

Eight studies[12,17–23] were included in the diagnostic review of high-throughput NIPT. There were three studies based in Bristol (UK).[12,17,18] The majority of included studies were judged as having a low risk of bias.

Meta-analyses found that high-throughput NIPT had very good diagnostic accuracy. In the primary analyses, in which women with inconclusive test results were treated as if positive, the summary FNR (women at risk of sensitisation) was 0.34% (95% CI 0.15% to 0.76%) and the FPR (women needlessly receiving anti-D) was 3.86% (95% CI 2.54% to 5.82%).

The three high-quality studies performed at Bristol,[12,17,18] which were most representative of UK practice, had a lower FNR of 0.21% (95% CI 0.09% to 0.48%), with a consequently higher FPR of 5.73% (95% CI 4.58% to 7.16%). This difference may be partly because the NIPT used in Bristol had a different test threshold to other countries to further reduce false-negative results.

The FPR found is mostly a consequence of treating women who have an inconclusive test result (approximately 7% of non-invasive prenatal tests in the UK) as if they had a positive test. Excluding these women from analysis gave a lower FPR of 1.26% (95% CI 0.87% to 1.83%). It may therefore be possible to reduce the FPR by further targeted testing of women with an initially inconclusive result.

The diagnostic accuracy performance of high-throughput NIPT varied by gestational age. The data suggest that high-throughput NIPT is insufficiently accurate before around 11 weeks' gestation (i.e. in first trimester) but is accurate at any time after the end of the first trimester. One study[12] also suggested that

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the number of inconclusive results may decline over time. Hence, NIPT cannot be recommended before the second trimester and may be best performed later in the second trimester.

Clinical effectiveness

Seven studies[18,20,22,24–27] were included in the clinical effectiveness review. Only two studies had a control group. All studies were judged as having a high risk of bias. As all except one were conducted in non-UK countries, the generalisability of their findings to the UK setting is limited because of variations in national guidelines and health policies between countries (e.g. prescription of RAADP). One large prospective cohort study[26] reported that use of high-throughput NIPT for targeted antenatal anti-D prophylaxis was associated with a significant risk reduction in sensitisation (adjusted odds ratio 0.41, 95% CI 0.22 to 0.87) compared with historical controls (routine management, postpartum anti-D only).

Uptake rates of NIPT were reported in seven studies, ranging from 70% in a pilot study conducted in England to > 95% in an established national programme in Denmark. Uptake rates of RAADP in women who accepted NIPT and received a positive result were moderate to high, ranging from 86% to 96.1% (four studies). Uptake rates of routine postnatal anti-D prophylaxis in women who accepted NIPT and received a positive result were reported in three studies and were generally high, ranging from 92% to 99.7%.

Three non-comparative studies evaluated changes in anti-D use following the implementation of NIPT. All found that the use of NIPT reduced the total use of anti-D immunoglobulin doses, which fell by 29% in one UK study[18], because around 35% of RhD-negative women avoided receiving anti-D unnecessarily.

As the quality of the clinical effectiveness evidence was limited, we performed a simulation study, based on the findings of our reviews, to assess the probable clinical consequences of implementing NIPT. Its results were broadly consistent with the review evidence. It suggested that NIPT, when compared with offering anti-D to all RhD-negative women, would substantially reduce the use for anti-D from 99% of women to 65.9%. The number of women receiving anti-D unnecessarily would fall from 38.9% to 5.7%. The number missing out on potentially beneficial anti-D (because of a false-negative test result or non-compliance) depends on the compliance rate but could increase from 0.6% to between 1.2% and 3.1%.

The impact of NIPT on sensitisation rates (compared with universal anti-D use) also depends on compliance. Sensitisation rates may increase by 3–15 sensitisations per 100,000 women if postpartum cord blood testing is continued, or 13–28 per 100,000 women if cord blood testing is withdrawn and postpartum anti-D given on the basis of the NIPT result. Ensuring that women who do not receive NIPT are still offered, and receive, antenatal anti-D will minimise the number of additional sensitisations.

Implementation

Twelve studies were included in the review of implementation. Most of the included studies were large cohort studies reporting implementation data along with diagnostic accuracy data, although one study was a UK-based survey. As most studies were conducted in non-UK countries, the generalisability of their findings to the UK settings is limited because of variations in national guidelines and health policies between countries. All the large cohort studies suggested that high-throughput RhD genotyping of fetuses in all RhD-negative women was feasible and should be recommended. A number of studies reported issues of implementation such as those relating to programme anti-D prophylaxis compliance. Some studies emphasised the importance of short transport times of samples and the need for good management of transporting samples. Some studies also identified the need for greater knowledge of NIPT among physicians, midwives and pregnant women.

Conclusions

High-throughput NIPT for fetal RhD status is an accurate diagnostic test, if performed after 11 weeks' gestation. It has a FNR (women remain at risk of sensitisation) of around 0.2% and a FPR (women receive unnecessary anti-D) of around 5.7%. The test gives an inconclusive result in around 7% of women in the

UK. Owing to limited evidence, the accuracy of NIPT in non-white women and multiple pregnancies is unclear. Treating inconclusive tests as if they were positive is the cause of most false-positive results. Giving antenatal anti-D immunoglobulin on the basis of NIPT, rather than to all RhD-negative women, will reduce the use of anti-D and largely eliminate unnecessary use of anti-D in women who do not need it because they have a RhD-negative fetus. Some women will, however, continue to receive anti-D unnecessarily because of an inconclusive test result.

Although the evidence was limited, it appears that using NIPT will lead, at worst, to only a small increase in the number of sensitisations compared with universal use of anti-D. The simulation suggested that achieving high compliance with both NIPT and antenatal anti-D (particularly in women who do not receive NIPT) is important in order to achieve good clinical effectiveness and to reduce the sensitisation rate. It may be clinically reasonable to withdraw postpartum cord blood testing and base postpartum anti-D administration on the results of NIPT. All large implementation studies suggested that high-throughput NIPT in all RhD-negative women was feasible and should be recommended. Key issues of implementation include ensuring anti-D prophylaxis compliance, effective management of transporting samples and greater knowledge of NIPT among physicians, midwives and pregnant women.

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Chapter 4 Systematic review of existing cost-effectiveness evidence

This chapter provides an overview of the existing cost-effectiveness evidence for the use of high-throughput NIPT for rhesus D status in RhD-negative women not known to be sensitised to the RhD antigen. We assessed the relevance of these data to inform UK practice and the current assessment, as set out in the NICE scoping documentation.[66] For each cost-effectiveness study we describe the manner in which NIPT is assumed to impact on the care pathway and summarise how existing cost-effectiveness studies have characterised the impact of NIPT on routine antenatal care costs, routine antenatal anti-D immunoglobulin administration, management of potentially sensitising events and postnatal administration of anti-D immunoglobulin. The findings from the review informed the development of a new decision-analytic model, reported in *Chapter 5*.

Methodology of the cost-effectiveness review

Searches

In addition to the searches conducted for the review of clinical evidence (see *Chapter 3*), the following databases were searched up to December 2015 for cost-effectiveness evidence: NHS EED, EconLit and IDEAS database via Research Papers in Economics (RePec). The bibliographies of relevant studies were also searched. Citations of identified studies were searched for any relevant publications published after the initial search.

Selection criteria

A broad range of studies was considered in the review, including economic evaluations conducted alongside trials, modelling studies and analyses of administrative databases. Only full economic evaluations that compared two or more options and considered both costs and consequences (i.e. cost-minimisation, cost-effectiveness, cost–utility and cost–benefit analyses) were included in the review.

Study selection

Relevant studies were then selected in two stages. Titles and abstracts identified by the search strategy were examined independently by two researchers (PS and SG) and screened for possible inclusion. Disagreements were resolved by discussion. Full texts of the potentially relevant studies were obtained. Two researchers (PS and SG) examined these independently for inclusion or exclusion, and disagreements were resolved by discussion.

Data extraction

One reviewer (PS) independently extracted details from full-text studies on objectives, setting, population, comparators, analytical approach, data on costs and outcomes (short- and long-term) and main results/conclusions. Another reviewer (SG) checked extracted data and disagreements were resolved by discussion.

Critical appraisal

A quality appraisal was carried out using the checklist of Drummond and Jefferson.[67] This checklist evaluates the extent to which each review result provides detail on different aspects, such as study design, data collected and their use in the economic evaluation and analysis and interpretation of results. One reviewer (PS) independently assessed the quality of all included studies according to all these domains. The quality assessment was checked by another reviewer (SG). Any disagreements were resolved by consensus.

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Results of the review of existing cost-effectiveness evidence

Quantity of research available

Number and type of studies included

The initial search of economic databases identified a total of 31 references. After the initial screening of titles and abstracts, 10 were considered to be potentially relevant and were ordered for full-text paper screening. Of those, seven met the selection criteria and were included in the review.[58,68–73] A flow diagram of the selection process is reported in *Figure 10*.

Number and type of studies excluded

A list of full-text papers that were excluded is given in *Appendix 9*. These papers were excluded because they failed to meet one or more of the inclusion criteria, including lack of full-text publications and ineligible study design.

Characteristics of included studies

The characteristics of the seven studies are summarised in *Table 15*. The large majority of studies specified the target population as being unsensitised RhD-negative pregnant women or RhD-negative pregnant women not known to be sensitised to the RhD antigen. Macher *et al.*[70] and Hawk *et al.*[72] stated that their analysis considered RhD-negative pregnant women but they were not clear about women's sensitisation status at study entry. Only two studies[68,73] explicitly stated that a high-throughput NIPT method was being used for the comparative assessment, although for the other studies this was considered implicit, as the test diagnostic performance was considered similar to the high-throughput studies. One study[71] explicitly focused on providing NIPT to all RhD-negative women, as the test for sensitisation was conducted only if the NIPT result was positive.

Most studies[58,68,71–73] evaluated the cost-effectiveness of introducing NIPT in the management pathway of RhD-negative pregnant women compared with alternative strategies. These studies explored a range of alternative strategies to prevent sensitisation. Except for Szczepura *et al.*[68] and Macher *et al.*,[70] two strategies were common across the studies: (non-targeted) RAADP at around 28–30 weeks to every (unsensitised) RhD-negative pregnant women; and use of NIPT for fetal RhD typing with prophylaxis guided by test results (targeted RAADP) for RhD-negative pregnant women. Duplantie *et al.*[71] also explored the immunological

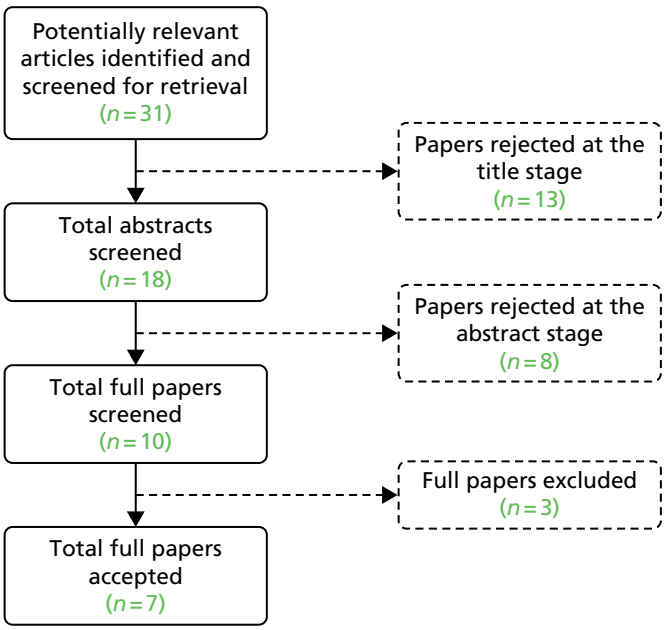

FIGURE 10 Assessment of cost-effectiveness: summary of study selection and exclusion.

TABLE 15 Cost-effectiveness study characteristics

Study	Objectives	Setting/ perspective	Population	Analytical approach	Diagnostic comparators	Outcomes	Main results
Szczepura *et al.*, 2011[68]	Cost-effectiveness analysis of NIPT implementation in England and Wales	English and Welsh NHS	Unsensitised RhD-negative pregnant women	Economic analysis of NIPT implementation. For each scenario a threshold analysis was performed to identify the circumstances under which NIPT might be considered cost saving compared with RAADP	Two scenarios compared: 1. Assumed that all RhD-negative women will routinely receive NIPT at approximately 28 weeks and that RAADP will be withheld if a RhD-negative fetus is identified (prophylactic anti-D for potentially sensitising events assumed withheld); postpartum testing and anti-D prophylaxis assumed to be unaffected 2. Assumed that, in addition to scenario 1, postdelivery blood cord serology and FMH test will be withheld if NIPT result has identified a RhD-negative fetus	Costs (including NIPT royalty fees), additional sensitisations/year	Analysis performed did not support routine implementation of NIPT in England and Wales for unsensitised RhD-negative pregnant women. Net financial benefit of implementing mass NIPT as an add-on (while maintaining current postnatal testing) was found to be negligible in England and Wales. NIPT implementation is unlikely to produce important clinical benefits: the number of sensitisations was estimated not to fall appreciably and the sensitisations are expected to rise if NIPT sensitivity is below 99.9%

continued

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TABLE 15 Cost-effectiveness study characteristics (*continued*)

Study	Objectives	Setting/ perspective	Population	Analytical approach	Diagnostic comparators	Outcomes	Main results
Benachi *et al.*, 2012[69]	Cost-minimisation analysis of NIPT on the costs of managing RhD-negative pregnant women, whether or not they are sensitised	French NHS	Unsensitised RhD-negative pregnant women	A prospective follow-up of RhD-negative women during their pregnancy	Four scenarios compared: 1. RAADP at 28–32 weeks' gestation 2. RAADP and additional 300-µg anti-D administration at 28 weeks' gestation 3. NIPT performed during the first trimester in order to detect women not at risk (i.e. carrying a RhD-negative fetus) 4. NIPT performed during the third trimester in order to offer RAADP only to women carrying a RhD-positive fetus For strategies 3 and 4 systematic (i.e. to all) and targeted (i.e. conditional on test results) newborn infant serology scenarios were explored	Costs, except for potentially sensitising events; no clinical outcomes were considered in the analysis	NIPT performed early during pregnancy (i.e. end of first trimester and beginning of second trimester) was found to be cost saving compared with RAADP during the third trimester
Macher *et al.*, 2012[70]	Cost-minimisation analysis of NIPT (multiplex real-time PCR assay for fetal cell-free DNA) in the plasma of pregnant women	Andalusian government, Spain	RhD-negative pregnant women	An analysis of feasibility of routine RhD status determination into the clinical setting using NIPT targeted towards two exons of the *RHD* gene and one exon of *SRY* gene	No diagnostic comparators were presented Three ways of detecting fetal RhD using NIPT were compared: 1. Exon 5 2. Exon 7 3. SRY Testing was performed on RhD-negative women in weeks 10–28 of pregnancy. The consequences of test results were not explored	Test accuracy; cost of assay per sample	The routine determination of fetal RhD status using NIPT is feasible. The use of multiplex real-time PCR allows the improvement of the response of the laboratory, saving time and reagent costs and opening the door to a complete automatisation of the process

Study	Objectives	Setting/ perspective	Population	Analytical approach	Diagnostic comparators	Outcomes	Main results
Duplantie *et al.*, 2013[71]	Cost-effectiveness analysis of strategies to prevent RhD alloimunisation	Public health-care system of Quebec, Canada	Unsensitised RhD-negative pregnant women	Computer-based simulation model with virtual population of 10,000 RhD-negative pregnant women 1. Two decision trees: 2. Applied to the first pregnancy of a RhD-negative woman 3. Applied to an eventual second pregnancy in 55% of those women	Four scenarios compared: 1. Systematic prophylaxis: RAADP at around 28 weeks' gestation (recommended by the Canadian guidelines) 2. NIPT at around 12 weeks' and/or at 28 weeks' gestation. RAADP and postpartum anti-D withheld for RhD-negative fetus result 3. Immunological determination of the father's Rh type 4. Mixed screening: immunological determination of the father's Rh type, followed, if the result is positive, by NIPT at around 15 weeks' gestation. RAADP and postpartum anti-D withheld for RhD-negative fetus result Prophylactic anti-D for potentially sensitising events not discussed but assumed withheld for a RhD-negative fetus result in scenarios 2 and 4	Clinical: 1. Number of babies without haemolytic disease 2. Number of surviving infants Economic: 1. Cost per 10,000 pregnancies 2. Cost per number of babies without haemolytic disease 3. Cost per number of surviving babies Outcomes obtained for first and second pregnancies	The four proposed strategies for prevention and treatment of sensitisation were found to be similar in terms of their effectiveness. In terms of cost-effectiveness, two options were found to be superior: RAADP and immunological Rh typing of the father. NIPT was found not to be a cost-effective option unless its cost is lowered RAADP remained the preferred option for the prevention of maternal sensitisation

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TABLE 15 Cost-effectiveness study characteristics (*continued*)

Study	Objectives	Setting/ perspective	Population	Analytical approach	Diagnostic comparators	Outcomes	Main results
Hawk *et al.*, 2013[72]	Cost-effectiveness of NIPT for targeted prophylaxis	US health system (Medicaid and Medicare)	RhD-negative women	Decision tree model using a decision tree structure comparing three relevant scenarios	Three scenarios compared: 1. RAADP at 28 weeks' gestation and postpartum prophylaxis guided by cord blood typing (current approach in most of the USA) 2. Non-invasive fetal RhD typing performed early in pregnancy (first trimester assumed) with prophylaxis (i.e. for potentially sensitising events, RAADP and postpartum anti-D administration) guided by test results 3. No screening or prophylaxis	Costs per RhD woman, morbidity and mortality attributable to haemolytic disease	Non-invasive fetal RhD testing was not found to provide any economic benefit for the management of RhD-negative women. RAADP and postpartum prophylaxis guided by cord blood typing remained the most cost-beneficial option for the management of RhD-negative women
Neovius *et al.*, 2016[58]	Cost-effectiveness of first-trimester NIPT for targeted antenatal vs. no RAADP or vs. non-targeted RAADP	Swedish health service	Unsensitised RhD-negative pregnant women	Decision-analytic model based on a population-based cohort study. Markov model with cohort simulation and three health states: 'not sensitised', 'sensitised during pregnancy' or 'sensitised from start of pregnancy'	Three scenarios compared: 1. First-trimester NIPT followed by targeted RAADP at 29 weeks' gestation as well targeted postpartum anti-D 2. Historical comparators of no RAADP, only postpartum anti-D in case of a RhD-positive baby 3. Non-targeted RAADP and postpartum anti-D prophylaxis guided by cord blood typing	Screening, pregnancy, delivery and future pregnancies related costs, additional costs per sensitisation averted	NIPT for targeted RAADP was found to be cost saving as well as more effective than no RAADP. Introduction of targeted prophylaxis was expected to save money, reduce sensitisations and avoid unnecessary exposure of pregnant women to a plasma product in short supply

Study	Objectives	Setting/ perspective	Population	Analytical approach	Diagnostic comparators	Outcomes	Main results
Teitelbaum *et al.*, 2015[73]	Cost-effectiveness of non-invasive fetal RhD determination	Canadian NHS	Unsensitised RhD-negative pregnant women	Decision-analytic modelling – decision trees to model costs and benefits of targeted vs. RAADP in Alberta over 1 year	Two scenarios compared: 1. RAADP for all unsensitised pregnant women – including the administration of anti-D at 28 weeks' gestation, at any potentially sensitising event and post partum for women whose infants were found to be RhD positive after delivery (current standard of care in Canada) 2. All RhD-negative women undergo NIPT for RhD genotyping at 12 weeks' gestation. If the fetus is found to be RhD negative, no prophylactic anti-D administration is required. Women with a RhD-positive fetus receive anti-D at 28 weeks' gestation, at any potentially sensitising event and post partum	Number of women sensitised in 1 year, doses of anti-D administered per pregnancy in 1 year, cost per pregnancy	Implementation of a programme of targeted anti-D prophylaxis using NIPT was found to be both feasible and cost saving with no increase in the risk of sensitisation. With higher sample throughput (i.e. in a national programme) the cost per patient was expected to decrease owing to economies of scale

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determination of the father's RhD type to target RAADP. Most studies considered the introduction of NIPT at a single time point, usually at first routine antenatal care appointment occurring between 8 and 12 weeks' gestation. Benachi *et al.*[69] compared alternative timings of NIPT by considering the cost consequences of performing NIPT during the first and the third gestation trimesters. With the exception of the Duplantie *et al.*[71] study, for which insufficient information is provided, all cost-effectiveness studies evaluated the consequences of introducing NIPT in terms of avoiding RAADP but also in terms of the impact it had on postpartum treatment.

Three studies[58,68,73] aimed to evaluate the short-term costs and consequences of sensitisation in RhD-negative women. Duplantie *et al.*[71] and Hawk *et al.*,[72] however, estimated long-term outcomes relating to morbidity and mortality attributable to haemolytic disease of the fetus and/or newborn infant. Furthermore, two studies[58,71] explicitly considered in their analysis women's first and subsequent pregnancies, presenting cost-effectiveness results for each scenario.

Benachi *et al.*[69] and Macher *et al.*[70] are cost-minimisation studies, as no health outcomes were considered, restricting their analysis to an evaluation of the impact of the test on the costs of managing the target population. A variety of cost components were considered across these two studies, such as anti-D immunoglobulin, genotyping, antibody testing.

The cost-effectiveness studies evaluated different strategies in different health systems, including England and Wales, Canada, Sweden and the USA. Except for Sweden, where only postpartum administration of anti-D (conditional on having a RhD-positive baby) is recommended, current guidance for the prevention of sensitisation in these countries is routine prophylactic administration of anti-D, with further prophylactic doses for potentially sensitising events and post partum. The two cost-minimisation studies[69,70] evaluated the cost implications of introducing NIPT in the French and Spanish (namely the Andalusia region) health-care settings. Current guidance on the prevention of sensitisation in these countries was not clearly stated. Macher *et al.*[70] focused mainly on addressing questions relating to the accuracy and implementation of different NIPT methodologies into current clinical practice in Spain.

Quality of included studies

A summary of the results of the quality appraisal of the seven included studies is provided in *Table 16*.

Study design

All studies stated their research question and provided a rationale for it. Most studies failed to clearly mention which economic approach was being taken; the ones that did only partially justified their choice. Five of the seven studies were cost-effectiveness analyses using a decision-analytic modelling approach, typically based on a decision tree. Most of these restricted their assessment to the more short-term outcome of sensitisation, although Duplantie *et al.*[71] and Hawk *et al.*[72] explicitly dealt not only with sensitisations, but also with a broader outcome set, such as the impact on infant health and/or on subsequent pregnancies. The remaining two studies were cost-minimisation studies, with no evidence cited to support this approach. None of the studies considered any adverse effects associated with the provision of NIPT or the administration of anti-D immunoglobulin. None of the studies considered the clinical effectiveness and/or cost-effectiveness of NIPT in ethnic minority groups. Except for one study,[68] most studies were not explicit in considering that most NIPT performance assessments have been undertaken in white European populations and, thus, its reliability in minorities is still to be fully demonstrated. Overall justifications and descriptions of the alternatives being compared were generally clear, with most studies comparing more than two alternative scenarios. The viewpoint of the analyses was mentioned in most studies and implicitly justified by the public health systems in which the studies were conducted.

Data

Studies utilised evidence on costs and/or effects from a variety of sources. Sources for the diagnostic accuracy of NIPT Fetal RhD genotyping were based mainly on diagnostic studies aimed at verifying test performance, including three studies[58,69,70] that considered evidence collected from subjects in the underlying

TABLE 16 Quality assessment of studies included in the economic review using the checklist of Drummond and Jefferson[67]

Criteria	Study						
	Szczepura *et al.*, 2011[68]	Benachi *et al.*, 2012[69]	Macher *et al.*, 2012[70]	Duplantie *et al.*, 2013[71]	Hawk *et al.*, 2013[72]	Neovius *et al.*, 2016[58]	Teitelbaum *et al.*, 2015[73]
Study design							
The research question is stated	Yes	Yes	Yes	Yes	Yes	Yes	Yes
The economic importance of the research question is stated	Yes	Yes	Yes	Yes	Yes	Yes	Yes
The viewpoint(s) of the analysis are clearly stated and justified	Yes	Yes	No	Yes	Partial	Yes	No
The rationale for choosing alternative programmes or interventions compared is stated	Yes	Yes	Yes	Yes	Yes	Yes	Yes
The alternatives being compared are clearly described	Yes	Yes	Yes	Yes	Yes	Yes	Yes
The form of economic evaluation used is stated	Partial	Partial	No	Yes	Partial	Yes	Partial
The choice of form of economic evaluation is justified in relation to the question addressed	No	No	No	Partial	No	Partial	No
Data collection							
The source(s) of effectiveness estimates used are stated	Yes	N/A	N/A	Yes	Yes	Yes	Yes
Details of the design and results of the effectiveness study are given (if based on a single study)	No	N/A	N/A	No	No	Yes	No
Details of the methods of synthesis or meta-analysis of estimates are given (if based on a synthesis of a number of effectiveness studies)	No	N/A	N/A	N/A	N/A	N/A	No
The primary outcome measure(s) for the economic evaluation are clearly stated	Yes	No	No	Partial	Partial	Yes	Yes
Methods to value benefits are stated	N/A	N/A	N/A	No	No	No	N/A
Details of the subjects from whom valuations were obtained are given	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Productivity changes (if included) are reported separately	No	No	No	No	No	No	No
The relevance of productivity changes to the study question is discussed	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Quantities of resource use are reported separately from their unit costs	Yes	Yes	Yes	Yes	No	Yes	Yes
Methods for the estimation of quantities and unit costs are described	Yes	No	Yes	Yes	Partial	No	Partial

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TABLE 16 Quality assessment of studies included in the economic review using the checklist of Drummond and Jefferson[67] (*continued*)

Criteria	Study						
	Szczepura *et al.*, 2011[68]	Benachi *et al.*, 2012[69]	Macher *et al.*, 2012[70]	Duplantie *et al.*, 2013[71]	Hawk *et al.*, 2013[72]	Neovius *et al.*, 2016[58]	Teitelbaum *et al.*, 2015[73]
Currency and price data are recorded	Yes	No	Yes	Yes	Partial	Yes	Partial
Details of currency of price adjustments for inflation or currency conversion are given	No	No	No	No	No	Partial	No
Details of any model used are given	No	No	No	Yes	Yes	Yes	Yes
The choice of model used and the key parameters on which it is based are justified	N/A	N/A	N/A	Partial	Partial	No	Partial
Analysis and interpretation of results							
Time horizon of costs and benefits is stated	No	No	No	Partial	No	Yes	No
The discount rate(s) are stated	No	No	No	N/A	No	Yes	No
The choice of discount rate(s) is justified	N/A	N/A	No	N/A	N/A	Yes	N/A

N/A, not applicable.

cohort studies. These types of observational studies are inherently prone to bias and tools exist to appraise them [e.g. Standards for Reporting Diagnostic accuracy studies,[74] Quality Assessment of Diagnostic Accuracy Studies (QUADAS)[75] or the more recent update QUADAS-2[14]]. To our knowledge, these tools were not used to appraise the study findings. Sources for the effectiveness of anti-D immunoglobulin varied across the different studies and were not based on systematic reviews but mainly on jurisdiction-specific sensitisation estimates. Studies that considered broader outcomes associated with sensitisation (i.e. haemolytic disease and impact of future pregnancies) populated these parameters with relevant published evidence.[76–78]

Three studies reported the methods of collecting health-care resource use data and the unit costs applied to them. The majority specified the currency and price date; however, almost all failed to provide details on whether or not any price and currency conversion adjustments were made. One study[72] did not report unit costs and quantities separately. No study valued health benefits or examined changes in productivity or its associated costs.

Two key aspects in these studies were the unit cost of the diagnostic test itself and the cost of the anti-D immunoglobulin treatment. The cost of NIPT varied significantly across studies from approximately €20.00[70] (2012 prices) to US$450[72] (2013 prices) per sample, with some including blood type, RhD determination and antibody screen. The NIPT cost range in the studies that explicitly stated that a high-throughput method was being assessed varied from £16.25[68] (2011 prices) to CA$34.45[73] (2015 prices). This may indicate that studies reporting a high unit cost for NIPT[71,72] were not based on a high-throughput process. The majority of studies that provided a reference for the NIPT cost figures obtained these from the government[58,71,73] or from laboratory genetic test companies.[72] A relevant consideration in relation to the cost of NIPT is whether or not the test is also subject to additional royalty fees that could affect the unit cost. For the majority of studies it is not clear if this fee was already included in the diagnostic test unit cost. Only the study by Szczepura *et al.*[68] explicitly considered this aspect by exploring the robustness of the results by varying the fee from zero to £46.50, the latter cost being the unit cost of a commercial

testing kit including the royalty fee. Significant variation was also found in the unit costs per dose of anti-D immunoglobulin, which varied from £33.50[68] (2011 prices) to US$462.00[72] (2013 prices). None of the studies considered the potential for further costs associated with the introduction of NIPT in terms of additional antenatal care appointments or counselling with regard to test implications.

Analysis and interpretation of results

The two cost-minimisation studies[69,70] took a simple approach and evaluated direct medical costs associated with the management of the RhD-negative pregnant women. Of the five cost-effectiveness studies, only one[58] explicitly stated the time horizon of costs and benefits and the discount rate used in the analysis. Uncertainty was assessed in the majority of studies[58,71–73] using deterministic sensitivity and scenario analysis. Only one of these[58] reflected the need to jointly consider uncertainty in all parameter inputs through probabilistic methods.

Except for Szczepura *et al.*,[68] all cost-effectiveness studies mentioned the timing for when NIPT was offered to pregnant women. This was generally assumed across studies to happen at around 12 weeks' gestation (typically at first routine antenatal care appointment). This assumption was largely supported by the fact that sufficiently high test diagnostic accuracy levels were expected at that stage of the pregnancy. Benachi *et al.*[69] found that greater cost savings were possible when NIPT was given in the first trimester than in the third trimester owing to the avoidance of costs associated with the management of potentially sensitising events in the intervening period. Their analysis shows that NIPT early in pregnancy (first trimester) was a cost-reduction strategy in comparison with performing the test later in pregnancy (third trimester), saving, on average, €38.00 per patient (2012 prices).

Teitelbaum *et al.*[73] and Szczepura *et al.*[68] were the only two research studies that, in their analyses, factored in the issue of NIPT fetal RhD genotyping producing inconclusive results and therefore performing SA over the inconclusive rate. Their analyses assumed that inconclusive test results would be treated as positive test results and, thus, women were assumed to receive RAADP.

Generally, the cost-effectiveness studies highlighted that the main limitations of their analysis were the external validity of the results, the uncertainty over the cost of the test and the associated royalty fee, the cost of clinically managing sensitisations, the fact the ethnic background of the target population had not been fully accounted for and the impact of this on the reliability of test assays.

Results of included studies

In terms of conclusions, conflicting results were reported across the existing economic studies. Three studies[68,71,72] reported NIPT fetal RhD genotyping not to be cost-effective or of no economic benefit. Hawk *et al.*[72] and Szczepura *et al.*[68] reported that the main factor driving these factors was the cost of the test itself (i.e. the clinical and economic benefits were not sufficient to offset the additional costs of the test). Szczepura *et al.*[68] also stated that the implementation of NIPT in the clinical pathway of the RhD-negative pregnant woman was not expected to produce important clinical benefits. Supporting this was an estimation of the potential rise in the number of sensitised women if NIPT sensitivity fell below 99.9%.

Two studies[58,69] reported that NIPT is cost saving compared with no RAADP (i.e. compared with postpartum anti-D only). Only one study[73] found NIPT for targeted RAADP to be cost saving compared with non-targeted RAADP, which also estimated no increase in the risk of sensitisation if NIPT were to be used. Duplantie *et al.*[71] found that targeting of RAADP based on the immunological RhD typing of the father is cost-effective compared with the use of NIPT.

Overall, the quality of the included studies' findings is uncertain because of a lack of reporting of the validity of the diagnostic accuracy outcomes used. Furthermore, although SA exercises were generally done over some key parameters, the degree of uncertainty in the cost-effectiveness estimates is generally difficult to establish.

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Relevance to the NHS and current decision problem

One of the key aspects of this review is to address how relevant study assumptions and findings are to the UK. None of the study approaches and findings reviewed was considered to be generalisable to the decision problem as set out in the NICE scope for the current diagnostic assessment. The scope for this decision problem includes an evaluation of the introduction of NIPT at different gestation points, the impact of the test result on the administration of anti-D immunoglobulin treatment routinely and post partum, and the impact of sensitisation on infant health and/or on subsequent pregnancies. Only one[68] of the seven economic studies reviewed directly relates to the UK. This study, however, did not explicitly explore how the introduction of NIPT could impact on costs relating to potentially sensitising events. In addition, it assumed that postpartum testing and treatment would be unaffected by NIPT results. Furthermore, no assessment of the timing of NIPT or any consideration of the impact on subsequent pregnancies was undertaken. Therefore, limited UK-specific information exists that explicitly relates to the decision problem as specified in the scope for this diagnostic appraisal. Although some studies are from Canada and the USA, countries in which similar guidance to that in the UK exists on the prevention of sensitisation, relevance to the UK and generalisability of findings can be questioned, as there are crucial health-care system differences and differences in how anti-D immunoglobulin policies have been implemented over recent decades.

Chapter 5 Independent economic assessment

Overview

A de novo independent economic model was developed to assess the cost-effectiveness of high-throughput NIPT to identify fetal rhesus D status in women who are RhD negative and not known to be sensitised to the RhD antigen. The conceptualisation and development of the de novo model was informed by existing economic modelling studies described in *Chapter 4*, *Methodology of the cost-effectiveness review* and the independent economic model used to inform NICE technology appraisal (TA)156 on the clinical and cost-effectiveness of RAADP.[62] The model provides a framework for the synthesis of diagnostic accuracy reported in *Chapter 3* with a range of other relevant parameters required to establish cost-effectiveness.

A decision-analytic model using a decision tree cohort approach was developed to estimate, based on best available data, the costs and health outcomes of the relevant testing and treatment strategies. The model was made up of two main elements: (1) an identification part reflecting the diagnostic performance and costs of the alternative identification strategies and (2) a treatment part that evaluated the subsequent costs and outcomes [expressed in quality-adjusted life-years (QALYs)] of alternative care pathways. The treatment part of the model was based closely on the economic model for NICE TA156 developed by researchers at the School of Health and Related Research (ScHARR), University of Sheffield.[62] This model was kindly provided on request and was subsequently modified and updated to accommodate all the required changes for the cost-effectiveness assessment of the introduction of high-throughput NIPT in pregnant RhD-negative women's clinical pathway, as outlined in *Appendix 10*.

The decision model is populated using the results from the systematic clinical review on the diagnostic accuracy of high-throughput NIPT as described in *Chapter 3* and other relevant parameters required to provide a link between the diagnostic accuracy of a given identification strategy, the impact on subsequent treatment decisions and the ultimate effect on health outcomes and costs. The determination of the RhD status of fetuses through high-throughput NIPT may impact the administration of anti-D immunoglobulin prophylactically following potentially sensitising events, routinely and at birth. Routine prophylactic anti-D immunoglobulin may be avoided by RhD-negative women who are indicated to be carrying a RhD-negative fetus. The use of fetal RhD status testing may also prevent further testing (i.e. FMH) as well as the administration of prophylactic anti-D immunoglobulin after a potentially sensitising event where the test result indicates a RhD-negative fetus. In addition, high-throughput NIPT for fetal RhD status determination may impact postpartum testing (i.e. cord blood typing and FMH) and postpartum anti-D immunoglobulin administration. As high-throughput NIPT is not a perfect test, women who receive inconclusive or false-positive test results will not avoid unnecessary use of anti-D immunoglobulin and the costs and consequences of suboptimal use of anti-D immunoglobulin prophylaxis in women who receive false-negative results need to be accounted for.

The following sections outline the decision problem and the structure of the model and also provide an overview of the key assumptions and data sources used to populate the model.

Overall aims and objectives of the independent economic assessment

The cost-effectiveness assessment of the use of high-throughput NIPT to identify fetal rhesus D status had the following overall main objectives:

- To produce a de novo cost-effectiveness model assessing the cost-effectiveness of high-throughput NIPT to identify fetal RhD status in RhD-negative women not known to be sensitised to the RhD antigen.
- To assess the impact of alternative scenarios related to the timing of the test and the impact of the test on the use of antenatal anti-D immunoglobulin prophylaxis for sensitising events and postdelivery testing and postpartum anti-D immunoglobulin administration.

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Intervention and comparator pathways

Current NICE clinical guidance on antenatal care[7] recommends that women be offered testing for blood group and rhesus D status in early pregnancy. All pregnant women identified as RhD-negative would be tested for the presence of RhD antibodies. Women identified as RhD-negative and found not to have RhD antibodies are not yet sensitised and form the population for this appraisal. In these women, anti-D immunoglobulin is recommended, both as prophylaxis and following potential sensitising events, to prevent sensitisation occurring.[2]

Routine antenatal anti-D prophylaxis is recommended to be given as two doses at weeks 28 and 34 of pregnancy, or as a single dose between 28 and 30 weeks. Supplementary doses of anti-D immunoglobulin should also be administered prophylactically after a potentially sensitising event.[2,8] Potentially sensitising events include those that may lead to FMH, such as medical interventions (e.g. chorionic villus sampling, amniocentesis or external cephalic version), terminations, late miscarriages, antepartum haemorrhage and abdominal trauma. Following a potential sensitisation event, the recommended minimum dosage of anti-D immunoglobulin increases with gestational age (i.e. a higher dose for > 20 weeks' gestation), and FMH testing is used to inform the actual dose after 20 weeks' gestation.

Following birth, RhD typing should be performed on a cord blood sample to determine the RhD status of the baby. If the baby is confirmed to be RhD positive, it is recommended that previously non-sensitised RhD-negative pregnant women receive anti-D immunoglobulin within 72 hours following delivery, with the actual dose guided by FMH results. This represents the pathway and current clinical practice of the management of RhD-negative pregnant women not known to be sensitised.

The intervention technology of this assessment is high-throughput NIPT for fetal rhesus D status. By analysing cell-free fetal DNA in the plasma of RhD-negative pregnant women, high-throughput NIPT is able to predict fetal RhD genotype. High-throughput NIPT for fetal RhD status may enable prophylactic anti-D immunoglobulin to be withheld from women who are RhD-negative and carrying a RhD-negative fetus. These women could avoid unnecessary treatment with anti-D immunoglobulin, along with the potential risk associated with blood products. The results of NIPT could impact on the care pathway in the following ways:

1. For women in whom the high-throughput NIPT indicates the presence of a RhD-negative fetus:

    - avoidance of RAADP
    - avoidance of prophylactic anti-D immunoglobulin and FMH tests following potentially sensitising events
    - avoidance of cord serology testing, fetal maternal haemorrhage test and administration of anti-D immunoglobulin following delivery.

2. For women in whom the high-throughput NIPT indicates the presence of a RhD-positive fetus:

    - avoidance of cord serology testing in favour of routine FMH testing and postpartum anti-D immunoglobulin following delivery.

Model structure

Modelling methodology and scope

A decision-analytic model using a decision tree structure simulates the experience of a hypothetical cohort of RhD-negative pregnant women not known to be sensitised to the RhD antigen, with and without the introduction of high-throughput NIPT for fetal RhD status. A pregnant woman enters the model after having been identified as RhD-negative and not yet sensitised based on the results of tests from bloods drawn either at first contact with the doctor or midwife (the date at which pregnancy is reported or established) or at the booking appointment (8–12 weeks' gestation). All further contacts between the

woman and the health service are informed by the recorded test results. At the routine 16-week visit the woman is informed about her RhD status, whether or not she is sensitised and how these results impact on further management. If the woman contacts the health service following any potentially sensitising event she may be offered anti-D immunoglobulin and, if after 20 weeks' gestation, a FMH test. Women provided with RAADP receive it at either or both of the routine visits at 28 and 34 weeks' gestation. At delivery, a sample of cord blood may be taken and the baby's RhD status established to guide the use of FMH tests and the administration of postpartum anti-D immunoglobulin.

All high-throughput NIPT are assumed to be performed early enough to determine the use of RAADP at 28 weeks' gestation. *Figure 11* shows the current schedule of routine antenatal care appointments and the potential placement of NIPT.

In addition to the first contact/8–12 weeks' gestation booking appointment, the points of routine contact at which blood could be drawn for NIPT are the 16-week visit and 18- to 20-week scan (at which outstanding routine screening tests are offered). Other opportunities may include attendance to receive the

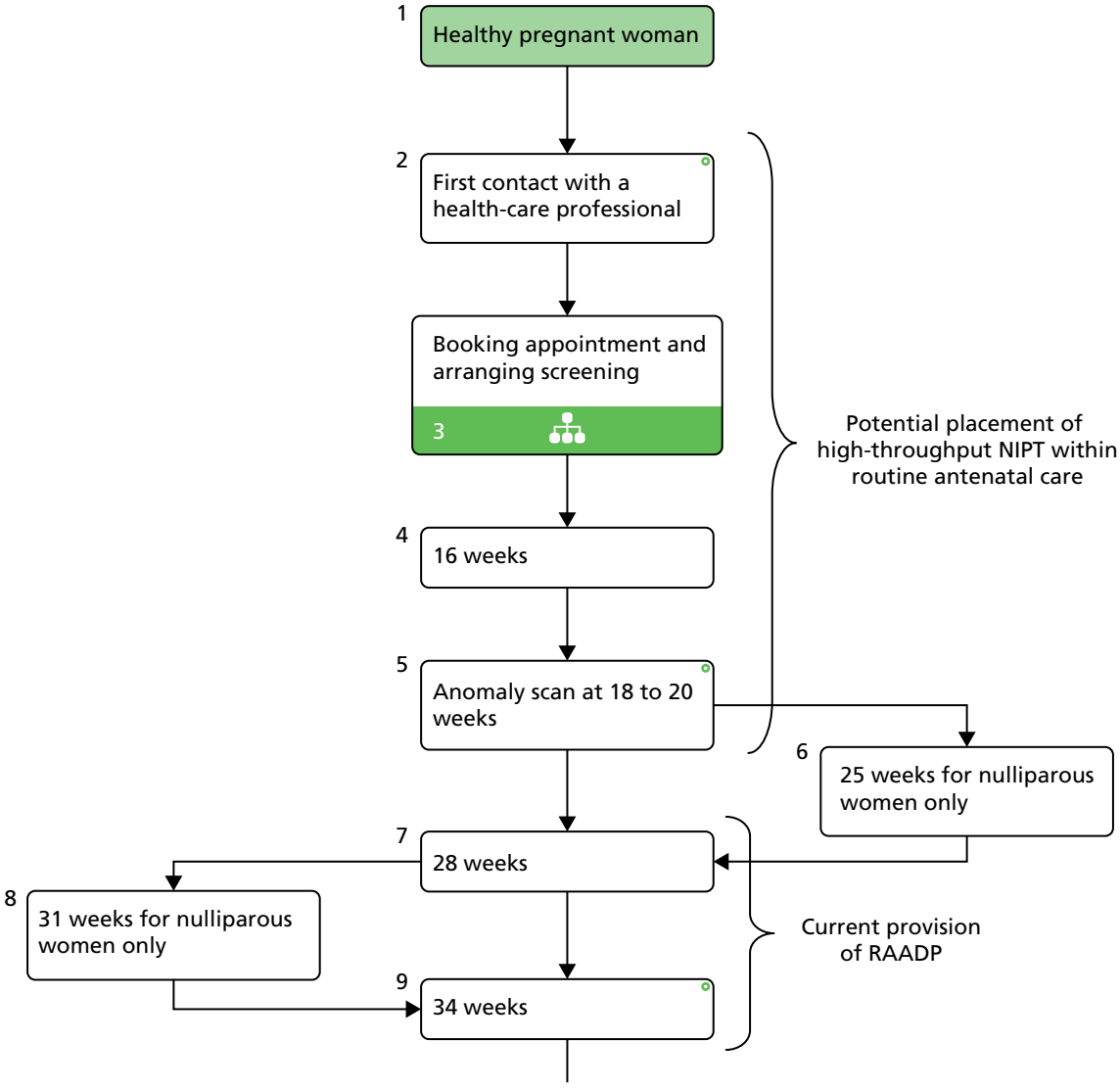

FIGURE 11 Excerpt from NICE schedule of appointments in routine antenatal care. © NICE 2016. All rights reserved. *Schedule of Appointments in Routine Care*.[79] Available from: http://beta.pathways.nice.org.uk/pathways/antenatal-care/schedule-of-appointments-in-routine-antenatal-care. NICE guidance is prepared for the National Health Service in England, and is subject to regular review and may be updated or withdrawn. NICE has not checked the use of its content in this report to confirm that it accurately reflects the NICE publication from which it is taken.

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whooping cough vaccine and the routine 25 weeks' gestation visit for first pregnancy only. Once the results of any high-throughput NIPT are known, they will be communicated to the woman and recorded with the potential to inform all further contacts and decisions regarding testing and treatment. We assume that RAADP and management for potentially sensitising events would be subsequently offered only to women in whom the test result indicates that their fetus is RhD positive and in whom the test result is inconclusive. For women in whom the high-throughput NIPT result is inconclusive, the existing care pathway will remain unchanged and they would receive the same management as women for whom the results of NIPT indicate a RhD-positive baby. We assume that provision of NIPT can be incorporated into routine antenatal care without requiring additional visits (to undertake the test or to communicate the results of test). Similarly, in the base case we do not model additional resources within existing antenatal care appointments to draw blood.

As previously mentioned, the model may be separated into two main elements: (1) an identification part reflecting the diagnostic performance and costs of the alternative identification strategies and (2) a treatment part evaluating the subsequent costs and outcomes (expressed in QALYs) of alternative care pathways. The main aim of the first model element is to divide the cohort according to fetal RhD status and treatment administered (i.e. routine anti-D immunoglobulin, FMH tests and anti-D immunoglobulin for potentially sensitising events, cord serology, FMH tests and postnatal anti-D immunoglobulin). This determines when receipt of anti-D immunoglobulin is appropriate (true positive in terms of NIPT result and/or postnatal cord serology and inconclusive result but pregnant with RhD-positive fetus), when avoidance of anti-D immunoglobulin is appropriate (true negative in terms of NIPT result), when anti-D immunoglobulin is unnecessary (false positive or inconclusive in terms of NIPT result and carrying a RhD-negative fetus) and when avoidance of anti-D immunoglobulin is potentially harmful (false negative in terms of NIPT result). Aspects such as the diagnostic test performance (including inconclusive results and results at different gestation timings), compliance with high-throughput NIPT and anti-D immunoglobulin treatment and the effectiveness of anti-D immunoglobulin all inform the estimation of the probability of sensitisation for each of these groups. The second model element (i.e. the treatment part) considers the short- and long-term consequences of sensitisations (i.e. fetal or neonatal death, minor and major development problems of the child) for the first, second, third and subsequent pregnancies. Costs and utilities are then evaluated for the different components and for each of the alternative pathways.

Four alternative ways in which the use of high-throughput NIPT may impact on the existing postpartum care pathway were considered.

1. NIPT postpartum scenario 1 (PP1): postpartum cord blood typing and FMH testing would continue to be performed, as per current guidelines, in all women regardless of the fetal RhD status identified through high-throughput NIPT.
2. NIPT postpartum scenario 2 (PP2): postpartum cord blood typing, FMH testing (and by implication anti-D immunoglobulin) would be withheld if high-throughput NIPT of fetal RhD status identifies a RhD-negative fetus but would continue to be performed if high-throughput NIPT was inconclusive or had identified a RhD-positive fetus.
3. NIPT postpartum scenario 3 (PP3): postpartum cord blood typing would be performed if high-throughput NIPT of fetal RhD status identifies a RhD-negative fetus. FMH testing and postdelivery anti-D immunoglobulin would be administered if high-throughput NIPT was inconclusive or identifies a RhD-positive fetus.
4. NIPT postpartum scenario 4 (PP4): postpartum cord blood typing not performed in any women. FMH testing and postdelivery anti-D immunoglobulin administered if high-throughput NIPT was inconclusive or had identified a RhD-positive fetus.

The impact that postdelivery testing has on the cost-effectiveness results is explored using separate scenarios in the model. In reality, these four separate scenarios actually represent separate and distinct testing and management strategies and, hence, could also be considered to represent relevant strategies that should be directly compared in the cost-effectiveness assessment.

The cost-effectiveness of high-throughput NIPT is determined by comparing with current practice (i.e. no use of high-throughput NIPT), which comprises (1) RAADP and supplementary anti-D immunoglobulin (as required based on potentially sensitising events) offered to all RhD-negative pregnant women and (2) further postpartum anti-D immunoglobulin offered to all RhD-negative women whose baby's RhD status is confirmed to be positive after cord blood typing.

A schematic representation of the model is provided in *Figure 12a* and *b*. Note that this figure does not provide a comprehensive representation of all components being considered in each alternative strategy, including the postpartum scenarios. The four postpartum scenarios for how the introduction of NIPT could impact on the use of cord serology, fetal maternal haemorrhage tests and anti-D immunoglobulin use following delivery are detailed in *Table 17*.

The model considers the total number of children who would be born to each RhD-negative woman in order to capture the effect of any sensitisation on all subsequent pregnancies based on national fertility rates. We assume that the consequences of sensitisation do not affect the pregnancy in which it occurs (with respect to treatments and tests administered, management and health outcomes of the resultant RhD-positive baby) but rather only subsequent pregnancies. Under current practice, a woman who is sensitised during pregnancy will be identified at the start of her next pregnancy, when she will be tested for antibodies to the RhD antigen. As a consequence of having been sensitised, the woman will be subject to more intense antenatal care in all subsequent pregnancies (see *Cost of management of sensitisation*) and any further RhD-positive fetuses are at risk of adverse health consequences (see *Cost of high-throughput non-invasive prenatal testing* and *Cost of management of sensitisation*). First and subsequent pregnancies together with long-term consequences of sensitisations, in terms of costs and utilities, are evaluated with a yearly cycle and a lifetime horizon. This lifetime horizon includes the full life expectancy of any fetus lost as a consequence of sensitisation. The decision model follows a NHS perspective, and all costs and effects are discounted at a rate of 3.5% each year. The main outcomes of interest within the model are the total lifetime costs and total lifetime QALYs for each of the alternative pathways. Other outcomes recorded in the model include:

- number of sensitisations and the associated costs
- number of affected fetuses following sensitisation
- number of fetuses lost and associated QALY loss
- cost per life-year gained.

What alternative scenarios have been modelled?

In addition to the five alternative pathways compared in the base-case analysis, we compare the inclusion of the high-throughput NIPT at specific gestational ages. These are determined based on available data that show how the diagnostic accuracy of the test varies with gestational age. The timing of the test is important not only in terms of diagnostic performance but also in terms of the cost of managing potentially sensitising events. Although the majority of these are thought to occur in the third trimester (weeks 29–40), any that occur prior to the use of the high-throughput NIPT will incur the cost of anti-D immunoglobulin for all women regardless of fetal RhD status. We further explore the impact of variation in compliance with anti-D immunoglobulin.

Under current guidance, more recent data on RAADP coverage indicate an uptake of approximately 99.0% in women who are still pregnant at 28 weeks and where the father is not established as RhD negative.[8] In addition, postpartum anti-D immunoglobulin current uptake is believed to be also close to 100%.[8] However, data relating to the uptake of routine and postpartum anti-D immunoglobulin in the presence of fetal RhD status identification are scarce (see *Chapter 3*). Finally, we consider alternative scenarios for the proportion of women in whom the NIPT result is inconclusive. The rate of inconclusive results may reach > 14% and these are typically managed as RhD-positive results (see *Chapter 3*, *Results: assessment of diagnostic accuracy*). However, women in whom the high-throughput NIPT result is inconclusive are likely to differ systematically from those in whom the test result is positive, with ethnicity being the most important factor.

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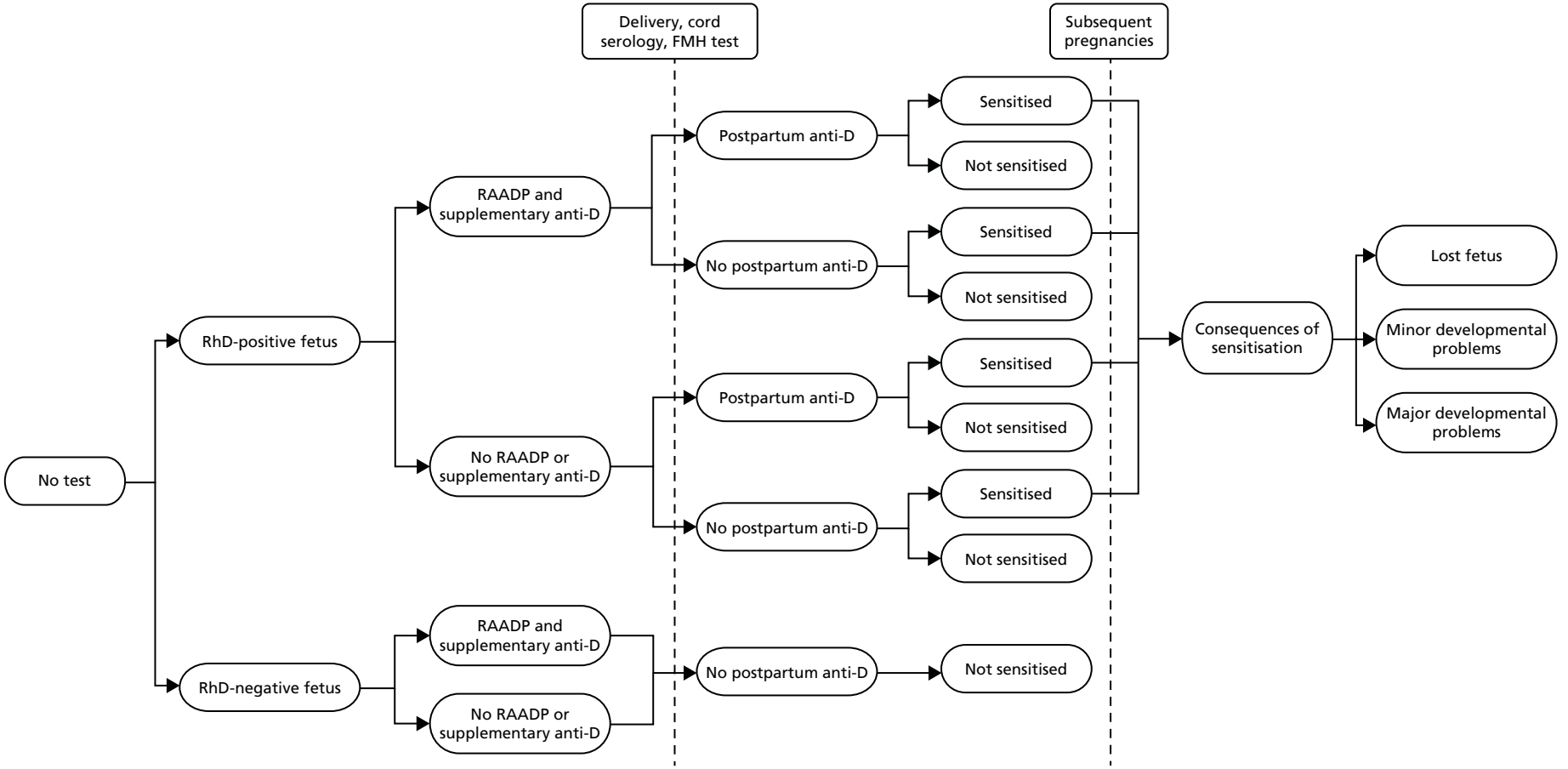

FIGURE 12 Decision-analytic model schematic representation of RhD-negative pregnant women pathways. (a) No high-throughput NIPT and RAADP (current practice, no test and RAADP); and (b) high-throughput NIPT and targeted RAADP. (*continued*)

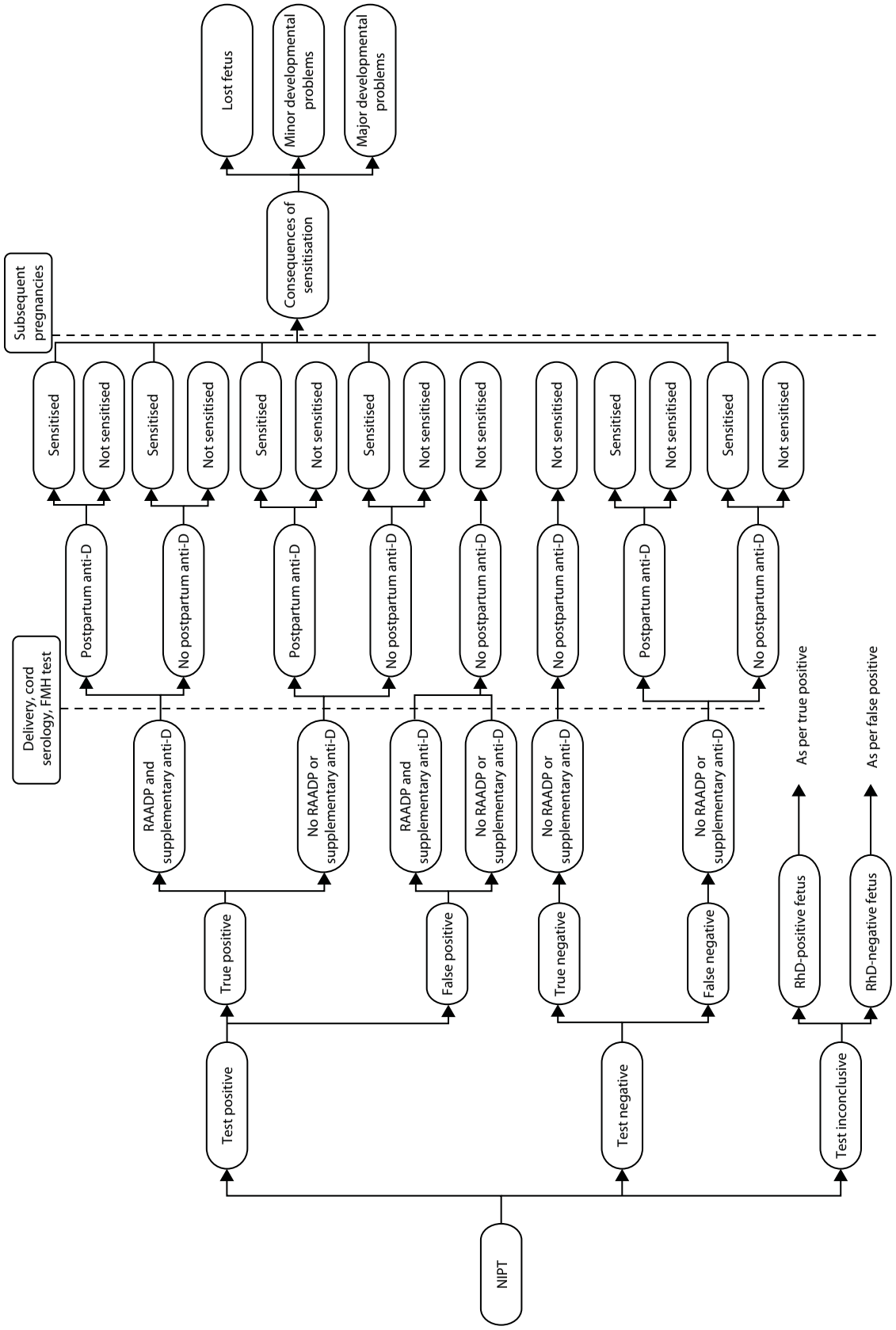

FIGURE 12 Decision-analytic model schematic representation of RhD-negative pregnant women pathways. (a) No high-throughput NIPT and RAADP (current practice, no test and RAADP); and (b) high-throughput NIPT and targeted RAADP.

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TABLE 17 Characteristics of the postpartum scenarios

Scenario	High-throughput NIPT result	Cord serology	FMH test	Postpartum anti-D
PP1	Any	Yes	Yes if CS+	As guided by CS and FMH test
PP2	T–	No	No	No
	T+, inc	Yes	Yes if CS+	As guided by CS and FMH test
PP3	T–	Yes	Yes if CS+	As guided by CS and FMH test
	T+, inc	No	Yes	Yes with additional dose per FMH test
PP4	T–	No	No	No
	T+, inc	No	Yes	Yes with additional dose per FMH test

CS, cord serology.
'–' indicates a negative high-throughput NIPT result; '+' indicates a positive high-throughput NIPT result; 'inc' indicates a inconclusive high-throughput NIPT result.

Model input parameters

This section provides a description of key model input parameters and the evidence used to inform these. A full list of parameters and their characteristics is given in *Table 23*.

Target population

The number of pregnancies in RhD-negative women in England was estimated to be 99,225 per year. This represents a cross-section of all pregnancies and the proportions of first, second, third and subsequent pregnancies are used to characterise the total fertility rate of a typical RhD-negative woman. This estimate was based on a birth rate of 12.2 per 1000 women per year[80] and assumes that 15% of the population is RhD negative.[3]

Proportion of RhD-positive babies born to RhD-negative women

The RhD status of babies does not depend solely on the zygosity of the mother but also of the father. The RhD-negative gene is recessive. Following Mendel's law on inheritance,[81] if the father is homozygous (i.e. he has two RhD-positive genes) all of his children will be RhD positive, but if he is heterozygous (i.e. he has one RhD-positive gene and one RhD-negative gene) his children will have a 50% chance of being RhD-negative. Therefore, as in the NICE TA156,[62] the model assumes that the proportion of RhD-positive babies born to RhD-negative women is a function of (1) the proportion of RhD-positive men (assumed to be identical to the proportion of RhD-positive women, thus, the complement of the proportion of RhD-negative women), (2) the proportion of heterozygous fathers and (3) the proportion of heterozygous fathers having RhD-positive babies. Although the probability of having a RhD-positive baby in subsequent pregnancies can be estimated conditional on knowledge of the RhD status of the first baby, we do not split the cohort in this way. The use of high-throughput NIPT among RhD-negative women not yet sensitised to the RhD antigen is not expected to be determined on the basis of the RhD status of previous offspring. It is therefore unnecessary to split the cohort according to this characteristic and so we apply the same overall rate of RhD-positive babies across all pregnancies. This equates to approximately 62%, as described in *Table 18*.

Diagnostic accuracy of non-invasive prenatal testing

Data on the diagnostic accuracy of high-throughput NIPT are based on the meta-analyses summarised in *Chapter 3*, *Results: assessment of diagnostic accuracy*. The base case utilises the pooled results for the subgroup of UK (Bristol-based) studies in which inconclusive results are considered as test positive. These were considered to be the most relevant to the English setting. Sensitivity, specificity (with 95% CIs) and the correlation between these two test accuracy dimensions (on the log-odds scale) were used to inform

TABLE 18 Probability of subsequent RhD-positive baby following the birth of a RhD-positive baby

Parameter	Mean value	Standard error	Distribution	Source/calculation
Total number of births	659,213	–	–	Office for National Statistics, 2013[82]
Proportion of pregnancies accounted for by Rh-negative women (a)	15.0%	–	–	NHS Digital, Hospital Episode Statistics, 2013–14[3]
Proportion of heterozygous fathers (b)	55.0%	10.0%	Normal	Roman and Pernell, 2002[83]
Proportion of heterozygous fathers having RhD-positive babies (c)	50.0%	–	–	Assumption
Proportion of RhD-positive babies in Rh-negative women (first baby) (d)	61.6%	–	Uncertainty captured from above (f)	Estimate based on information above [= (1 – a) – ((1 – a) × b × c)]
Probability that baby will be RhD-positive in second, third and subsequent pregnancies	61.6%	–	Uncertainty captured from above (f)	Assumed the same as the proportion of RhD-positive babies in Rh-negative women (first baby) (d)

log-normal distributions within the decision model. Note that the correlation estimate for the UK (Bristol) approach was based on only three studies (*Table 19*). SAs were performed based on pooled results from all studies and when inconclusive results were not considered as test positive. In general, high-throughput NIPT accuracy is consistently high across the different approaches to the diagnostic meta-analysis. The subgroup of only UK studies shows a lower FNR and a slightly higher FPR than other scenarios.

Only one study[12] extensively examined the test performance at multiple gestation time points. In scenario analysis these results were used to assess the cost and consequences of introducing high-throughput NIPT at different gestation ages (*Table 20*). We considered that high-throughput NIPT might be targeted at more specific gestational ages from 11 weeks' gestation and not after 24 weeks' gestation, and thus, in the model, we compared the diagnostic accuracy reported for 11–13 weeks, 14–17 weeks and 18–23 weeks (see *Sensitivity analyses results*).

TABLE 19 Summary results of alternative scenarios of high-throughput NIPT RhD diagnostic testing using bivariate models

Pooled NIPT accuracy from bivariate synthesis model	Sensitivity (mean, 95% CI)	Specificity (mean, 95% CI)	Correlation between sensitivity and 1 – specificity (log-odds scale)	Distribution
All studies (excluding inconclusive results)	0.996 (0.991 to 0.999)	0.987 (0.981 to 0.991)	0.461	Log-normal
All studies (treating inconclusive results as if testing positive)	0.997 (0.992 to 0.999)	0.962 (0.943 to 0.975)	–0.316	Log-normal
Only studies reporting inconclusive results[a] (treating inconclusive results as if testing positive)	0.996 (0.989 to 0.998)	0.957 (0.932 to 0.972)	–0.074	Log-normal
UK Bristol studies only (treating inconclusive results as if testing positive)	0.998 (0.992 to 0.999)	0.942 (0.92 to 0.959)	–1.000	Log-normal

a Excluding Thurik *et al.*[21] and Grande *et al.*[22]

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TABLE 20 High-throughput NIPT RhD diagnostic test performance at multiple time points and when including and excluding inconclusive test results

NIPT accuracy per gestational age (weeks)[12]	Sensitivity (mean, standard error)	Specificity (mean, standard error)	Distribution
Treating inconclusive results as if testing positive			
< 11	0.9685 (0.0079)	0.9440 (0.0123)	Log-normal
11–13	0.9983 (0.0023)	0.9525 (0.0114)	Log-normal
14–17	0.9967 (0.0045)	0.9534 (0.0141)	Log-normal
18–23	0.9982 (0.0003)	0.9304 (0.0138)	Log-normal
> 24	1.0000 (0.0010)	0.9574 (0.0076)	Log-normal
Excluding inconclusive results			
< 11	0.9615 (0.0079[a])	0.9970 (0.0123[a])	Log-normal
11–13	0.9981 (0.0023[a])	0.9884 (0.0114[a])	Log-normal
14–17	0.9963 (0.0045[a])	0.9956 (0.0141[a])	Log-normal
18–23	0.9980 (0.0003[a])	0.9847 (0.0138[a])	Log-normal
> 24	1.0000 (0.0010[a])	0.9900 (0.0076[a])	Log-normal

a In the absence of information the standard errors were assumed to be the same as in the approach in which inconclusive results were treated as positive results.

Non-invasive prenatal testing inconclusive results

In the UK studies that inform the base case for the decision model, the pooled proportion of inconclusive NIPT results was 6.7%. Across all diagnostic studies that report the number of inconclusive results this proportion is lower at 4.0%. The results of the diagnostic accuracy studies suggest that the probability of a RhD-positive baby is higher among women in whom the high-throughput NIPT is inconclusive than in all RhD-negative women (see *Chapter 3*, *Inconclusive test results*). In *Proportion of RhD-positive babies born to RhD-negative women,* it was estimated that the probability of RhD-negative women having RhD-positive babies in the first and subsequent pregnancies was 61.6%. In the presence of high-throughput NIPT inconclusive results it is estimated that this probability is 70.1%, irrespective of the pregnancy. This probability is slightly reduced (70.7%) if only UK studies are considered. These last two probabilities are used to estimate the positive predictive value of NIPT, and in SA around the postpartum management of women with inconclusive NIPT results (SA8).

Effectiveness of anti-D immunoglobulin

The introduction of high-throughput NIPT into the care pathway will be used to determine the level of use of anti-D immunoglobulin. Anti-D immunoglobulin affects the rate of sensitisation in women carrying RhD-positive fetuses and carries a potential risk of adverse effects as it is derived from blood products. The costs and consequences of the introduction of high-throughput NIPT are therefore determined by:

- the efficacy of anti-D immunoglobulin in preventing sensitisation, as this determines the health and cost implications for women from whom this is incorrectly withheld because of a false-negative high-throughput NIPT result
- the costs and adverse effects associated with administration of anti-D immunoglobulin.

The clinical effectiveness and cost-effectiveness of RAADP in RhD-negative women has been previously established in NICE TA41[84] and most recently in NICE TA156.[62] No new systematic reviews of RAADP with studies not considered in TA156 were identified. We maintain consistency between the NICE TA process and the diagnostics assessment of high-throughput NIPT for fetal rhesus D status by utilising the RAADP

efficacy estimated based on the same set of clinical effectiveness studies that were considered to be most representative of the UK within NICE TA156. The parameter estimates applied in our base-case analyses are based on the synthesis presented within NICE TA156. The impact of using alternative estimates reported in a related publication by Turner *et al.*,[63] published after NICE TA156 had been completed, is explored within a separate SA. Evidence for the clinical effectiveness of the postpartum use of anti-D immunoglobulin was sourced from a previous Cochrane review.[65] The clinical effectiveness estimates of RAADP and postpartum use of anti-D immunoglobulin reported across these separate sources are reported in *Table 21*.

National Institute for Health and Care Excellence technology appraisal on routine antenatal anti-D prophylaxis

The NICE TA156 found 10 relevant studies that evaluated the clinical effectiveness of RAADP. These studies varied in terms of their patient selection criteria and dosage regimens. Despite this apparent heterogeneity across studies, overall consistency of results was obtained when synthesising relevant data from different subsets of the evidence base. The result of a fixed-effect meta-analysis of two non-randomised community-based UK studies that used a dosage regimen of 500 international units (IUs) at 28 weeks and 34 weeks were considered to be most relevant to the UK. Based on these results, the introduction of RAADP, in addition to the use of anti-D immunoglobulin for potentially sensitising events and post partum, was assumed to reduce the sensitisation rate from 0.95% (95% CI 0.18% to 1.71%) to 0.35% (95% CI 0.29% to 0.40%). These sensitisation rates are conditional on anti-D immunoglobulin treatment being provided at potentially sensitising events also. This gives an odds ratio for the risk of sensitisation of 0.37 (95% CI 0.21 to 0.65) for RAADP compared with no RAADP and an absolute reduction in risk of sensitisation in RhD-negative mothers at risk (i.e. of carrying a RhD-positive child) of 0.6%. These estimates were used in the economic model, which informed the NICE TA156 and are also used to inform the base-case analysis for the de novo model presented here.

Turner *et al.*[63]

Following the publication of the NICE TA156, Turner *et al.*[63] revisited the effectiveness of RAADP for preventing sensitisation in pregnant RhD-negative women. This publication used alternative meta-analytic

TABLE 21 Effectiveness of anti-D immunoglobulin when routinely administered and post partum

	Sensitisation, odds ratio (95% CI)		Sensitisation rate, % (95% CI)		
Source	RAADP[a]	At birth, follow-up up to 6 months, with postpartum anti-D[b]	(Baseline) no RAADP[a]	RAADP (pooled using meta-analysis)	No RAADP and no postpartum anti-D
NICE TA156[62]	0.37 (0.21 to 0.65)	–	0.95 (0.18 to 1.71)	0.35 (0.29 to 0.40)	–
Turner *et al.*[63]	0.31 (0.17 to 0.56)	–	0.95[c] (0.18 to 1.71)	0.40 (0.16 to 0.70)	–
Turner *et al.*,[63] (single dose[d])	0.42 (0.17 to 0.73)	–	0.95[c] (0.18 to 1.71)	0.30 (0.16 to 0.53)	–
Turner *et al.*,[63] (two dose[e])	0.31 (0.09 to 0.65)	–	0.95[c] (0.18 to 1.71)	0.31 (0.09 to 0.62)	–
[f]Crowther *et al.*[65]	–	0.08 (0.06 to 0.11)	0.95[c] (0.18 to 1.71)	–	10.7 (8.0 to 13.8)

a Versus no RAADP, conditional on receiving postpartum anti-D immunoglobulin.
b Versus no postpartum anti-D immunoglobulin, conditional on no RAADP.
c Baseline sensitisation rate of no RAADP assumed the same.
d Single dose (1500 IU) at 28–30 weeks, conditional on receiving postpartum anti-D immunoglobulin.
e Two doses (500 IU) at 28 and 34 weeks, conditional on receiving postpartum anti-D immunoglobulin.
f Sensitisation 6 months after delivery, irrespective of ABO status.

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methods, which allow for the adjustment of both methodological limitations (internal biases) in the set of studies to be combined and differences in study design relative to the research question of interest (external biases). The impact of differences in dose regimen, follow-up times and study populations were evaluated by clinical experts ('assessors') with knowledge of anti-D immunoglobulin prophylaxis, and the impact of methodological flaws in the studies was evaluated by assessors with quantitative expertise. Elicited evidence on the bias for each study was used to adjust the study effect estimates and standard errors, while acknowledging uncertainty in the extent of bias.

After adjusting for differences in study quality and design, the pooled odds ratio for sensitisation was estimated to be 0.31 (95% CI 0.17 to 0.56), with no evidence of heterogeneity ($I^2 = 0\%$). Pooled results were similar to the ones obtained from the NICE TA156 meta-analysis, which included only two studies. Thus, this result substantiated the already existing evidence on the effectiveness of RAADP in preventing sensitisation of pregnant RhD-negative women. This odds ratio is applied in a SA for the de novo model presented here.

Postpartum use of anti-D

Current anti-D immunoglobulin postpartum prophylaxis states that following a baby's birth, ABO and RhD typing should be performed on a cord blood sample. If the baby is confirmed to be RhD positive, all RhD-negative, previously non-sensitised, women should receive a minimum of 500 IU of anti-D within 72 hours of delivery. Maternal samples should be tested for FMH and additional dose(s) given as guided by FMH tests.[2,7]

A Cochrane systematic review was identified that assessed the effectiveness of anti-D immunoglobulin in RhD-negative women who had given birth to RhD-positive babies.[65] Data on six eligible studies, comparing postpartum anti-D immunoglobulin prophylaxis with no treatment or placebo, were synthesised. The estimated odds ratio for sensitisation 6 months after birth with postpartum anti-D immunoglobulin was 0.08 (95% CI 0.06 to 0.11). The estimated odds ratio for sensitisation in subsequent pregnancies with postpartum anti-D immunoglobulin was 0.12 (95% CI 0.07 to 0.19). The former was estimated on five studies with approximately 7500 participants and the latter was based on four studies with approximately 1000 patients. Thus, on the basis of a larger sample size we assumed the former estimate to be the most representative of the effectiveness of postpartum anti-D immunoglobulin in the target population (reported in the last row of results in *Table 20*). Estimated benefits of postpartum anti-D immunoglobulin administration were observed irrespective of the ABO status of mother and child.

Potentially sensitising events

Following potentially sensitising events, the administration and dosage of anti-D immunoglobulin is conditional to the pregnancy stage in which the event occurs. Current guidance[7] recommends that only in extraordinary sensitising events (such as ectopic pregnancy, molar pregnancy or therapeutic termination of pregnancy) should anti-D immunoglobulin be administered at < 12 weeks' gestation. A minimum dose of 250 IU of anti-D immunoglobulin within 72 hours of the event is recommended to be administered if it occurs between 12 and 20 weeks' gestation. For potentially sensitising events after 20 weeks' gestation, a minimum anti-D immunoglobulin dose of 500 IU should be administered within 72 hours, with additional doses as guided by a test for FMH.

Evidence on the reported number of potentially sensitising events was found in the recent audit on anti-D immunoglobulin prophylaxis.[8] The probability of women having at least one (reported) potentially sensitising event was estimated to be 15.5%. Of these, 69.3% women were estimated to have had a FMH test and 95.8% women were estimated to have been treated with anti-D immunoglobulin following the event. It was estimated that approximately 80% of these events happened after 20 weeks' gestation. We assume that these 80% of sensitising events are treated with the minimum required dose of 500 IU of anti-D immunoglobulin. For the remaining 20% of events (pre 20 weeks' gestation events), we assumed that women received the minimum required dose of 250 IU of anti-D immunoglobulin.

The audit on anti-D immunoglobulin prophylaxis[8] also provided information on the type of potentially sensitising event. It was estimated that the probability of women having a miscarriage (including stillbirth and intrauterine death) was 4.7%. We assumed that these fetal deaths were not a consequence of sensitisation and they are incorporated in the model only to adjust the amount of postpartum health resource consumption following delivery.

In contrast to women in whom the high-throughput NIPT result indicates that their fetus is RhD positive, women in whom the test shows that the fetus is RhD negative will not be offered prophylactic anti-D immunoglobulin treatment and will not be subject to FMH testing. This is an issue particularly for the false negatives (RhD-negative women with a RhD-positive fetus but for whom the test result was negative), as these women will, at most, receive only postpartum treatment. For women with false-negative NIPT results who receive only postpartum anti-D immunoglobulin, the model assumes a rate of sensitisation of 0.95%. This is likely to be an underestimate, as it includes receipt of anti-D immunoglobulin for potentially sensitising events. However, the only other estimate for the rate of sensitisation without RAADP is that based on no anti-D immunoglobulin at all, including no postpartum treatment (10.7%), which is likely to be a large overestimate, as the majority of events occur at birth (see *Table 20*). The true rate of sensitisation is likely to lie between 0.95% and 10.7%, but it appears reasonable that this rate will be closer to 0.95%.

Compliance with routine antenatal anti-D prophylaxis and postpartum anti-D immunoglobulin

The National Comparative Audit of Blood Transfusion 2013 on Anti-D Immunoglobulin Prophylaxis[8] reported that, out of all eligible women, 99% received at least one RAADP injection. Full compliance (i.e. correct dose at the correct time) was found to be higher in the single-dose regime (90%) than in the two-dose regime (59%). In addition, the audit shows that a very high proportion of eligible women (98.4%) received postpartum anti-D immunoglobulin prophylaxis. Finally, for documented potentially sensitising events, it showed that approximately 96% of eligible women having these events received anti-D immunoglobulin.

Following the recent audit findings, within the de novo economic model it has been assumed that compliance with RAADP is 99.0%. This value was assumed for the base case and subject to scenario analysis, assuming a rate of 87.5% (i.e. the proportion receiving the correct dose at the correct time). Evidence from the audit points to higher compliance with the single-dose regimen than with the two-dose regimen for a number of reasons (e.g. cost, manufacturer supply, etc.) and there is a move towards the use of the single dose, over the two dose, with its market share reaching approximately 93%.[8] Thus, we did not adjust the compliance rate across RAADP regimen. In the model it has been also assumed that postpartum anti-D immunoglobulin compliance rate is 98.4%, again following evidence from the recent audit.[8] This value was subject to scenario analysis by assuming a rate of 91.6% (i.e. the proportion receiving the correct dose at the correct time).

Compliance with non-invasive prenatal testing given routine antenatal anti-D prophylaxis and postpartum anti-D immunoglobulin

The evidence for compliance with high-throughput NIPT is scarce, particularly in health systems in which the test is introduced after RAADP guidance is in place (see *Chapter 3*, *Results: assessment of clinical effectiveness*). In the absence of such evidence, and based on the already high rates of compliance assumed for current practice (99.0% for RAADP and 98.4% of women received at least one dose of anti-D immunoglobulin at RAADP and post-partum, respectively), we subsequently assume that the use of high-throughput NIPT has no additional impact on compliance. Therefore, it has been assumed that RAADP and postpartum anti-D immunoglobulin compliance is 99.0% and 98.4%, respectively, the same as in the no high-throughput NIPT scenarios.

Sensitisation outcomes

As for the independent economic model developed for NICE TA156 on RAADP, the current economic model considered a set of input parameters directly related to the consequences of sensitisation towards

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the fetus and the newborn infant, namely the implications of haemolytic disease. Three of these model input parameters were key to an appropriate representation of the possible health states, namely (1) the fetal loss rate per RhD-negative women at risk (i.e. carrying a RhD-positive baby), (2) the proportion of babies affected by haemolytic disease that resulted in minor developmental problems (these include, for instance, myopia, squint or delay in language and fine motor skills) and (3) the proportion of babies affected by haemolytic disease that resulted in major developmental problems (these include, for instance, severe permanent neurodevelopmental delay, such as cerebral palsy). Given the long-term consequences of these two last parameters, it was also important to consider the average duration of minor development problems and the life expectancy of an individual with major development problems.

A pragmatic literature search was performed to identify evidence sources for the outcomes associated with haemolytic disease of the fetus and newborn infant, in addition to the ones found in the NICE TA156. The literature review focused particularly on the anti-D immunoglobulin systematic reviews[64,65,85] and the high-throughput NIPT diagnostic accuracy studies (see *Chapter 3*, *Results: assessment of clinical effectiveness*) as potential sources of data associated with the consequences of sensitisation. Apart from the study published by Finning *et al.*,[17] no other relevant evidence was found. Evidence from this study relating to the proportion of fetal or neonatal deaths (5%) and to the proportion of babies affected with mild/severe development problems (5%) was used to populate the model. In the absence of more recent data for parameters relating to the proportion of babies affected with minor development problems and the duration of these problems and relating to the life expectancy of people with major developmental problems, we used the same evidence as NICE TA156 with updated costs. It should be noted that owing to the small number of haemolytic disease-related events, the corresponding model estimates are subject to considerable uncertainty.

In the absence of more recent or relevant data, the health-related quality of life evidence used relating to the utilities of minor (0.85) and major (0.42) development problems and the associated uncertainty was assumed to be the same as those used in NICE TA156.[62]

Cost of high-throughput non-invasive prenatal testing

For the base-case analysis the cost of high-throughput NIPT per sample was estimated to be (confidential information has been removed). This unit cost takes into account consumables, staffing, equipment, indirect costs and overhead costs. This is the company's estimated cost of testing at full capacity, that is, dealing with at least 100,000 samples. An estimated royalty payment of (confidential information has been removed) of the test cost is assumed to be added to the unit cost of the test, bringing the base-case estimate of the cost of the test to (confidential information has been removed). The cost of high-throughput NIPT is discounted according to the pregnancy number in which it is being performed, accounting for an expected median time between pregnancies of around 3.2 years. The unit cost per sample may, however, fluctuate, as it is a function of capacity and predicted level of usage of each testing machine annually. The cost applied in the base-case analysis does not include transport costs for the delivery of blood samples for testing. Szczepura *et al.*[68] included a postage cost of £1.10 per sample in their analysis, although they recognised that the cost would be much reduced if the existing NHS transport service system was to be used.

Cost of routine antenatal anti-D prophylaxis and of anti-D immunoglobulin for potentially sensitising events and post partum

The cost of anti-D immunoglobulin was taken from the *British National Formulary* (BNF).[86] Currently, two brands [D-Gam® (Bio Products Laboratory Ltd, Elstree, Hertfordshire, UK) and Rhophylac®(CSL Behring LLC, Kankakee, IL, USA)] and four doses (250-, 500-, 1500- and 2500-unit vials) are available. At current prices the cost of anti-D immunoglobulin is £23.75 for D-Gam 250 IU, £33.75 for D-Gam 500 IU and £39.52 for Rhophylac. Note that current market prices of anti-D immunoglobulin may vary with supply and demand. Regional and local price negotiations exist that may make the cost anti-D immunoglobulin lower than the values indicated above.

The cost of anti-D immunoglobulin for potentially sensitising events was estimated to be £31.69, representing a weighted average of the cost of anti-D immunoglobulin 250 IU and 500 IU (minimum

required) doses and their expected utilisation before and after 20 weeks' gestation based on evidence from a recent audit.[8] The cost of RAADP was estimated to be £41.58, representing a weighted average of single-dose (1500 IU) and two-dose (2 × 500 IU) regimens and their associated market share, 92.6% versus 7.4%, respectively.[8] Similarly, the cost of anti-D immunoglobulin administered post partum was estimated to be £35.69, which reflects the expected utilisation of 'standard' doses: 500 IU (66.3%) and 1500 IU (33.7%).[8] Costs applied in the current economic model were discounted according to the timing of the pregnancy (the pregnancy number) in which the treatments are administered. As in NICE TA156,[62] an administration cost of anti-D immunoglobulin was set to £5.

Cost of postpartum health resources used

Following birth, in current practice a cord serology test should be performed to confirm the baby's RhD type. In addition, maternal blood samples should be tested for FMH. The costs, updated to 2015 prices, for postpartum serology (£4.18) and associated phlebotomy (£3.32) were obtained from Szczepura *et al.*[68] The cost of FMH testing was provided by personal communication with a NHS Blood and Transplant Manager and estimated to be £128.10 (for test by flow cytometry, NHS Blood and Transport Red Cell Immunohaemotology) (Erika Rutherford, NHS Blood and Transplant, 2016, personal communication). This cost was subject to SA, as Szczepura *et al.*[68] report a much lower value of £3.17 for a Kleihauer test (when updated to 2015 prices). All costs were discounted according to the timing of the pregnancy in which the resources were consumed.

Cost of management of sensitisation

The list of relevant interventions in the management of maternal and neonatal sensitisation was taken from the previous NICE TA156.[62] The proportion of individuals requiring each intervention, the estimated average number of interventions required per individual and the estimated average number of days were considered to be the same as in NICE TA156[62] (*Table 22*). Utilisation of these resources was validated by our clinical experts, who highlighted that no significant changes in clinical practice have occurred since 2009. Similarly, the estimated annual costs for minor (£111) and major (£574) development problems was assumed to be the same as in NICE TA156 but updated to 2015 prices. Unit costs were sourced from the NHS reference costs 2014–15.[87] The total average cost per sensitisation is estimated to be £3167. Note that, owing to the multiplicity of factors affecting sensitisation and its management, the uncertainty associated with this parameter was taken from NICE TA156[62] and assumed to be substantial (standard error £700).

Model parameters and main assumptions

The parameters used within the de novo economic model, and their characteristics, as described in the preceding sections, are outlined in *Table 23*. Costs refer to 2015 prices.

Within the model the following assumptions are consistent with NICE TA156:[62]

- Sensitisations do not affect the pregnancy in which they occur.
- Anti-D immunoglobulin used within one pregnancy has no effect on reducing sensitisations during the next pregnancy.
- The proportion of RhD-negative women is based on the white European population given that this group makes up > 90% of the population of England and Wales.

Furthermore, the following assumptions were made:

- The proportion of RhD-positive babies in Rh-negative women is the same irrespective of pregnancy number.
- The probability of having a RhD-positive baby in the general population of Rh-negative women (61.6%) is combined with the diagnostic accuracy results in terms of sensitivity and specificity (in which inconclusive results are treated as test positive) to determine the number of Rh-positive babies in the model.
- The probability of having a RhD-positive baby in women with inconclusive test results is based on the pooled probability in the study populations used to inform the diagnostic accuracy estimates.

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TABLE 22 Cost of management of sensitisation

Intervention	Management element	Percentage of sensitised mothers/babies requiring intervention	Average number required per person	Average days per treatment	Unit cost of intervention (£)	Total cost (£)	Listed *NHS Reference Costs 2014–15*[87] used for the unit costs
Management of maternal sensitisation	Blood tests, bilirubin, monitoring, etc.	100	6	1	195	1172	Code NZ19B – Ante-Natal Major Disorders with CC Score 0–1 – Regular Day or Night Admissions
	Doppler scanning	90	4	1	109	392	Code NZ21Z – Ante-Natal Standard Ultrasound Scan – Outpatient Procedures
	In utero transfusion	5	3	1	195	29	Code NZ19B – Ante-Natal Major Disorders with CC Score 0–1 – Regular Day or Night Admissions
Management of the sensitised baby	Phototherapy	71	1	3	526	1121	Code PB04D; PB05C; PB06F; PB06M (average) – Neonatal Diagnoses – Non-elective Inpatients – Short Stay
	Exchange transfusion	5	2	1	526	53	Code PB04D; PB05C; PB06F; PB06M (average) – Neonatal Diagnoses – Non-elective Inpatients – Short Stay
	Neonatal follow-up visits	10	2	1	526	105	Code PB04D; PB05C; PB06F; PB06M (average) – Neonatal Diagnoses – Non-elective Inpatients – Short Stay
	Neonatal intensive care unit	5	1	5	1176	294	Code XA01Z – Neonatal Critical Care, Intensive Care – Critical Care
Total						3167	

CC, Complication and Comorbidity.

TABLE 23 Model parameters

Parameter	Mean value	Standard error	Distribution	Source/calculation
Discounting				
Discount rate for utilities	3.5%	–	–	NICE methods guidance[88]
Discount rate for costs	3.5%	–	–	NICE methods guidance[88]
Target population characteristics				
Population of England (a)	54,316,600	–	–	Office for National Statistics, *Annual Mid-year Population Estimates, 2014*[89]
Crude birth rate in England: all births per 1000 population of all ages (b)	12.18	–	–	Office for National Statistics, *Births in England and Wales, 2014*[80]
Proportion of pregnancies accounted for by Rh-negative women (c) – reiterated from *Table 17*	15.0%	–	–	NHS Digital, *Hospital Episode Statistics: NHS Maternity Statistics*, 2013–14[3]
Number of women requiring treatment	99,225	–	–	Estimate based on information above [= (a × (b/1000) × c)]
Proportion of first pregnancies proceeding to next pregnancy	91.4%	–	–	Office for National Statistics, *Birth Summary Tables, England and Wales*[90] – *Characteristics of Mother 2, England and Wales – Average 2009 to 2013*
Proportion of second pregnancies proceeding to next pregnancy	40.5%	–	–	Office for National Statistics, *Birth Summary Tables, England and Wales*[90] – *Characteristics of Mother 2, England and Wales – Average 2009 to 2013*
Proportion of third pregnancies proceeding to next pregnancy	58.3%	–	–	Office for National Statistics, *Birth Summary Tables, England and Wales*[90] – *Characteristics of Mother 2, England and Wales – Average 2009 to 2013*
Median time between pregnancies (years)	3.17	–	–	Office for National Statistics, *Birth Summary Tables, England and Wales 2014*[90] – *Characteristics of Mother 2, England and Wales, 2013*
Compliance				
Compliance with RAADP	99.0%	0.1%	Beta	NHS Blood and Transplant, *National Comparative Audit of Blood Transfusion – 2013 Audit of Anti-D Immunoglobulin Prophylaxis*[8]
Compliance with RAADP if high-throughput NIPT performed	99.0%	0.1%	Beta	Assumed the same as compliance with RAADP
Compliance with postpartum Anti-D immunoglobulin (dose of at least 500 IU given within 3 days of delivery)	98.0%	0.2%	Beta	NHS Blood and Transplant, *National Comparative Audit of Blood Transfusion – 2013 Audit of Anti-D Immunoglobulin Prophylaxis*[8]

continued

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TABLE 23 Model parameters (*continued*)

Parameter	Mean value	Standard error	Distribution	Source/calculation
High-throughput NIPT inconclusive results				
Proportion of high-throughput NIPT inconclusive results: all studies reporting inconclusives	6.7%	0.4%	Beta	Diagnostic accuracy review (see *Chapter 3*)
Proportion of high-throughput NIPT inconclusive results: UK Bristol studies	4.0%	0.1%	Beta	Diagnostic accuracy review (see *Chapter 3*)
Proportion of RhD-positive babies in high-throughput NIPT inconclusive results: all studies reporting inconclusives	70.1%	0.7%	Beta	Diagnostic accuracy review (see *Chapter 3*)
Proportion of RhD-positive babies in high-throughput NIPT inconclusive results: UK Bristol studies	70.7%	0.3%	Beta	Diagnostic accuracy review (see *Chapter 3*)
Sensitisation events				
Probability of having at least one potentially sensitising event	15.5%	0.5%	Beta	NHS Blood and Transplant, *National Comparative Audit of Blood Transfusion – 2013 Audit of Anti-D Immunoglobulin Prophylaxis*[8]
Probability of performing a FMH test given at least one potentially sensitising event	69.3%	1.4%	Beta	NHS Blood and Transplant, *National Comparative Audit of Blood Transfusion – 2013 Audit of Anti-D Immunoglobulin Prophylaxis*[8]
Probability of receiving anti-D after having at least one potentially sensitising event	95.8%	0.6%	Beta	NHS Blood and Transplant, *National Comparative Audit of Blood Transfusion – 2013 Audit of Anti-D Immunoglobulin Prophylaxis*[8]
Probability of women having a miscarriage (including stillbirth and intrauterine death)	4.7%	0.3%	Beta	NHS Blood and Transplant, *National Comparative Audit of Blood Transfusion – 2013 Audit of Anti-D Immunoglobulin Prophylaxis*[8]
Consequences of sensitisation				
Fetal loss rate per woman at risk	5.0%	1.0%	Beta	Finning *et al.* (2008)[17] and the previous NICE assessment (TA156)[62]
Proportion of babies affected by HDN with minor developmental problems	6.0%	2.0%	Beta	Previous NICE assessment (TA156)[62]
Duration of minor developmental problems (years)	16	5	Beta	Previous NICE assessment (TA156)[62]
Proportion of babies affected by HDN with major developmental problems	5.0%	1.0%	Beta	Finning *et al.* (2008)[17] and the previous NICE assessment (TA156)[62]
Life expectancy for person with major developmental problems	59.5	Range 40–79	Uniform	Previous NICE assessment (TA156)[62]

TABLE 23 Model parameters (*continued*)

Parameter	Mean value	Standard error	Distribution	Source/calculation
Utilities				
Utility for 'normal' person	0.88	0.02	Beta	Previous NICE assessment (TA156)[62]
Utility for minor development problems	0.85	0.02	Beta	Previous NICE assessment (TA156)[62]
Utility for major development problems	0.42	0.03	Beta	Previous NICE assessment (TA156)[62]
Costs				
Cost of high-throughput NIPT	(Confidential information has been removed)	–	–	(Confidential information has been removed)
Royalty fee of high-throughput NIPT	(Confidential information has been removed)	–	–	(Confidential information has been removed)
Cost of RAADP	£41.58	–	–	*BNF*[86] and NHS Blood and Transplant, *National Comparative Audit of Blood Transfusion – 2013 Audit of Anti-D Immunoglobulin Prophylaxis*[8] – weighted average of single- and two-dose anti-D regimen costs and their market share
Cost of anti-D immunoglobulin for potentially sensitising events	£31.69	–	–	*BNF*[86] and NHS Blood and Transplant, *National Comparative Audit of Blood Transfusion – 2013 Audit of Anti-D Immunoglobulin Prophylaxis*[8] – weighted average of dose anti-D regimen cost and the likelihood of pre and post 20 weeks events
Cost of postpartum anti-D immunoglobulin	£35.69	–	–	*BNF*[86] and NHS Blood and Transplant, *National Comparative Audit of Blood Transfusion – 2013 Audit of Anti-D Immunoglobulin Prophylaxis*[8] – weighted average of dose anti-D regimen cost and their market share
Cost of anti-D immunoglobulin administration per RhD-negative woman treated	£5.00	£2.00	Gamma	Previous NICE assessment (TA156)[62]
Cost of postpartum blood cord serology	£4.18	–	–	Szczepura *et al.*,[68] updated to 2015
Cost of FMH testing	£128.10	–	–	Provided by clinical experts
Cost of phlebotomy	£3.32	–	–	Szczepura *et al.*,[68] updated to 2015 prices
Cost of management of a sensitised woman and sensitised neonate	£3166.72	£700.00	Gamma	Previous NICE assessment (TA156)[62]
Yearly cost of minor developmental problems	£110.58	£35.00	Gamma	Previous NICE assessment (TA156),[62] updated to 2015 prices
Yearly cost of major developmental problems	£573.72	£405.73	Gamma	Previous NICE assessment (TA156),[62] updated to 2015 prices

HDN, Haemolytic Disease of the Newborn.

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- All NIPT is performed early enough to determine the use of RAADP at 28 weeks' gestation.
- Routine and prophylactic anti-D immunoglobulin is offered only to women whose NIPT result indicates that their fetus is RhD positive or whose results are inconclusive.
- In women with an inconclusive NIPT result the existing care pathway is unchanged and they are treated the same as women who test positive in terms of RAADP, anti-D immunoglobulin and associated tests.
- Women identified to receive RAADP will receive supplementary anti-D immunoglobulin at the minimum dose required for any potentially sensitising events.
- Potentially sensitising events that involve fetal death were independent of previous sensitisation within the same pregnancy.
- Women with false-negative test results but who are provided with cord serology and postpartum anti-D immunoglobulin have a sensitisation rate of 0.95% despite forgoing anti-D immunoglobulin treatment for potentially sensitising events.
- Compliance with RAADP is same with and without NIPT; similarly, compliance for postpartum anti-D immunoglobulin is assumed to be the same with or without NIPT.

Analytic methods

In exploring the alternative means by which the introduction of high-throughput NIPT could impact on the postpartum care pathway, we first present results for each postpartum scenario separately compared with 'no test and RAADP'. Thereafter, we combine them and compare them directly in a full incremental analysis.

The decision-analytic model was evaluated using 10,000 Monte Carlo simulations to reflect the joint uncertainty across all of the inputs according to the probability distributions assigned to each, as shown in *Table 22*. All results are presented in terms of the average over 10,000 simulations, as these provide an unbiased estimate of the expected model outcomes. The existing model non-linearity means that the deterministic results are not an accurate estimate of the mean costs and QALYs in each strategy. This non-linearity is likely to be attributable to the model being structured around the specificity and sensitivity of NIPT and the rate of sensitisation, all characterised by skewed distributions and all with baseline values close to the upper bound of 1 (sensitivity and specificity) or lower bound of 0 (rate of sensitisation). The primary results are the total expected costs and expected QALYs for each alternative strategy. Population net health benefits (NHBs) are used to summarise the cost-effectiveness results in addition to the cost-effectiveness ratio. NHBs are calculated for cost-effectiveness thresholds of £20,000 and £30,000 as shown in the equation below:

$$\text{Net health benefit} = \text{QALYs} - \frac{\text{Costs}}{\text{Cost-effectiveness threshold}}. \quad (1)$$

For a given cost-effectiveness threshold, the strategy with the highest net benefit is the same strategy that would be considered cost-effective when comparing incremental cost-effectiveness ratios (ICERs) against the threshold. They are useful to summarise results when there are small differences in health between strategies and when the new intervention may be less effective and less costly than current practice. In these circumstances, ICERs can be very volatile and sensitive to small changes in the denominator. Further to this, the ICER for a less costly and less effective new intervention actually represents the cost per QALY gain of introducing current practice, and this can lead to some confusion in interpretation. The introduction of the high-throughput NIPT is not expected to produce large differences in clinical outcomes and may result in lower health outcomes than RAADP if the rate of sensitisations is increased.

Results are expressed per pregnancy and for the cross-section of 100,000 pregnancies, as described in *Target population*. It should be noted that for the population-level results, the total number of pregnancies is distributed across time and, therefore, not all test costs or consequences are experienced in year 1. Results were initially calculated for the comparison of 'no test and RAADP' with 'no test and no RAADP' in order to illustrate the impact of the adjustments made to the model used in NICE TA156[62] and to establish

the baseline comparability in terms of the cost-effectiveness of the current practice, 'no test and RAADP'. This was necessary because the benefits of a diagnostic test are reliant on there being a cost-effective treatment available. The results of this analysis are shown in *Appendix 10*. Throughout the main body of this diagnostic assessment report we omit the 'no test and no RAADP' strategy, as this is not relevant to UK current practice.

Cost-effectiveness acceptability curves are used to show the probability that each alternative strategy is cost-effective for a range of cost-effectiveness threshold. We also calculate the health consequences of the total amount of parameter uncertainty in terms of the potential health benefits that could be gained if all uncertainty were eliminated. This is the expected value of perfect information and it represents an upper bound for the value of any further research to reduce parameter uncertainty. The maximum value of further research was calculated as the difference between the expected value of basing a decision about the use of NIPT on perfect information (i.e. with no probability of error) and the expected value of that decision made on the basis of existing evidence (i.e. subject to uncertainty). This value is expressed in terms for the cross-section of 100,000 pregnancies multiplied over 10 years, as the further research may inform decisions beyond the immediate cohort of pregnancies considered in this model.

Uncertainty regarding the appropriate source of data, the appropriate assumptions or model structure and other scenarios are explored using one- and two-way SA, as described further in *Sensitivity analyses*.

Base-case analysis

The set of main assumptions used in the base-case analysis are shown in *Table 24*.

Sensitivity analyses

A series of scenario analyses and SAs was also conducted. We focused on parameters and assumptions to which we expected that the ICER would be the most sensitive and where the available evidence was limited. The SAs are described in detail but also summarised in *Table 24*. We focus on the comparison of current practice with the best performing postpartum scenario in all cases unless the results of the SA

TABLE 24 Main base-case assumptions

Parameter	Assumption/evidence source
High-throughput NIPT accuracy	Bivariate meta-analysis of UK (Bristol) studies, see *Chapter 3*; the diagnostic test was assumed to be performed at first contact with health services
Effectiveness of RAADP (vs. no RAADP)	Sensitisation rate = 0.35% (NICE TA156[62])
Uptake of RAADP (with and without high-throughput NIPT performed)	99.0% (NHS Blood and Transplant, *National Comparative Audit of Blood Transfusion – 2013 Audit of Anti-D Immunoglobulin Prophylaxis*[8])
Uptake of postpartum anti-D immunoglobulin (with and without high-throughput NIPT performed)	98.4% (NHS Blood and Transplant, *National Comparative Audit of Blood Transfusion – 2013 Audit of Anti-D Immunoglobulin Prophylaxis*[8])
High-throughput NIPT inconclusive results	Inconclusive rate of 6.2% treated as positive test results
Cost of high-throughput NIPT	Base-case unit cost of (confidential information has been removed) with a (confidential information has been removed) royalty fee added: (confidential information has been removed)
Cost of anti-D immunoglobulin	Potentially sensitising event, £31.69; RAADP, £41.58; postpartum, £35.69
Cost of FMH test	£128.10 (Erika Rutherford, Business Development Manager, Red Cell Immunohaematology, NHS Blood and Transplant, 2016, personal communication)
Further postpartum scenario on the management of high-throughput NIPT inconclusive results	Inconclusive results are treated post delivery as positive test results

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affect the rank order of postpartum scenarios or suggest that multiple postpartum scenarios could potentially provide the highest NHB.

Sensitivity analysis 1

We explored alternative sources for the diagnostic performance of high-throughput NIPT. The base-case analysis utilises the results from the UK (Bristol) studies, as these are thought to be most generalisable to a UK setting. We also show the results utilising all available studies, regardless of geography. For lower estimates of sensitivity, high-throughput NIPT is expected to result in more false-negative results, which are associated with adverse health consequences in terms of additional sensitisations. For lower estimates of specificity, high-throughput NIPT is expected to result in more false-positive results, which reduce the amount of unnecessary anti-D immunoglobulin and associated management costs that is avoided.

Sensitivity analysis 2

We explored the use of high-throughput NIPT at different gestation periods. Performance results from a recent UK study[12] were used to assess the cost and consequences of introducing high-throughput NIPT at 11–13 weeks, 14–17 weeks and 18–23 weeks. Note that the economic model does not incorporate the timing of a potentially sensitising event and so a threshold analysis is performed to determine the percentage of the costs that would have to occur prior to NIPT in order for the ICER to cross a threshold of £20,000 per QALY.

Sensitivity analysis 3

The base-case analysis utilised the same rate of sensitisation with 'no test and RAADP' as was used in the NICE TA156.[62] Subsequent to NICE TA156 a further meta-analysis was performed by Turner *et al.*,[63] which suggests that anti-D immunoglobulin could be marginally more effective if all studies are taken into account, reducing the rate of sensitisation with 'no test and RAADP' from 0.35% to 0.30%. The increased efficacy of RAADP will increase the health costs associated with false-negative results of high-throughput NIPT, as women will have incorrectly forgone a more effective treatment.

Sensitivity analysis 4

We explore the impact of an overall change in uptake of anti-D immunoglobulin. Lower uptake of RAADP will reduce the cost savings possible from avoiding unnecessary RAADP but will also affect the health consequences of additional sensitisations. However, we did not explore an effect of high-throughput NIPT on uptake. The base-case analysis assumes that the introduction of high-throughput NIPT will not alter the proportion of women who comply with the administration of anti-D immunoglobulin. Currently, few women in the UK refuse RAADP, so there is little scope for an increase in uptake. We consider that it may be possible that women who would refuse RAADP would also refuse high-throughput NIPT, but this should not impact on the cost-effectiveness of NIPT, only on throughput. Although the clinical effectiveness review identified studies that reported the rate of uptake of anti-D immunoglobulin among women provided with high-throughput NIPT, none provided a comparison with what uptake would have been in those same women without provision of high-throughput NIPT. We therefore assumed that women informed that they are carrying a RhD-positive fetus would be no more or less likely to uptake anti-D immunoglobulin than they would be if offered RAADP. Some women who are told that they are carrying a RhD-negative fetus may still demand RAADP, and this cost is not incorporated in the model. We conduct a two-way SA in which the uptake of RAADP is decreased or increased alongside the reduction of the uptake of postpartum anti-D immunoglobulin.

Sensitivity analysis 5

The base-case analysis incorporates the rate of inconclusive high-throughput NIPT results found in the UK (Bristol) studies.[12,17,18] The rate of inconclusive results will vary according to the local population demography because they are more likely in certain ethnic groups, such as in those of African ethnic origin. The rate of inconclusive results may also vary if the operation of NIPT is different in a trial setting compared with in routine use, for example if less time is spent on reprocessing initially inconclusive test results. Increasing the rate of inconclusive test results when these are treated as test positive will increase

the rate of false-positive results and reduce the specificity of NIPT. This will, in turn, reduce the amount of unnecessary anti-D immunoglobulin and associated management costs that can be avoided through the use of high-throughput NIPT.

Sensitivity analysis 6

We conduct a two-way SA in which the cost per dose of anti-D immunoglobulin therapy is varied alongside the cost per high-throughput NIPT. The cost of high-throughput NIPT to the NHS is uncertain for a number of reasons: (1) the unit cost varies by throughput and so will depend on the total uptake of NIPT, (2) the unit cost of the test must be considered alongside other potential additional costs relating to the transportation of blood samples for testing, to whether or not additional antenatal visits are required to draw blood and to the delivery of test counselling and results and (3) the royalty fee charged to the NHS in addition to the unit cost of the test is uncertain. The base-case analysis includes a test cost of (confidential information has been removed) and a royalty fee of (confidential information has been removed). The base case assumes that high-throughput NIPT can be incorporated in to routine antenatal care without imposing further marginal costs to the NHS, which is likely to be favourable to any 'test and RAADP' strategies. We calculate the threshold NHS cost per high-throughput NIPT at which the ICER for any strategy incorporating NIPT falls below £20,000 and £30,000 per QALY. We also show how the ICER varies as the cost per test is varied between £13.20 and £24.20. The cost of anti-D immunoglobulin may be subject to discounts from the list prices utilised in the base-case analysis. We show how the cost-effectiveness results vary to –20%, –10%, +10% and +20% of list price. The cost-effectiveness of any high-throughput NIPT will be reduced as the price of anti-D immunoglobulin falls because the savings from avoiding unnecessary RAADP will be lower.

Sensitivity analysis 7

Since the introduction of RAADP there has been a move from the two-dose to the single-dose regimen for a variety of reasons, as indicated in the recent anti-D immunoglobulin prophylaxis audit. We conducted a SA that assumes a 100% use of the cheaper of the two regimens, that is, the single dose.

Sensitivity analysis 8

A further alternative way in which the use of high-throughput NIPT may impact on the existing postpartum care pathway is considered. This strategy, rather than grouping high-throughput NIPT inconclusive results with positive results, regards them as distinct from those for whom NIPT indicated a RhD-positive fetus. In this scenario postpartum cord blood typing would be performed if high-throughput NIPT of fetal RhD status identifies a RhD-negative fetus or if the test result is inconclusive. FMH testing and postdelivery anti-D immunoglobulin would be administered if a RhD-positive fetus is identified either in the positive test result group or in the inconclusive test result group.

A summary of the SA performed is listed in *Table 25*.

Model validation

Pedro Saramago developed the model and Susan Griffin checked the model for errors. Comparisons across strategies were done to identify inconsistencies. Comparisons with the previous NICE TA156 were also done to identify the sources of any potential discrepancy.

Results of the independent economic assessment

This section reports the results of the de novo economic model developed to assess the cost-effectiveness of high-throughput NIPT to identify fetal RhD status in women who are RhD-negative and not known to be sensitised to the RhD antigen. The base-case results for the different postpartum strategies are shown first, followed by the results of performing SA on key model input parameters. All results are based on the probabilistic analysis. Detailed characteristics of each postpartum scenario are provided in *Table 16*.

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TABLE 25 Summary of SA performed

Parameter	Assumption/evidence source
High-throughput NIPT accuracy	SA1: bivariate meta-analysis of all studies (see *Chapter 3*)
	SA2: high-throughput NIPT performance assessed at different gestation periods, using evidence from Chitty *et al.*[12]
Effectiveness of RAADP (vs. no RAADP)	SA3: sensitisation rate = 0.30% (Turner *et al.*[63])
Compliance with RAADP (with and without high-throughput NIPT performed)	SA4a: 87.5% (NHS Blood and Transplant, *National Comparative Audit of Blood Transfusion – 2013 Audit of Anti-D Immunoglobulin Prophylaxis*[8])
Compliance with postpartum anti-D immunoglobulin (with and without high-throughput NIPT performed)	SA4b: 91.6% (NHS Blood and Transplant, *National Comparative Audit of Blood Transfusion – 2013 Audit of Anti-D Immunoglobulin Prophylaxis*[8])
High-throughput NIPT inconclusive results	SA5: pooled estimates for the sensitivity and specificity replaced with the individual study results
Cost of high-throughput NIPT	SA6a: varied between £13.20 and £24.20 (confidential information has been removed)
Cost of anti-D immunoglobulin	SA6b: all varied ± 20%
Cost of FMH test	SA7: £3.17 (Szczepura *et al.*,[68] updated to 2015 prices)
Further postpartum scenario on the management of high-throughput NIPT inconclusive results	SA8:

NIPT result	Cord serology	FMH test	Postpartum anti-D
T–	Yes	Yes if CS+	As guided by CS and FMH test
T+	No	Yes	Yes with additional dose per FMH test
Inconclusive	Yes	Yes if CS+	As guided by CS and FMH test

CS, cord serology.

Base-case results

Table 26 presents the results for each postpartum testing scenario separately against current practice of 'no test and RAADP'. Total costs, total QALYs, incremental costs and incremental QALYs are presented together with incremental cost per QALY gained (ICER) and population NHBs at £20,000 and £30,000 threshold values. The results of the model suggest that for each additional sensitisation there is a loss of approximately 0.9 QALYs. Any difference in QALYs between strategies is attributable wholly to the difference in the number of sensitisations.

Non-invasive prenatal testing PP1 describes the use of NIPT to guide RAADP only, with all women continuing to receive cord serology with FMH testing and postpartum anti-D immunoglobulin as required, irrespective of NIPT result. This is estimated to reduce costs by £584,000 per 100,000 pregnancies and to result in lower health benefits (0.5 QALYs) than current practice.

Non-invasive prenatal testing PP2 (NIPT PP2) describes the use of NIPT to guide both RAADP and postpartum care to women who test positive or in whom the results are inconclusive, when cord serology is provided only in these women to guide FMH testing and postpartum anti-D immunoglobulin as required. This is estimated to reduce costs compared with current practice by approximately £671,000 but to result in a loss of 19.1 QALYs per 100,000 pregnancies.

Non-invasive prenatal testing PP3 (NIPT PP3) describes the use of NIPT to guide RAADP and postpartum anti-D immunoglobulin to women who test positive or inconclusive and when cord serology is used to guide FMH testing and postpartum anti-D immunoglobulin as required only to women whom NIPT

TABLE 26 Incremental cost-effectiveness outcomes associated with high-throughput NIPT vs. other strategies (base-case postpartum scenarios): probabilistic results

Strategies	Total costs (£)	Total QALYs	Incremental costs (£)	Incremental QALYs	ICER (£/QALY gained)	Population NHB (λ = £20,000)	Population NHB (λ = £30,000)
Current clinical practice							
No test and RAADP	15,983,725	2,433,756	–	–	–	2,432,957	2,433,223
NIPT PP1							
Test and RAADP (T+ only) vs. no test and RAADP	15,400,187	2,433,756	–583,538	–0.46	1,269,050	2,432,986	2,433,242
NIPT PP2							
Test and RAADP (T+ only) vs. no test and RAADP	15,312,630	2,433,737	–671,095	–19.13	35,087	2,432,972	2,433,227
NIPT PP3							
Test and RAADP (T+ only) vs. no test and RAADP	15,498,942	2,433,756	–484,783	–0.46	1,054,281	2,432,981	2,433,239
NIPT PP4							
Test and RAADP (T+ only) vs. no test and RAADP	15,410,610	2,433,737	–573,114	–19.13	29,964	2,432,967	2,433,223

Estimates of ICERs shown relate to the comparison No test and RAADP vs. any strategy involving NIPT.

indicates have a RhD-negative fetus. This is estimated to reduce costs compared with current practice by £485,000 but to result in a loss of 0.5 QALYs per 100,000 pregnancies.

Non-invasive prenatal testing PP4 (NIPT PP4) describes the use of NIPT to guide both RAADP and postpartum FMH testing and anti-D immunoglobulin to women who test positive or inconclusive and when cord serology is not provided. This is estimated to reduce costs compared with current practice by approximately £573,000 but results in a loss of 19.1 QALYs per 100,000 pregnancies.

All postpartum scenarios are cost saving but also less effective than no test and RAADP, placing them on the south-west quadrant of the cost-effectiveness plane (*Figure 13*). The least effective strategies are those that omit cord serology for women who test negative on NIPT. Without cord serology false negatives are not picked up at delivery and are not provided with postpartum anti-D immunoglobulin. In the model, the additional health gains are determined by the management of high-throughput NIPT false-negative test results.

Owing to these NIPT strategies being less costly and less effective than no test and RAADP, the ICERs calculated in *Table 25* (and *Figure 13*) show the cost per QALY gained with current practice compared with high-throughput NIPT. Hence, when the ICER is above the cost-effectiveness threshold this would support the use of NIPT (no test and RAADP vs. NIPT PP1, ICER approximately £1,270,000 per QALY gained). The cost-effectiveness threshold can be used to present results in terms of NHBs, in which case the comparison is more straightforward, as the strategy with the highest NHB is preferred. All NIPT strategies have an expected NHB higher than no test and RAADP, both at threshold values of £20,000 and £30,000. Compared with no test and RAADP, NIPT PP1 has greater NHB (incremental NHB at £20,000 of approximately 14; incremental NHB at £30,000 of approximately 16, vs. no test and RAADP).

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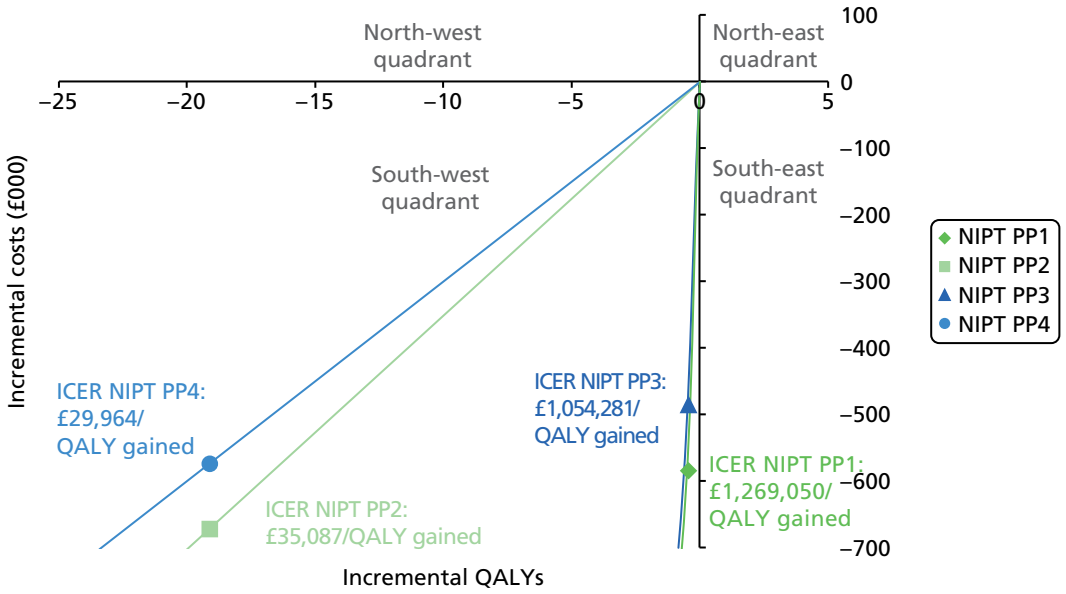

FIGURE 13 Cost-effectiveness plane of current practice (no test and RAADP) and alternative NIPT scenarios (PP1 to PP4).

The base-case analysis assumes no adverse health impacts from use of a blood-based product, such as anti-D immunoglobulin. This is in line with the fact that widespread global use of anti-D immunoglobulin has yet to produce evidence of any adverse consequences. We illustrate how sensitive the ICER is to changes in these assumptions. Using the net benefit framework, it is possible to interpret the results of the SA around the price of anti-D immunoglobulin in terms of health impact. An increase of 20% in the cost of anti-D immunoglobulin represents a cost of £39.50 × 0.2 = £7.90. At a cost-effectiveness threshold of £20,000 per QALY, this is equivalent to assuming a health cost of 7.9/20,000 = 0.0004 QALYs per administration, or a loss of 3.5 hours of full lifetime health from every woman per dose of anti-D immunoglobulin they receive.

The incremental costs of introducing NIPT can be broken down into the cost of NIPT, the cost of managing potentially sensitising events, the cost of RAADP, the cost of postpartum tests and anti-D immunoglobulin and the cost consequences of sensitisations, and these are shown in *Table 27*. Although the added NIPT cost is similar across strategies (at approximately £1,585,000 per 100,000 pregnancies) it is accumulated over multiple pregnancies and so is affected by the performance of strategy in terms of the number of sensitisations. Strategies with more sensitisations (NIPT PP2 and NIPT PP4) have marginally less test cost, as sensitised women do not receive NIPT to guide RAADP in subsequent pregnancies (however, it is worth noting that it is recommended that NIPT be used in women who are sensitised in order to guide antenatal care). Similarly, all strategies save similar levels of costs from avoiding RAADP (approximately £1,544,000 per 100,000 pregnancies) and the management of potentially sensitising events (approximately £626,000 per 100,000 pregnancies). The NIPT strategies vary more markedly in their impact on postpartum testing

TABLE 27 Breakdown of incremental costs of high-throughput NIPT strategies vs. no test and RAADP

Cost item (£)	NIPT PP1	NIPT PP2	NIPT PP3	NIPT PP4
NIPT cost	1,585,117	1,584,861	1,585,117	1,584,861
Potentially Sensitising Event management costs	–626,165	–627,470	–626,165	–627,470
RAADP costs	–1,544,149	–1,544,887	–1,544,149	–1,544,887
Postpartum test and anti-D costs	–43	–152,771	98,712	–54,790
Sensitisation costs	1703	69,173	1703	69,173
Total incremental cost	–583,538	–671,095	–484,783	–573,114

and anti-D immunoglobulin costs. Here, NIPT PP1 is essentially the same as current practice, except for the small reduction in costs attributable to increased sensitisations, which makes women ineligible for FMH testing and anti-D immunoglobulin. NIPT PP2 decreases postpartum care costs by avoiding cord serology for women who test negative, but this comes at an increased cost of managing sensitisations, as false negatives are not picked up at delivery and women testing negative falsely are not provided with postpartum FMH tests and anti-D immunoglobulin. NIPT PP3 increases postpartum care costs because, although cord serology is avoided for those who test positive, this results in unnecessary use of FMH tests and anti-D immunoglobulin among women who test false positive (which includes those who test inconclusive but carry a RhD-negative baby). NIPT PP4 decreases postpartum care costs relative to current practice by avoiding cord serology for all women and is a combination of NIPT PP2 and NIPT PP3. As might be expected, the added cost of managing sensitisations and their associated health consequences is largest for the strategies with more sensitisations (NIPT PP2 and NIPT PP4) and is very small for strategies NIPT PP1 and NIPT PP3 (approximately £1700 per 100,000 pregnancies).

The assumption that the results of NIPT can be used to avoid all costs associated with the management of potentially sensitising events is favourable to NIPT and £626,000 represents the maximum cost saving in this regard. If this cost saving is reduced to £52,000, that is, if 92% of potentially sensitising events occur prior to the results of the NIPT being known, the ICER for no test and RAADP compared with NIPT PP1 would fall below £20,000 per QALY. The results of the audit indicate that 80% of potentially sensitising events occur after 20 weeks' gestation. This suggests that incorporating NIPT into routine antenatal care when it would be provided in week 20 or earlier (see *Figure 11* for schedule of appointments) could avoid upwards of 80% of the cost of managing potentially sensitising events.

We calculated the probability that each strategy would be cost-effective compared with no test and RAADP for each pair-wise comparison. NIPT PP1 and NIPT PP3 both have 99% probability of being cost-effective at a threshold of £20,000 per QALY. NIPT PP2 and NIPT PP4 have a lower probability of being cost-effective at £20,000 per QALY, at no higher than 73% compared with no test and RAADP.

Table 28 presents the fully incremental cost-effectiveness probabilistic results for high-throughput NIPT versus other strategies. Fully incremental results do not compare each NIPT strategy with current practice (i.e. no test and RAADP) but compare all NIPT scenarios simultaneously as competing alternative strategies. In this table, strategies are ranked by total costs and total QALYs, with the cheapest strategy coming first (NIPT PP2). Dominated strategies (those that have higher costs than more effective strategies) are in the bottom rows of the table. Incremental costs, incremental QALYs and, consequently, the ICER are incremental to the next cheapest, non-dominated strategy. This means that they represent the difference between the strategy in the current row compared with the strategy in the row above, as the table is ordered from least to most costly. The same applies to the incremental NHBs at £20,000 and £30,000 threshold values.

TABLE 28 Fully incremental cost-effectiveness outcomes associated with high-throughput NIPT vs. other strategies (base-case postpartum scenarios): probabilistic results

Strategies	Total costs (£)	Total QALYs	Incremental costs (£)	Incremental QALYs	ICER (£/QALY gained)	Population incremental NHBs (λ = £20,000)	Population incremental NHBs (λ = £30,000)
NIPT PP2	15,312,630	2,433,737	–	–	–	–	–
NIPT PP1	15,400,187	2,433,756	87,557	18.67	4690	14	16
No test and RAADP	15,983,725	2,433,756	583,538	0.46	1,269,050	–29	–19
NIPT PP4	15,410,610	2,433,737	–	–	Dominated	–	–
NIPT PP3	15,498,942	2,433,756	–	–	Dominated	–	–

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In NIPT PP2 cord serology is used to identify false-positive results, thereby avoiding unnecessary FMH testing and anti-D immunoglobulin in these women, but this is withheld in women for whom NIPT indicates a RhD-negative fetus. Using the negative results of high-throughput NIPT to rule out postpartum cord serology, FMH and anti-D immunoglobulin (NIPT PP2 and NIPT PP4) has lower QALYs than no test and RAADP, NIPT PP1 and NIPT PP3. Although there are further cost savings from avoiding postpartum cord serology and anti-D immunoglobulin, the majority of sensitisations occur and can be prevented by the administration of anti-D immunoglobulin at delivery. NIPT PP2 is the cheapest strategy and provides the same QALYs as NIPT PP4. Hence, NIPT PP4 is dominated by NIPT PP2.

Providing cord serology to all women, as with NIPT PP1, will identify both the false-positive results (the small number of false positives and the proportion of women with inconclusive results who are carrying RhD-negative babies) and false-negative results. Although NIPT PP1 has higher costs than NIPT PP2 because of the additional cord serology tests, these are offset somewhat by cost savings from avoiding sensitisations in false negatives. Compared with NIPT PP2, NIPT PP1 is estimated to provide approximately 19 additional QALYs per 100,000 pregnancies, at approximately £88,000 in additional costs, corresponding to an ICER of around £5000 per QALY gained.

In NIPT PP3 cord serology is used to identify false-negative results but this is withheld in women with inconclusive results or for whom NIPT indicates a RhD-positive fetus (in favour of FMH testing and anti-D immunoglobulin). Compared with NIPT PP1, the QALY gain is not affected as the model assumes no adverse health benefits from unnecessary use of anti-D immunoglobulin. As NIPT PP3 is more costly than NIPT PP1, in the base case it is dominated by NIPT PP1.

No test and RAADP is more costly than NIPT PP1 and is the most effective strategy. The administration of RAADP and supplementary anti-D immunoglobulin for potentially sensitising events among the false negatives leads to an additional 0.5 QALYs per 100,000 pregnancies compared with NIPT PP1, at an additional cost of £584,000. This means that the ICER for no test and RAADP compared with NIPT PP1 is £1,270,000. Using high-throughput NIPT and performing cord serology irrespective of the result (NIPT PP1) has higher NHB than any other strategy.

The decision uncertainty can be shown graphically with a cost-effectiveness acceptability curve. *Figure 14* shows the cost-effectiveness acceptability curves for the different scenarios being compared (i.e. no test and RAADP and alternative high-throughput NIPT scenarios – PP1 to PP4) in which we can depict the probability that each strategy is cost-effective for a range of threshold values. When all strategies are

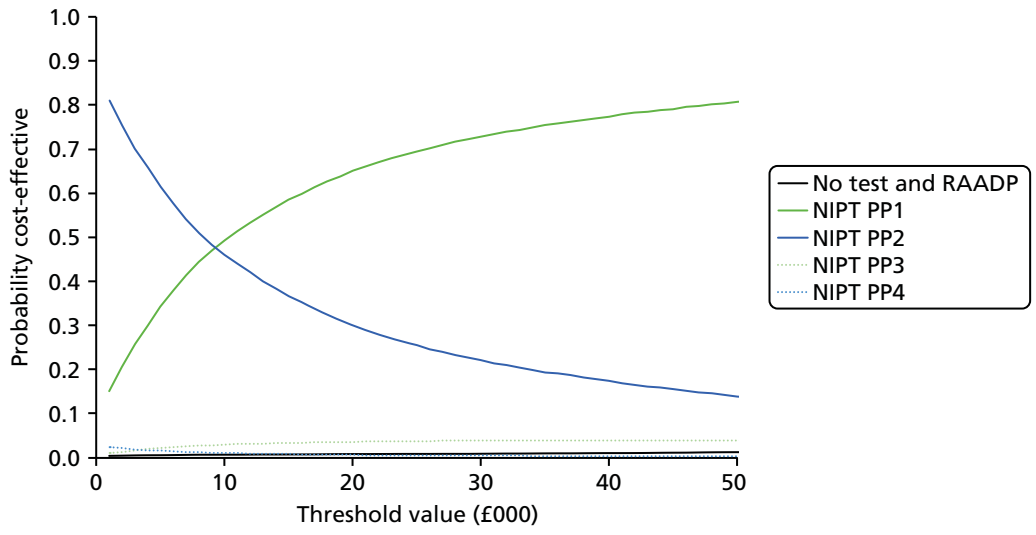

FIGURE 14 Cost-effectiveness acceptability curves of current practice (no test and RAADP) and alternative NIPT scenarios (PP1 to PP4).

simultaneously compared, for threshold values of £20,000 and £30,000, the highest probability of being cost-effective is obtained by NIPT PP1 with 0.65 and 0.73, respectively. For the same threshold values, the probability of NIPT PP2 being cost-effective is 0.30 and 0.22, respectively. NIPT PP1 is the alternative with the highest probability of being cost-effective and also the expected cost-effective alternative for thresholds above £10,000. An estimate of the maximum value of further research, the expected value of perfect information, is estimated to be approximately £203,000 considering 10 years of cohorts of 100,000 pregnancies and using a cost-effectiveness threshold of £20,000 per QALY. If research to reduce uncertainty in the model values would cost > £203,000 this suggests that it would not represent a good investment.

Sensitivity analyses results

Several SAs were carried out to assess the sensitivity of the base-case cost per QALY findings, as detailed in *Table 24*. We assessed the impact of using pooled evidence from all relevant NIPT accuracy evidence rather than UK Bristol studies only and, by using recent evidence from a UK study,[12] assessed the performance of high-throughput NIPT at different gestation periods. An analysis of the NIPT inconclusive results was also performed by replacing the pooled estimates for the sensitivity and specificity with the individual study results. SA was performed on the effectiveness of RAADP by using a different sensitisation rate pooled from a larger number of studies. An assessment was also carried out for the uptake rates for RAADP and postpartum anti-D immunoglobulin, with and without NIPT, decreasing these to the circumstances when the correct dose at the correct time was administered according to recent evidence.[8] In addition, we analysed the impact of altering the cost of the diagnostic test and the cost of treatment, two key components of this assessment as highlighted in the relevant literature. Finally, we have evaluated the impact of reducing the cost of the FMH test and, under an alternative postpartum scenario, assessed the management of high-throughput inconclusive results separately to the positive test results. The following sections look closely at each of these analyses and provide interpretations of obtained results relative to the base-case findings.

Sensitivity analysis 1: sensitivity analysis over the non-invasive prenatal testing accuracy using all relevant evidence

Table 29 shows the results when diagnostic accuracy for high-throughput NIPT accuracy is based on all available studies as opposed to UK (Bristol) studies only. This increases the pooled specificity by 2%, although the pooled sensitivity levels are reduced by only 0.2% (see *Chapter 3*, *Results: assessment of diagnostic accuracy*). Compared with the base case, the 2% reduction in false-positive results allows for more avoidance of anti-D immunoglobulin and associated tests, reducing total costs across all NIPT strategies by between £20,000 and £150,000 per 100,000 pregnancies. Total QALYs are marginally affected by the small 0.2% increase in false negatives, with NIPT PP2 and NIPT PP4 being the most affected, as these assume no use of cord serology post partum for women with negative results. Compared with the base case, this results in a further loss of approximately 12 QALYs per 100,000 pregnancies. Compared with no test and RAADP, NIPT PP2 and NIPT PP3 are still found to be cost saving (approximately £630,000–690,000 per 100,000 pregnancies) but NIPT PP3 is associated with a loss of approximately 1 QALY per 100,000 pregnancies compared with a loss of 31 with NIPT PP2. NIPT PP1 and NIPT PP3 are the only strategies to offer increased NHBs compared with no test and RAADP, with ICERs for no test and RAADP of approximately £830,000.

Sensitivity analysis 2: sensitivity analysis over the non-invasive prenatal testing accuracy at different timings using Chitty *et al.*[12]

Table 30 presents the results of providing high-throughput NIPT at different gestation periods. These are based on the analysis by Chitty *et al.*[12] (see *Chapter 3*, *Results: assessment of diagnostic accuracy*), with the sensitivity and specificity repeated for information. In this analysis, only the diagnostic accuracy is varied from the base-case values of 0.998 for sensitivity and 0.942 for specificity, which impacts on the probability of sensitisation. The sensitivity estimate is least favourable at 14–17 weeks' gestation and the specificity estimate is least favourable at 18–23 weeks' gestation, although these differences may be a result of random chance rather than systematic variation between these time points. Although this analysis does not directly take into consideration the impact of the test timing on the potential to avoid costs

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TABLE 29 Incremental cost-effectiveness outcomes associated with high-throughput NIPT vs. other strategies: probabilistic results – all NIPT accuracy evidence

Strategies	Total costs (£)	Total QALYs	Incremental costs (£)	Incremental QALYs	ICER (£/QALY gained)	Population NHB (λ = £20,000)	Population NHB (λ = £30,000)
Current clinical practice							
No test and RAADP	15,983,725	2,433,756	–	–	–	2,432,957	2,433,223
NIPT PP1							
Test and RAADP (T+ only) vs. no test and RAADP	15,353,678	2,433,756	–630,047	–0.76	829,196	2,432,988	2,433,244
NIPT PP2							
Test and RAADP (T+ only) vs. no test and RAADP	15,291,035	2,433,725	–692,690	–31.13	22,253	2,432,961	2,433,215
NIPT PP3							
Test and RAADP (T+ only) vs. no test and RAADP	15,351,238	2,433,756	–632,487	–0.76	832,406	2,432,988	2,433,244
NIPT PP4							
Test and RAADP (T+ only) vs. no test and RAADP	15,286,779	2,433,725	–696,946	–31.13	22,390	2,432,961	2,433,216

associated with the management of a potentially sensitising events, we estimate the threshold amount of these costs that would have to occur prior to NIPT in order for the ICER to cross the threshold of £20,000 per QALY gained. Thus, results are shown only for the best NIPT strategy within each period.

As for the base case, the introduction of high-throughput NIPT results in lower health benefits than no test and RAADP. This happens irrespective of the timing at which the test is carried out. The QALY loss is slightly greater when performing NIPT at 14–17 weeks' gestation because of the very small drop in sensitivity of 0.002, leading to more false negatives and a loss of approximately 1 QALY per 100,000 pregnancies compared with current practice, rather than a loss of approximately 0.4 QALYs if NIPT is provided at 11–13 weeks' or 18–23 weeks' gestation. The cost saving is greatest at 14–17 weeks because of the increase in specificity, as fewer false-positive results result in less unnecessary treatment.

The base-case results suggest that NIPT PP1 provides savings of £626,000 from avoiding the costs of managing potentially sensitising events. The audit[8] indicates that 80% of potentially sensitising events occur after week 20. If NIPT PP1 is provided between 18 and 23 weeks' gestation and £547,000 or 87% of the cost of managing potentially sensitising events occurs prior to the test, the ICER for no test and RAADP would fall below £20,000 per QALY gained. If NIPT PP3 is provided between 11 and 13 weeks' or 14 and 17 weeks' gestation, then approximately £598,000 or 95% of the cost of managing potentially sensitising events would have to occur prior to the test in order for the ICER for no test and RAADP to fall below £20,000 per QALY gained.

Sensitivity analysis 3: sensitivity analysis on the effectiveness of routine antenatal anti-D prophylaxis using Turner *et al.*

Findings from Turner *et al.*[63] estimated a pooled odds ratio estimate for sensitisation under RAADP (vs. no RAADP, only postpartum anti-D immunoglobulin) of 0.31 rather than 0.37 as in NICE TA156[62] (*Table 31*).

TABLE 30 Incremental cost-effectiveness outcomes associated with high-throughput NIPT at different timings vs. other strategies (postpartum scenarios): probabilistic results, based on Chitty *et al.*[12]

Strategies	Sensitivity	Specificity	Total costs (£)	Total QALYs	Incremental costs (£)	Incremental QALYs	ICER (£/QALY gained)	Population NHB (λ = £20,000)	Population NHB (λ = £30,000)
Current clinical practice – irrespective of NIPT timing									
No test and RAADP	–	–	15,983,725	2,433,756	–	–	–	2,432,957	2,433,223
Best postpartum scenario when NIPT performed at 11–13 weeks' gestation									
NIPT PP1 (vs. no test and RAADP)	0.9983	0.9525	15,378,009	2,433,756	–605,716	–0.39	1,536,731	2,432,987	2,433,243
Best postpartum scenario when NIPT performed at 14–17 weeks' gestation									
NIPT PP1 (vs. no test and RAADP)	0.9967	0.9534	15,370,718	2,433,756	–613,007	–0.77	797,046	2,432,987	2,433,243
Best postpartum scenario when NIPT performed at 18–23 weeks' gestation									
NIPT PP1 (vs. no test and RAADP)	0.9982	0.9304	15,429,067	2,433,756	–554,658	–0.36	1,529,418	2,432,984	2,433,242

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TABLE 31 Incremental cost-effectiveness outcomes associated with high-throughput NIPT vs. other strategies (postpartum scenarios): probabilistic results – pooled RAADP effectiveness, based on Turner *et al.*[63]

Strategies	Total costs (£)	Total QALYs	Incremental costs (£)	Incremental QALYs	ICER (£/QALY gained)	Population NHB (λ = £20,000)	Population NHB (λ = £30,000)
Current clinical practice							
No test and RAADP	15,923,756	2,433,774	–	–	–	2,432,978	2,433,243
NIPT PP1							
Test and RAADP (T+ only) vs. no test and RAADP	15,339,945	2,433,773	–583,811	–0.50	1,164,285	2,433,006	2,433,262
NIPT PP2							
Test and RAADP (T+ only) vs. no test and RAADP	15,252,388	2,433,755	–671,369	–19.17	35,018	2,432,992	2,433,246
NIPT PP3							
Test and RAADP (T+ only) vs. no test and RAADP	15,438,716	2,433,773	–485,040	–0.50	967,307	2,433,001	2,433,259
NIPT PP4							
Test and RAADP (T+ only) vs. no test and RAADP	15,350,384	2,433,755	–573,372	–19.17	29,906	2,432,987	2,433,243

Compared with base-case results (see *Table 25*) the marginal reduction in the sensitisation rate (0.05% less) brings minimal changes to the total costs and QALYs estimates, as expected. The increase in effectiveness of RAADP provides reductions in total costs for all strategies and minor changes in the QALY loss associated with NIPT.

Sensitivity analysis 4: sensitivity analysis on the uptake of routine antenatal anti-D prophylaxis and postpartum anti-D immunoglobulin

In the base-case analysis our estimates of compliance are based on the use of anti-D immunoglobulin in women who are eligible in terms of RhD status and ignorance of the father's status, and who remain pregnant, to receive RAADP. The National Comparative Audit of Blood Transfusion 2013 on Anti-D Immunoglobulin Prophylaxis[8] reported that, out of all RhD-negative women, 87.5% received the correct dose at the correct time of RAADP. Furthermore, it reported that 91.6% received the correct dose at the correct time of postpartum anti-D immunoglobulin prophylaxis. We made use of these estimates to provide a lower bound for compliance with anti-D immunoglobulin. As for the base case, it was assumed that the use of high-throughput NIPT does not influence the uptake with anti-D immunoglobulin, that is, the uptake rate is the same irrespective of whether NIPT was previously accepted/administered.

Table 32 presents the incremental cost-effectiveness outcomes for each alternative scenario when different RAADP and postpartum anti-D immunoglobulin uptake rates are used. As the SA does not impact on the rank order of the alternative postpartum scenarios, the results are shown for NIPT PP1 only, that is, out of the five alternatives being compared, the results for the best strategy are shown together with current practice. Base-case results correspond to 99.0% and 98.4% uptake with RAADP and postpartum anti-D immunoglobulin, respectively. Overall, the results are robust to reduced compliance and there is little impact on incremental comparison between NIPT PP1 and no test and RAADP. The cost for all strategies is increased if compliance with a cost-effective treatment, such as RAADP, is reduced, although the QALY loss associated with additional sensitisations is slightly reduced.

TABLE 32 Incremental cost-effectiveness outcomes associated with high-throughput NIPT vs. other strategies (postpartum scenarios): different anti-D immunoglobulin uptake rates of RAADP and postpartum anti-D immunoglobulin – probabilistic results of the two best strategies for each analysis are shown

Strategies	Total costs (£)	Total QALYs	Incremental costs (£)	Incremental QALYs	ICER (£/QALY gained)	Population NHB (λ = £20,000)	Population NHB (λ = £30,000)
RAADP at 99.0% and post partum at 98.4% (base case)							
No test and RAADP	15,983,725	2,433,756	–	–	–	2,432,957	2,433,223
NIPT PP1 (vs. no test and RAADP)	15,400,187	2,433,756	–583,538	–0.46	1,269,050	2,432,986	2,433,242
RAADP at 87.5% and post partum at 98.4%							
No test and RAADP	16,060,984	2,433,733	–	–	–	2,432,930	2,433,198
NIPT PP1 (vs. no test and RAADP)	15,477,810	2,433,733	–583,174	–0.41	1,430,198	2,432,959	2,433,217
RAADP at 99.0% and post partum at 91.6%							
No test and RAADP	16,029,705	2,433,743	–	–	–	2,432,941	2,433,208
NIPT PP1 (vs. no test and RAADP)	15,446,384	2,433,742	–583,321	–0.43	1,360,214	2,432,970	2,433,227
RAADP at 87.5% and post partum at 91.6%							
No test and RAADP	16,101,601	2,433,721	–	–	–	2,432,916	2,433,185
NIPT PP1 (vs. no test and RAADP)	15,518,619	2,433,721	–582,982	–0.38	1,532,578	2,432,945	2,433,204

Sensitivity analysis 5: sensitivity analysis on non-invasive prenatal testing inconclusive results

The cost saving achievable by using the high-throughput NIPT to guide anti-D immunoglobulin will depend on the rate of inconclusive test results, as for these women the current care pathway is unchanged. That is, all inconclusive results are managed as if they were test positive and, hence, unnecessary anti-D immunoglobulin continues to be provided in these women carrying a RhD-negative fetus. In order to undertake a SA around the rate of inconclusives, we replaced the pooled estimates for the sensitivity and specificity with the individual study results. *Figure 15* shows how the specificity varies with the rate of inconclusives within each study. In general, a higher rate of inconclusive results will lead to a larger number of false positives and, correspondingly, a lower specificity. The cost saving achievable by using high-throughput NIPT to guide anti-D immunoglobulin will depend on the rate of inconclusive test results, as for these women the current care pathway is unchanged, that is, all inconclusive results are managed as if they were test positive, and, hence, unnecessary antenatal anti-D immunoglobulin continues to be provided in those women carrying a RhD-negative fetus.

One study produced no inconclusive results and no false-negative results and so we omitted this from the SA.[22] In general, the NHBs associated with the NIPT strategies fall as the rate of inconclusive results increases, but at no point do the NHBs from NIPT PP1 or NIPT PP3 fall below those offered with no test and RAADP. *Figure 16* shows the NHBs for all of the NIPT strategies. When the rate of inconclusive results is low, NIPT PP3 offers the highest NHB. This is because the amount of unnecessary postpartum FMH testing and anti-D immunoglobulin is reduced when the number of false-positive results falls. When the rate of inconclusives is high, NIPT PP1 is preferred. If the rate of inconclusives was very high, no test and

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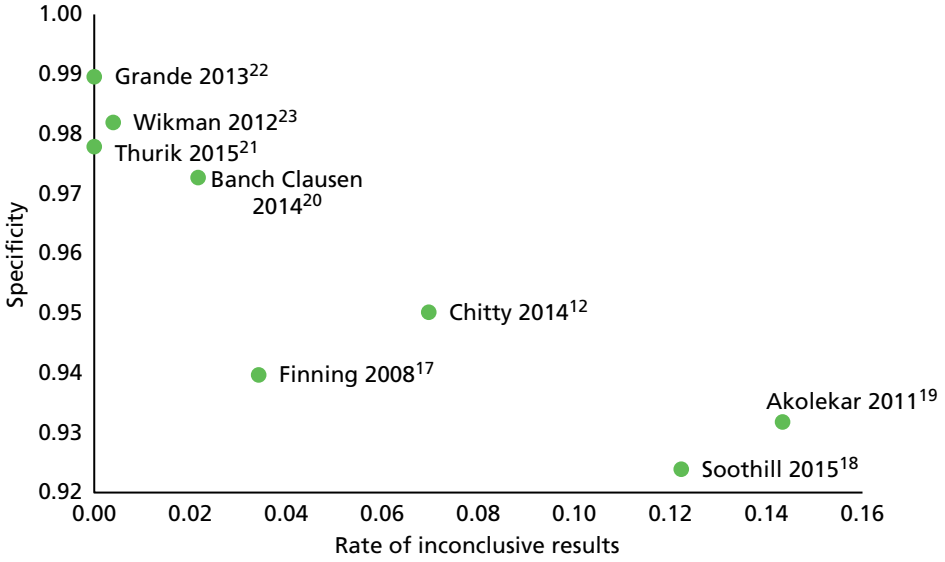

FIGURE 15 Specificity by rate of high-throughput NIPT inconclusive results per study.

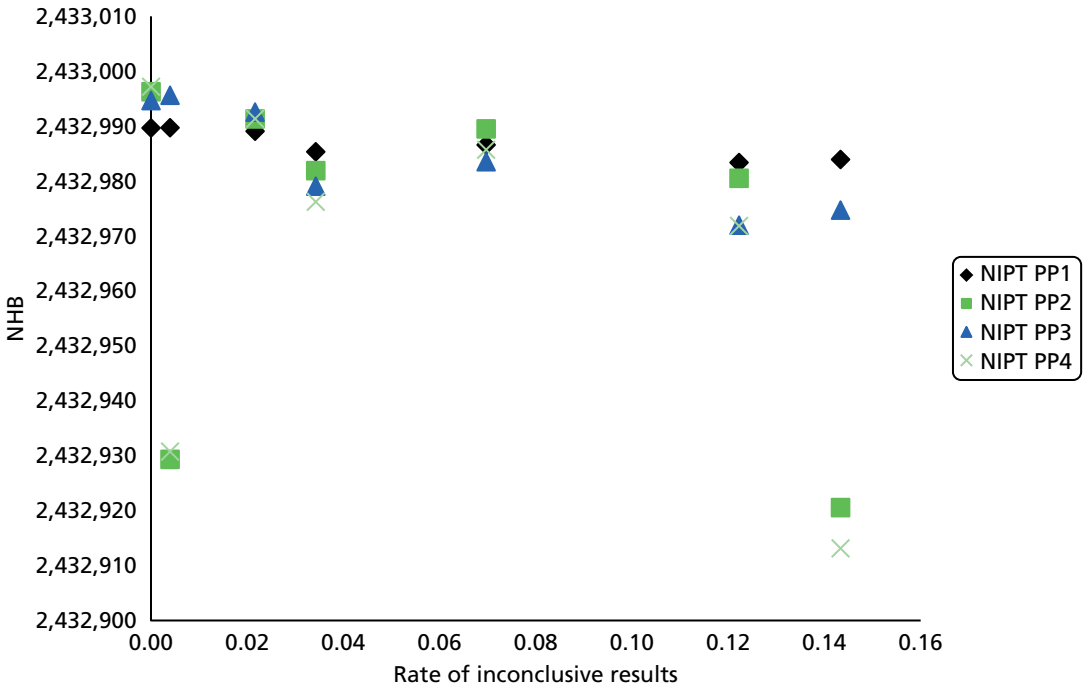

FIGURE 16 Population NHBs for all NIPT strategies by rate of NIPT inconclusive results per study.

RAADP would be preferred. However, the rate would have to be much higher than that observed in the set of studies underlying the evidence synthesis. Akolekar *et al.*[91] and Wikman *et al.*[23] diverge from the remaining studies in terms of the number of false-negative results and sensitivity and this impacts on the NHBs of the strategies that do not identify false negatives through cord serology (NIPT PP2 and NIPT PP4). For these two strategies, the NHB falls below that offered by current practice when the results associated with these two studies are used. *Figure 17* shows how the NHBs for NIPT PP1 vary only with the rate of inconclusives.

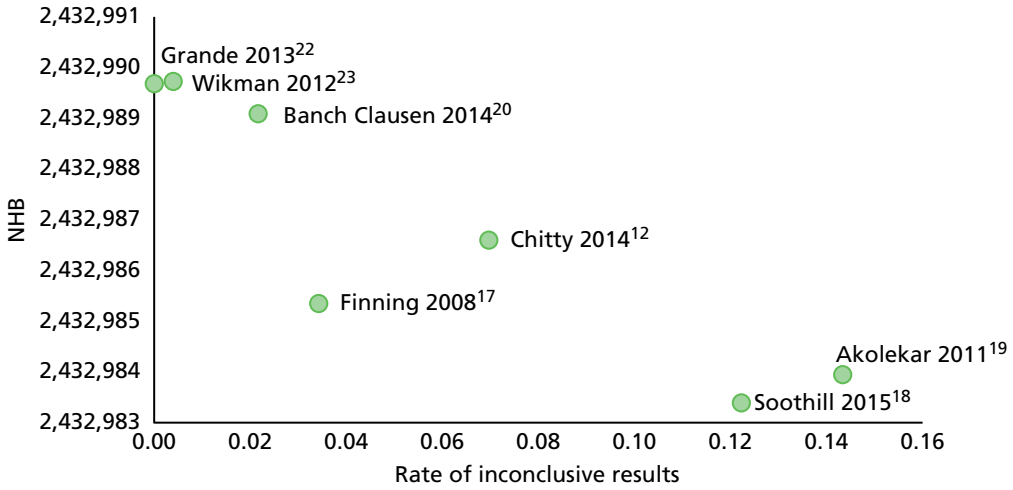

FIGURE 17 Population NHBs for NIPT PP1 by rate of NIPT inconclusive results per study.

Sensitivity analysis 6: sensitivity analysis on non-invasive prenatal testing and Anti-D costs

The unit cost of NIPT is subject to some uncertainty as it depends on throughput (the total number of samples per year) and the level of the royalty fee. The throughput determines how many machines must be bought and at what capacity they are utilised. The base-case analysis assumed sufficient machines to process all pregnancies in England in a given year. Further to this, the introduction of NIPT may impose additional costs in routine antenatal care in terms or appointments and staff time. Similarly, the cost of anti-D immunoglobulin may depart from the list price on the basis of negotiated discounts.

The results of a two-way analysis around these unit costs reported in *Figure 18* show that the base case is very sensitive to both the price of NIPT and the price of anti-D. The *x*-axis represents the range of anti-D immunoglobulin cost from –20% to +20%. This increase/decrease in the cost of anti-D immunoglobulin is applied to all occasions in which the treatment is administered and, thus, the RAADP cost shown is indicative only, as the estimated costs of anti-D for potentially sensitising events and post partum, as described in *Cost of postpartum health resources used*, are omitted. The *y*-axis represents the range of costs per high-throughput NIPT from £17.60 to £28.60 [which may, for example (confidential information has been removed)].

A price increase would raise the costs associated with all strategies that provide NIPT and does not affect the ranking of the strategies. The postpartum strategy that provides the lowest NHB will be associated with the lowest threshold cost, and the postpartum strategy that provides the highest NHB will be associated with the highest threshold cost.

The threshold cost for NIPT PP1, the strategy with the highest NHB, is £24.64 (confidential information has been removed). That is, raising the cost per high-throughput NIPT to £24.64 implies that NIPT PP1 no longer offers the highest population NHB, switching to no test and RAADP. Similar results were found when the cost-effectiveness threshold was £20,000 or £30,000. NIPT PP1 strategy is always preferred over other postpartum strategies (PP2, PP3 or PP4). At no point would the price of anti-D immunoglobulin be high enough to make the omission of postpartum anti-D immunoglobulin (NIPT PP2 and NIPT PP4) look cost-effective.

FIGURE 18 (Confidential information has been removed.)

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Sensitivity analysis 7: sensitivity analysis over the fetal–maternal haemorrhage test cost

Reducing the cost of the FMH test to £3.17 (Szczepura *et al.*,[68] updated to 2015 prices) halves the estimated total costs of all strategies compared with the total costs of the base-case scenarios (*Table 33*). Estimated total QALYs are similar to base-case findings. NIPT PP1 is now less cost saving than current practice. This is explained by the use of the FMH test in the management of potentially sensitising events. When the cost of the FMH test is reduced, the savings from avoiding the management of potentially sensitising events are reduced. All NIPT strategies still reduce costs compared with no test and RAADP but by a lesser amount. This causes the ICER for no test and RAADP compared with NIPT PP2 and NIPT PP4 to fall below £20,000 per QALY.

Sensitivity analysis 8: sensitivity analysis on postpartum management of inconclusive results

The postpartum scenarios specified in the decision problem applied cord serology, FMH testing and postpartum anti-D immunoglobulin according to whether or not the results of NIPT were positive or negative. In this regard, we grouped inconclusive results with NIPT positive results. However, in terms of postpartum management, it may be worthwhile to regard those with inconclusive results as distinct from those on whom NIPT indicates a RhD-positive fetus. This would allow cord serology to be provided to women with negative results in order to identify false negatives and cord serology to be provided to women with inconclusive results in order to identify false positives, but for it to be withheld in women in whom NIPT indicates a RhD-positive fetus. This would result in total costs of £15,230,372 and 2,433,756 QALYs per 100,000 pregnancies. This postpartum approach would dominate all other NIPT strategies, and the ICER for no test and RAADP compared with this strategy would be £1,638,356 per QALY gained.

Table 34 summarises the results of the base-case analysis and the key SAs.

TABLE 33 Incremental cost-effectiveness outcomes associated with high-throughput NIPT vs. other strategies (postpartum scenarios): probabilistic results – FMH test cost reduced

Strategies	Total costs (£)	Total QALYs	Incremental costs (£)	Incremental QALYs	ICER (£/QALY gained)	Population NHB (λ = £20,000)	Population NHB (λ = £30,000)
Current clinical practice							
No test and RAADP	8,132,447	2,433,756	–	–	–	2,433,350	2,433,485
NIPT PP1							
Test and RAADP (T+ only) vs. no test and RAADP	7,986,460	2,433,756	–145,987	–0.46	317,485	2,433,356	2,433,490
NIPT PP2							
Test and RAADP (T+ only) vs. no test and RAADP	7,915,559	2,433,737	–216,888	–19.13	11,339	2,433,341	2,433,473
NIPT PP3							
Test and RAADP (T+ only) vs. no test and RAADP	7,846,684	2,433,756	–285,763	–0.46	621,464	2,433,363	2,433,494
NIPT PP4							
Test and RAADP (T+ only) vs. no test and RAADP	7,775,584	2,433,737	–356,862	–19.13	18,658	2,433,348	2,433,478

TABLE 34 Summary of base-case analysis and key SA results

	Total		Compared with no test and RAADP (current practice)	Compared with next best strategy	
Analysis	Cost (£)	QALYs	ICER (£)	ICER (£)	Comparator
Base case					
No test and RAADP	15,983,725	2,433,756	–	1,269,050	NIPT PP1
NIPT PP1	15,400,187	2,433,756	1,269,050	4690	NIPT PP2
NIPT PP2	15,312,630	2,433,737	35,087	–	–
NIPT PP3	15,498,942	2,433,756	1,054,281	–	–
NIPT PP4	15,410,610	2,433,737	29,964	–	–
SA1: bivariate meta-analysis of all studies					
No test and RAADP	15,983,725	2,433,756	–	834,396	NIPT PP3
NIPT PP1	15,353,677	2,433,756	831,178	–	–
NIPT PP2	15,291,034	2,433,725	22,255	–	–
NIPT PP3	15,351,238	2,433,756	834,396	2123	NIPT PP4
NIPT PP4	15,286,779	2,433,725	22,391	–	–
SA2: high-throughput NIPT performance assessed at different gestation periods (Chitty* et al.*[12])					
11–13 weeks' gestation					
No test and RAADP	15,983,725	2,433,756	–	1,536,731	NIPT PP1
NIPT PP1	15,378,008	2,765,228	1,165,229	3190	NIPT PP4
NIPT PP2	15,283,278	2,765,206	31,462	–	–
NIPT PP3	15,420,079	2,765,228	1,084,295	–	–
NIPT PP4	15,325,344	2,765,206	29,573	–	–
14–17 weeks' gestation					
No test and RAADP	15,983,725	2,433,756	–	797,046	NIPT PP1
NIPT PP1	15,370,717	2,433,756	604,062	678	NIPT PP4
NIPT PP2	15,310,563	2,433,724	15,604	–	–
NIPT PP3	15,409,227	2,433,756	566,114	–	–
NIPT PP4	15,349,062	2,433,724	14,712	–	–
18–23 weeks' gestation					
No test and RAADP	15,983,725	2,433,756	–	1,529,418	NIPT PP1
NIPT PP1	15,429,066	2,433,756	1,162,227	6209	NIPT PP2
NIPT PP2	15,334,643	2,433,741	31,744	–	–
NIPT PP3	15,593,754	2,433,756	817,141	–	–
NIPT PP4	15,499,308	2,433,741	23,691	–	–
SA3: sensitisation rate (Turner* et al.*[63])					
No test and RAADP	15,923,756	2,433,774	–	1,164,285	NIPT PP1
NIPT PP1	15,339,945	2,433,773	1,164,285	4690	NIPT PP2
NIPT PP2	15,252,387	2,433,755	35,021	–	–
NIPT PP3	15,438,716	2,433,773	970,788	–	–
NIPT PP4	15,350,383	2,433,755	29,909	–	–

continued

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TABLE 34 Summary of base-case analysis and key SA results (*continued*)

Analysis	Total		Compared with no test and RAADP (current practice)	Compared with next best strategy	
	Cost (£)	QALYs	ICER (£)	ICER (£)	Comparator
SA4: uptake with RAADP (with and without high-throughput NIPT performed)					
Uptake of RAADP at 87.5%					
No test and RAADP	16,060,984	2,433,733	–	1,430,198	NIPT PP1
NIPT PP1	15,477,810	2,433,733	1,430,198	4691	NIPT PP2
NIPT PP2	15,390,257	2,433,714	35,171	–	–
NIPT PP3	15,576,545	2,433,733	1,188,057	–	–
NIPT PP4	15,488,218	2,433,714	30,035	–	–
Uptake of postpartum anti-D immunoglobulin at 91.6%					
No test and RAADP	16,029,705	2,433,743	–	1,360,214	NIPT PP1
NIPT PP1	15,446,384	2,433,742	1,360,214	4691	NIPT PP2
NIPT PP2	15,358,829	2,433,724	35,137	–	–
NIPT PP3	15,545,127	2,433,742	1,129,960	–	–
NIPT PP4	15,456,798	2,433,724	30,006	–	–
Uptake of RAADP at 87.5% and postpartum anti-D immunoglobulin at 91.6%					
No test and RAADP	16,101,601	2,433,721	–	1,532,578	NIPT PP1
NIPT PP1	15,518,619	2,433,721	1,532,578	4692	NIPT PP2
NIPT PP2	15,431,068	2,433,702	35,216	–	–
NIPT PP3	15,617,343	2,433,721	1,273,046	–	–
NIPT PP4	15,529,017	2,433,702	30,072	–	–
SA5: high-throughput NIPT inconclusive results rate					
Please see *Sensitivity analysis 5: sensitivity analysis on non-invasive prenatal testing inconclusive results*					
SA6: cost of high-throughput NIPT and anti-D immunoglobulin					
Please see *Sensitivity analysis 6: sensitivity analysis on non-invasive prenatal testing and Anti-D costs*					
SA7: cost of FMH test					
No test and RAADP	8,132,446	2,433,756	–	621,464	NIPT PP3
NIPT PP1	7,986,460	2,433,756	317,485	–	–
NIPT PP2	7,915,559	2,433,737	11,340	–	–
NIPT PP3	7,846,683	2,433,756	621,464	3809	NIPT PP4
NIPT PP4	7,775,584	2,433,737	18,658	–	–
SA8: postpartum management of high-throughput NIPT inconclusive results					
Please see *Sensitivity analysis 8: sensitivity analysis on postpartum management of inconclusive results*					

Discussion of the independent economic assessment

The evidence to support the diagnostic accuracy of NIPT is of good quality. We can combine this with established evidence for the efficacy of RAADP and postpartum anti-D immunoglobulin in order to estimate the impact of introducing NIPT on the number of sensitisations. However, there is little evidence as to the impact of sensitisations in terms of their long-term health and cost consequences. Our model suggests that each additional sensitisation costs the NHS £3167 and is associated with a loss of approximately 0.9 QALYs, but these estimates are subject to uncertainty and incorporate expert opinion.

There is uncertainty regarding the cost of introducing high-throughput NIPT. The unit cost will vary with throughput and may be subject to an additional royalty fee. Unless NIPT can be incorporated seamlessly into routine antenatal care, it may result in additional costs for blood draw, transportation of samples and antenatal care visits to administer the test and deliver counselling and results. We conducted extensive SAs to address this uncertainty and to identify the threshold cost per NIPT. The cost of high-throughput NIPT has to increase by only (confidential information has been removed) above that modelled in the base case in order for no test and RAADP to be the preferred strategy. The unit cost of high-throughput NIPT to the NHS is the most important parameter in determining cost-effectiveness. Although there is uncertainty as regards the timing of the test, our analysis suggests that this is not influential in determining the cost-effectiveness results either in terms of diagnostic accuracy or in terms of the extent of management costs for potentially sensitising events that can be avoided.

As might be expected, the potential NHBs of using NIPT to target care are reduced, as the rate of inconclusive results is increased. However, our SA indicates that, even with high-throughput NIPT inconclusive results as high as 14.3%, the introduction of NIPT compares favourably to current practice. The ability of the NIPT result to avoid unnecessary use of anti-D immunoglobulin varies systematically according to ethnicity. Although this may not be an equality issue, it should be noted that following the introduction of NIPT, any unnecessary use of anti-D immunoglobulin will be proportionately higher in ethnic groups, for example in those of African origin. We can conclude that the identification of the false-positive results is key to the estimation of the cost-effectiveness outcomes, negatively impacting the results if this rate is higher and altering the postpartum strategy that would offer the highest NHB.

There are numerous ways in which the results of high-throughput NIPT could be used to guide postpartum testing and administration of anti-D immunoglobulin. We have compared four alternative postpartum scenarios, and the results indicate that cord serology testing should be retained in women for whom NIPT indicates a RhD-negative fetus. This use of cord serology to capture false-negative results has the potential to undermine the implementation of the test if it impacts on the confidence in the NIPT results. A postpartum strategy that distinguishes between inconclusive results and positive results offers the greatest cost savings.

If the cost of the FMH test is high relative to cord serology, then it would make sense to apply cord serology to women with positive and inconclusive NIPT results. This allows for the low-cost cord serology test to avoid both the unnecessary use of a much more expensive FMH test and unnecessary postpartum anti-D immunoglobulin. It is likely that these benefits are almost entirely obtained by applying cord serology in women with inconclusive results, as 30–40% of these women would be revealed to be carrying a RhD-negative fetus. In contrast, when the results of NIPT indicate a RhD-positive fetus, the rate of false positives is very low. In the base-case analysis, women who receive inconclusive results are managed as if they test positive, but there may be potential for further cost savings if these are treated as a distinct group in terms of postpartum care. This would allow for a postpartum scenario in which cord serology was applied to women who test negative and to those who test inconclusive but for whom FMH tests and anti-D immunoglobulin are provided without cord serology in women who test positive.

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Conclusions of the cost-effectiveness section

The use of high-throughput NIPT to guide the provision of anti-D immunoglobulin prophylaxis is estimated to be cost saving compared with current practice of providing RAADP to all women who are RhD-negative. The extent of the cost saving is highly sensitive to the cost of NIPT itself to the NHS, which comprises the base unit cost per test, the level of any royalty fee and any increase in antenatal care costs required to accommodate an additional test. In the base-case analysis, the extent of the cost saving is sufficient to outweigh the small increase in sensitisations and the associated small QALY loss through using NIPT. However, even a small increase in the cost imposed on the NHS of (confidential information has been removed) or more per test would cause the ICER for no test and RAADP to reduce below £20,000 per QALY.

Chapter 6 Discussion

Statement of principal findings

Diagnostic accuracy

Eight studies were included in the diagnostic review of high-throughput NIPT. There were three studies based at Bristol (UK). The majority of included studies were judged as having a low risk of bias.

Meta-analyses showed very high diagnostic accuracy of high-throughput NIPT. In the primary analyses, for which women with inconclusive test results were treated as being testing positive, the summary FNR (i.e. women at risk of sensitisation) was 0.34% (95% CI 0.15% to 0.76%) and the FPR (i.e. women needlessly receiving anti-D) was 3.86% (95% CI 2.54% to 5.82%). SAs did not materially alter the overall result.

A subgroup analysis of three high-quality studies based at Bristol (UK) showed a slightly lower FNR of 0.21% (95% CI 0.09% to 0.48%) and a higher FPR of 5.73% (95% CI 4.58% to 7.16%). This suggests that the Bristol NIPT approach may be using a different threshold for the detection algorithm that further reduces false-negative error rates, consequently increasing the false-positive error rate. The FPR found was mostly as a result of treating the roughly 7% of women (in the UK) who have an inconclusive test result as if they had a positive test. Excluding these women from analysis resulted in a lower FPR of 1.26% (95% CI 0.87% to 1.83%). We were unable to conduct the subgroup analysis based on ethnicity because of lack of relevant data from included studies.

The diagnostic accuracy performance of high-throughput NIPT varied by gestational age. The data suggest that high-throughput NIPT is insufficiently accurate before around 11 weeks' gestation (i.e. in first trimester) but is consistently accurate at any time after 11 weeks' gestation. This may be because of a low concentration of cell-free fetal DNA in early pregnancy[92] but an increased concentration of cell-free fetal DNA after the end of the first trimester.[93]

Clinical effectiveness

Seven studies[18,20,22,24–27] were included in the clinical effectiveness review. Only two studies[20,26] had a control group, but both studies were judged as having a high risk of bias. One large prospective cohort study[26] reported that use of high-throughput NIPT for targeted antenatal anti-D prophylaxis was associated with a significant risk reduction in sensitisation (adjusted odds ratio 0.41, 95% CI 0.22 to 0.87) compared with historical controls (routine management, postpartum anti-D only).

Three non-comparative studies[18,20,22] reported outcome measures relating to anti-D doses administrated. All studies found that the use of NIPT reduced the total use of anti-D immunoglobulin doses (decreasing by 29% in one UK study by Soothill *et al.*[18]) because around 35% of RhD-negative women avoided unnecessary anti-D administration.

Four studies[20,26,27,49] reported moderate to high compliance with antenatal anti-D immunoglobulin administration. The compliance with antenatal anti-D administration after a positive NIPT result ranged from 86% to 96.1% (four studies[20,26,27,49]). High-throughput NIPT uptake rates ranged from 70% to > 95% (seven studies.[12,18,20,22,25–27]).

The results from the simulation study suggested that the use of NIPT to determine antenatal anti-D use would substantially reduce the number of women receiving anti-D unnecessarily from 38.9% to 5.7%. Results were sensitive to the rate of compliance. NIPT use could increase sensitisation rates by up to 15 sensitisations per 100,000 women if postpartum cord blood testing is continued or up to 28 per 100,000 women if cord blood testing is withdrawn and postpartum anti-D given on the basis of the NIPT result.

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Sensitisation rates are minimised by ensuring that women who do not receive NIPT are still offered, and receive, antenatal anti-D. The results suggest that NIPT results (if available and conclusive) could potentially be used in place of cord blood testing for administration of postpartum anti-D, if the small increase in sensitisations rates can be considered ethically acceptable.

Implementation

Twelve studies[13,17,18,20–28] were included in the review of implementation. Most of the included studies were large cohort studies[13,17,20,21,23–27] reporting implementation data along with diagnostic accuracy data, although one study was a survey that was based in the UK (London).[28] All the large cohort studies reported high diagnostic accuracy of high-throughput NIPT and suggested that high-throughput RhD genotyping of fetuses in all RhD-negative women was feasible and should be recommended. A number of studies reported potential issues of implementation such as those relating to programme anti-D prophylaxis compliance.[20,27] Some studies highlighted the importance of short transport times of samples and the need for effective management of transporting samples.[13,17,24] Some studies also identified the need for greater knowledge of NIPT among physicians, midwives and pregnant women.[27,28]

Cost-effectiveness

Seven cost-effectiveness studies[58,68–73] were included in the review. Conflicting results were identified across the existing economic studies, with three of the studies[68,71,72] reporting that NIPT fetal RhD genotyping did not appear to be cost-effective. The unit cost of the test was consistently identified as a key driver of the cost-effectiveness results and the potential for the use of NIPT to result in overall cost savings. Only one of the studies[68] was undertaken in a UK context, but this study did not explicitly explore how the introduction of NIPT could impact on costs relating to potentially sensitising events. For the studies undertaken outside the UK, differences in health-care systems and in implementation of anti-D immunoglobulin policies limit their relevance to UK practice. In conclusion, none of the existing studies was considered to be sufficiently generalisable to inform the specific decision problem as set out in the NICE scope for the current assessment.

A de novo independent economic model was developed to assess the cost-effectiveness of high-throughput NIPT to identify fetal rhesus D status in women who are RhD negative and not known to be sensitised to the RhD antigen. The model was made up of two main elements: (1) an identification part reflecting the diagnostic performance and costs of the alternative identification strategies and (2) a treatment part that evaluated the subsequent costs and outcomes (expressed in QALYs) of alternative care pathways. Four alternative ways in which the use of high-throughput NIPT may impact on the existing postpartum care pathway were evaluated (cord serology, FMH testing and postpartum anti-D immunoglobulin). These included scenarios in which the result of NIPT was used to guide RAADP only (with all women continuing to receive cord serology with FMH testing and postpartum anti-D immunoglobulin as required, irrespective of NIPT result) and scenarios for which the NIPT result guided both RAADP and separate aspects of postpartum care. A series of additional sensitivity and scenario analyses was also performed.

Our de novo economic model indicated that the use of high-throughput NIPT to guide the prenatal and postpartum provision of anti-D immunoglobulin prophylaxis is estimated to be cost saving compared with the current practice of providing RAADP to all women who are RhD negative. The magnitude of the cost saving appears to be highly sensitive to the cost of NIPT itself to the NHS, which comprises the base unit cost per test, the level of any royalty fee and any increase in antenatal care costs required to accommodate an additional test. In the base-case analysis, the extent of the cost saving appears sufficient to outweigh the small increase in sensitisations and the associated small QALY loss through using NIPT compared with current practice. However, even a small increase in the cost imposed on the NHS of (confidential information has been removed) or more per test would alter these conclusions.

In the base-case analysis, all four separate postpartum scenarios were estimated to be cost saving but also less effective than current practice. Based on a cross-section of 100,000 pregnancies, the magnitude of cost savings varied between approximately £485,000 and £671,000. The magnitude of the QALY loss varied between 0.5 QALYs and 19.1 QALYs (per 100,000 pregnancies). Although the magnitude of the

cost savings was sufficient to outweigh the associated QALY loss when each postpartum scenario was separately compared with current practice, these four separate scenarios potentially represent separate and distinct testing and management strategies that should be directly compared. In the base-case analysis, the strategy in which the NIPT result is used to guide RAADP only (i.e. all women continuing to receive cord serology with FMH testing and postpartum anti-D immunoglobulin) was associated with the highest NHB and had the highest probability of being cost-effective for threshold values of £20,000 and £30,000 per QALY (probability of 0.65 and 0.73, respectively). However, the use of cord serology to capture false-negative results has the potential to undermine the implementation of the test if it impacts on the confidence in the NIPT results. The most efficient postpartum strategy was also shown to vary across several of the main SAs.

A postpartum strategy that distinguishes between inconclusive results and positive results offers the greatest cost savings. In the base-case analysis, women who receive inconclusive results were assumed to be managed as if they test positive, but there may be potential for further cost savings if these are treated as a distinct group in terms of postpartum care. This could allow for a postpartum scenario in which cord serology was applied to women who test negative and who test inconclusive but in which FMH tests and anti-D immunoglobulin are provided without cord serology in women who test positive.

Strengths and limitations of the assessment

Clinical effectiveness

Extensive literature searches were conducted in an attempt to maximise retrieval of potentially relevant studies. These included electronic searches of a variety of bibliographic databases as well as screening of clinical trial registers and conference proceedings to identify unpublished studies. The search strategy did not restrict by study design. The review process followed recommended methods to minimise the potential for error and/or bias. The quality of the included studies was assessed and accounted for when interpreting the review results. Appropriate synthesis methods were employed by taking into account the heterogeneity of study characteristics.

There was some evidence of inconsistency in the meta-analysis of diagnostic accuracy studies. The observed heterogeneity may be explained by variations in methods used in the high-throughput NIPT approach (including diagnostic accuracy thresholds and number and types of exons targeted), gestational age at the time of testing and different methods of handling inconclusive test results. There were also variations in the reporting of included studies. Particularly, two studies[19,21] did not report the number of inconclusive results of the test and some studies[12,18,22] did not report detailed reasons for inconclusive results.

There was very limited evidence relating to the clinical effectiveness of high-throughput NIPT. No studies were identified reporting adverse effects of high-throughput NIPT.

Owing to limited evidence, the generalisability of the review findings to non-white women and multiple pregnancies is unclear.

Cost-effectiveness

The de novo economic model was specifically developed to address the limitations of existing studies and concerns regarding the generalisability to current UK practice. The main strength of the decision model is the linkage between the diagnostic accuracy of a given identification strategy, the impact on subsequent treatment decisions and the ultimate effect on health outcomes and costs. A key element of the model is based on the previous economic model underpinning NICE TA156[62] on RAADP ensuring consistency between the separate diagnostic and TAs. A broad range of scenario analyses and SAs were undertaken to address key assumptions and uncertainties.

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Uncertainties

Clinical effectiveness

In this assessment we identified very limited data on the evaluation of clinical effectiveness for using high-throughput NIPT to detect fetal RhD status in RhD-negative women. Therefore, the potential role of high-throughput NIPT in terms of its clinical impact on the care pathway and adverse effects to the mother and fetus remains unclear. In particular, we did not identify any studies reporting comparative data relating to patient-related outcomes, such as quality-of-life measures.

Owing to a lack of sufficient data from included studies, we were unable to conduct subgroup analyses based on ethnicity. Therefore, whether or not the diagnostic performance of high-throughput NIPT differs between different ethnic groups remains unclear.

In terms of implementing high-throughput NIPT in health-care settings, no studies were identified reporting compliance rates to prenatal anti-D treatment in UK settings. Although a few non-UK studies reported compliance rates to prenatal anti-D treatment, the generalisability of their findings to the UK settings remains uncertain because of variations in national guidelines and health policies between different countries.

Cost-effectiveness

There is uncertainty regarding the cost of introducing high-throughput NIPT. The unit cost will vary with throughput and may be subject to an additional royalty fee. Unless NIPT can be incorporated seamlessly into routine antenatal care, it may result in additional costs for blood draw, transportation of samples and antenatal care visits to administer the test and deliver counselling and results. We conducted extensive SAs to address this uncertainty and to identify the threshold cost per NIPT. The cost of high-throughput NIPT has to increase by only (confidential information has been removed) above that modelled in the base case in order for current practice to be the preferred strategy.

Although there remains uncertainty as regards the timing of the test, our analysis suggests that this does not appear to be influential in determining the cost-effectiveness results either in terms of diagnostic accuracy or in terms of the extent of management costs for potentially sensitising events that can be avoided.

Although the evidence to support the diagnostic accuracy of NIPT is of good quality, existing evidence informing the impact of sensitisations in terms of their long-term health and cost consequences are more limited and highly uncertain.

Other relevant factors

Owing to a lack of relevant evidence, we have not considered any adverse health impacts from the provision of a blood-based product. Although widespread global use of anti-D immunoglobulin would suggest that is it safe, there remains uncertainty as regards the potential for risk associated with prion disease or other unknown pathogens. There may also be ethical considerations concerning the unnecessary administration of a blood-based product.

We also have not considered any adverse consequences from the introduction of the high-throughput NIPT over and above the slight increase in risk of sensitisation. Women who know that they are sensitised may factor this into their family planning decisions but we have assumed no such impact within the model. It is possible that NIPT could inadvertently reveal mistaken paternity of the child in cases in which a woman's partner knows that he is RhD negative and the baby is revealed to be RhD positive. Concerns about revealed paternity have been noted in relation to testing the father's blood type in order to target anti-D immunoglobulin only to those women with RhD-positive partners. The inclusion of an additional prenatal test could potentially have adverse impacts on the uptake of other antenatal care if the overall quality of care is compromised by the additional test burden.

Chapter 7 Conclusions

Implications for service provision

The findings from this assessment demonstrated high diagnostic performance of high-throughput NIPT for the detection of fetal RhD status in RhD-negative women from 11 weeks' gestation, with very low FPR and FNR. About 0.7% of women will have an incorrect test result and approximately 7% will have an inconclusive result. SAs did not materially alter the results. These findings have important implications for service provision.

The use of high-throughput NIPT as a routine screening test for fetal RhD status in RhD-negative women can largely remove unnecessary exposure to prophylactic anti-D treatment, without substantially altering the rate of sensitisations. However, there will be a very small number of women (about 0.1%) with a false-negative test result who are at increased risk of sensitisation because they do not receive antenatal anti-D prophylaxis. This risk will be increased if postnatal cord blood testing is withdrawn from clinical practice. However, the numbers of additional sensitisations is likely to be very small.

Based on a cross-section of 100,000 pregnancies, the magnitude of expected cost savings is estimated to range between £296,000 and £409,000 depending on the impact of high-throughput NIPT on postpartum management.

Suggested research priorities

For future research priorities, evidence on the diagnostic accuracy of high-throughput NIPT in women of non-white ethnicity is needed, for which large prospective cohort studies collecting diagnostic accuracy data will be required. This is of particular concern as non-white women are more likely to have less accurate test results. For example, in people with African ethnicity, because of the presence of the *RHD* pseudogene,[5] the prenatal detection of fetal RhD type from maternal blood would lead to higher rates of false-positive results in this particular population. Future diagnostic accuracy studies should systematically record and report the number of and reasons for inconclusive results and how these were dealt with when deriving estimates of diagnostic accuracy.

Given the limited evidence on the clinical impact of NIPT, further cohort studies comparing the use of high-throughput NIPT with universal antenatal anti-D administration are required. Such studies would ideally include a consecutive and representative sample of pregnant women in the UK. These should focus on recording relevant clinical outcomes (such as sensitisation rates, test and anti-D compliance and costs and quality of life) and adjust for relevant and clearly defined confounders (such as compliance with anti-D, timing of anti-D uptake and gestational age at time of NIPT). There is also limited existing evidence on the impact of sensitisations in terms of their long-term health and cost consequences. Although well-conducted cohort studies that comprehensively assess the full impact of sensitisations over mothers and children would be ideal, the complexity and cost associated with such studies means that promoting more systematic reporting and good-quality national audit data collection may be preferred. Surveys conducted in representative samples of women that assess the impact on quality of life of NIPT appear to be warranted.

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Acknowledgements

We would like to thank the following for providing advice: Dr Finning Kirstin, NHS Blood and Transplant; Professor Peter Soothill, Emeritus Professor at the University of Bristol; and Professor Lyn Chitty, Institute of Child Health, University College London.

Contributions of authors

Pedro Saramago (Research Fellow) was responsible for the cost-effectiveness section, protocol development, study selection, data extraction, development of the economic model and writing the economic sections of the report.

Huiqin Yang (Research Fellow) commented on the protocol, conducted study selection, data extraction, validity assessment, interpretation of evidence and wrote the clinical sections of the report.

Alexis Llewellyn (Research Fellow) contributed to the clinical effectiveness section, drafted the protocol, conducted study selection, data extraction and validity assessment, commented on drafts of the report and provided input to clinical sections.

Ruth Walker (Research Student) contributed to the clinical effectiveness section and conducted study selection, data extraction and validity assessment.

Melissa Harden (Information Specialist) devised the search strategy, carried out the literature searches and wrote the search section.

Stephen Palmer (Professor of Health Economics) provided project management, commented on drafts of the report and contributed to all aspects of the project.

Susan Griffin (Senior Research Fellow) contributed to the cost-effectiveness section, study selection, data extraction, development of the economic model and writing the economic sections of the report and had overall responsibility for the cost-effectiveness section of the report.

Mark Simmonds (Research Fellow) provided project management, performed the statistical analysis and wrote the simulation section, commented on drafts of the report and contributed to all aspects of the project and had overall responsibility for the clinical effectiveness section of the report.

Publication

Saramago P, Yang H, Llewellyn A, Palmer S, Simmonds M, Griffin S. High-throughput, non-invasive prenatal testing for fetal RHD genotype to guide antenatal prophylaxis with anti-D immunoglobin: a cost-effectiveness analysis [published online ahead of print February 7 2018]. *BJOG* 2018.

Data sharing statement

The data used in the analyses in this report are predominantly drawn from published and publicly available sources, as cited throughout the report. Summaries of the non-confidential data and of the models used are available on request from the corresponding author.

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Appendix 1 Search strategies

MEDLINE (via Ovid, http://ovidsp.ovid.com/)

Date range searched: 1946 to October Week 5 2015.

Date searched: 5 November 2015.

Records retrieved: 1815.

The search was updated on 26 February 2016, retrieving 77 records from MEDLINE and 40 records from MEDLINE In-Process & other Non-Indexed Citations.

1. Rh-Hr Blood-Group System/ (10,006)
2. (RhD or "rhesus D" or "Rh(D)" or "Rh-(D)" or Rh D).ti,ab. (3323)
3. (Rh-negative or Rh-positive).ti,ab. (898)
4. (Rhesus negative or Rhesus positive).ti,ab. (228)
5. ((rh or rhesus) adj2 (factor or factors or antigen$ or system or group)).ti,ab. (3438)
6. or/1-5 (13,812)
7. Rh Isoimmunization/ (1505)
8. ((isoimmuni$ or iso-immuni$ or isoimmune or iso-immune) adj6 (rh or rhesus or maternal or pregnan$)).ti,ab. (1164)
9. ((alloimmuni$ or allo-immuni$ or alloimmune or allo-immune) adj6 (rh or rhesus or maternal or pregnan$)).ti,ab. (870)
10. ((unsensiti#ed or un-sensiti#ed or non-sensiti#ed) adj6 (rh or rhesus or maternal or pregnan$)).ti,ab. (25)
11. ((sensiti#ation$ or sensiti#ed) adj6 (rh or rhesus or maternal or pregnan$)).ti,ab. (1074)
12. ((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) adj2 immuni#ation).ti,ab. (80)
13. ((rh or rhesus) adj2 (immuni#ation or autoimmuni#ation)).ti,ab. (695)
14. or/7-13 (4428)
15. exp Erythroblastosis, Fetal/ (11,006)
16. ((hemolytic or haemolytic) adj2 (disease$ or disorder$)).ti,ab. (4465)
17. HDFN.ti,ab. (95)
18. ((rhesus or rh) adj2 (disease$ or disorder$)).ti,ab. (742)
19. ((rhesus or rh or RhD) adj2 (incompatib$ or antagonism)).ti,ab. (750)
20. ((erythroblastoses or erythroblastosis) adj2 f?etal$).ti,ab. (760)
21. or/15-20 (13,551)
22. 6 or 14 or 21 (25,723)
23. Prenatal Diagnosis/ (33,273)
24. Maternal Serum Screening Tests/ (153)
25. Hematologic Tests/ (5564)
26. ((prenatal or pre-natal or antenatal or ante-natal) adj3 (test$ or screen$ or diagnos$ or determin$ or detect$)).ti,ab. (32,925)
27. ((fetal or foetal or fetus$ or foetus$) adj3 (test$ or screen$ or diagnos$ or determin$ or detect$)).ti,ab. (20,036)
28. (NIPD or NIPT).ti,ab. (328)
29. or/23-28 (69,981)
30. Genotyping Techniques/ (2761)
31. ((genotype$ or genotyping) adj2 (fetal or foetal or fetus$ or foetus$ or prenatal or pre-natal or antenatal or ante-natal)).ti,ab. (606)
32. ((genotype$ or genotyping) adj2 (maternal or pregnan$)).ti,ab. (789)

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33. ((genotype$ or genotyping) adj2 (noninvasive or non-invasive)).ti,ab. (71)
34. cell-free f?etal DNA.ti,ab. (489)
35. cffDNA.ti,ab. (87)
36. or/30-35 (4483)
37. 22 and 29 (1795)
38. 22 and 36 (276)
39. 37 or 38 (1869)
40. (editorial or comment).pt. (946,538)
41. 39 not 40 (1824)
42. exp animals/ not humans/ (4,137,930)
43. 41 not 42 (1815)

Key

/ = indexing term [medical subject heading (MeSH) heading]

exp = exploded indexing term (MeSH heading)

$ = truncation

= mandated wildcard – stands for one character

? = optional wildcard – stands for zero or one character

.ti,ab. = terms in either title or abstract fields

.pt. = publication type

adj = terms next to each other (order specified)

adj2 = terms within two words of each other (any order)

Cumulative Index to Nursing & Allied Health (via EBSCOhost, www.ebscohost.com)

Date range searched: inception to 5 November 2015.

Date searched: 6 November 2015.

Records retrieved: 290.

The search was updated on 26 February 2016, retrieving 31 records.

#	Query	Results
S39	S37 OR S38	290
S38	S22 AND S36	73
S37	S22 AND S29	268
S36	S30 OR S31 OR S33 OR S34 OR S35	2737
S35	TI cffDNA OR AB cffDNA	20
S34	TI "cell-free f#etal DNA" OR AB "cell-free f#etal DNA"	124

#	Query	Results
S33	TI (((genotype* or genotyping) N2 (noninvasive or non-invasive))) OR AB ((genotype* or genotyping) N2 (noninvasive or non-invasive)))	21
S32	TI (((genotype* or genotyping) N2 (maternal or pregnan*))) OR AB (((genotype* or genotyping) N2 (maternal or pregnan*)))	105
S31	TI (((genotype* or genotyping) N2 (fetal or foetal or fetus* or foetus* or prenatal or pre-natal or antenatal or ante-natal))) OR AB (((genotype* or genotyping) N2 (fetal or foetal or fetus* or foetus* or prenatal or pre-natal or antenatal or ante-natal)))	103
S30	MM "Genetic Techniques"	2529
S29	S23 or S24 or S25 or S26 or S27 or S28	22,920
S28	TI ((NIPD or NIPT)) OR AB ((NIPD or NIPT))	93
S27	TI ((fetal or foetal or fetus* or foetus*) N3 (test* or screen* or diagnos* or determin* or detect*)) OR AB ((fetal or foetal or fetus* or foetus*) N3 (test* or screen* or diagnos* or determin* or detect*))	2644
S26	TI ((prenatal or pre-natal or antenatal or ante-natal) N3 (test* or screen* or diagnos* or determin* or detect*)) OR AB ((prenatal or pre-natal or antenatal or ante-natal) N3 (test* or screen* or diagnos* or determin* or detect*))	5033
S25	(MH "Noninvasive Procedures")	1538
S24	(MH "Hematologic Tests")	11,530
S23	(MH "Prenatal Diagnosis")	5562
S22	S6 OR S14 OR S21	1924
S21	S15 OR S16 OR S17 OR S18 OR S19 OR S20	998
S20	TI ((erythroblastoses or erythroblastosis) N2 (fetal* or foetal*)) OR AB ((erythroblastoses or erythroblastosis) N2 (fetal* or foetal*))	16
S19	TI ((rhesus or rh or RhD) N2 (incompatib* or antagonism)) OR AB ((rhesus or rh or RhD) N2 (incompatib* or antagonism))	45
S18	TI ((rhesus or rh) N2 (disease* or disorder*)) OR AB ((rhesus or rh) N2 (disease* or disorder*))	76
S17	TI HDFN OR AB HDFN	20
S16	TI ((hemolytic or haemolytic) N2 (disease* or disorder*)) OR AB ((hemolytic or haemolytic) N2 (disease* or disorder*))	298
S15	(MH "Erythroblastosis, Fetal+")	775
S14	S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13	446
S13	TI ((rh or rhesus) N2 (immuni?ation or autoimmuni?ation)) OR AB ((rh or rhesus) N2 (immuni?ation or autoimmuni?ation))	17
S12	TI ((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) N2 immuni?ation) OR AB ((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) N2 immuni?ation)	2
S11	TI ((sensiti?ation* or sensiti?ed) N6 (rh or rhesus or maternal or pregnan*)) OR AB ((sensiti?ation* or sensiti?ed) N6 (rh or rhesus or maternal or pregnan*))	61
S10	TI ((unsensiti?ed or un-sensiti?ed or non-sensiti?ed) N6 (rh or rhesus or maternal or pregnan*)) OR AB ((unsensiti?ed or un-sensiti?ed or non-sensiti?ed) N6 (rh or rhesus or maternal or pregnan*))	3
S9	TI ((alloimmuni* or allo-immuni* or alloimmune or allo-immune) N6 (rh or rhesus or maternal or pregnan*)) OR AB ((alloimmuni* or allo-immuni* or alloimmune or allo-immune) N6 (rh or rhesus or maternal or pregnan*))	126
S8	TI ((isoimmuni* or iso-immuni* or isoimmune or iso-immune) N6 (rh or rhesus or maternal or pregnan*)) OR AB ((isoimmuni* or iso-immuni* or isoimmune or iso-immune) N6 (rh or rhesus or maternal or pregnan*))	47
S7	(MH "RH Isoimmunization")	297
S6	S1 OR S2 OR S3 OR S4 OR S5	870

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#	Query	Results
S5	TI ((rh or rhesus) N2 (factor or factors or antigen* or system or group)) OR AB ((rh or rhesus) N2 (factor or factors or antigen* or system or group))	167
S4	TI ("Rhesus negative" or "Rhesus positive") OR AB ("Rhesus negative" or "Rhesus positive")	24
S3	TI (Rh-negative or Rh-positive) OR AB (Rh-negative or Rh-positive)	53
S2	TI (RhD or "rhesus D" or "Rh(D)" or "Rh-(D)" or Rh D) OR AB (RhD or "rhesus D" or "Rh(D)" or "Rh-(D)" or "Rh D" or "Rh-D")	492
S1	(MH "Rh-Hr Blood-Group System")	458

Key

MH = indexing term (CINAHL heading)

* = truncation

? = wildcard – stands for one character

= optional wildcard – stands for zero or one character

TI = words in the title

AB = words in the abstract

" " = phrase search

N2 = terms within two words of each other (any order)

PT = publication type

Cochrane Central Register of Controlled Trials (via Wiley Online Library, http://onlinelibrary.wiley.com/)

Issue 10 of 12, October 2015.

Date searched: 6 November 2015.

Records retrieved: 16.

The search was updated on 26 February 2016, retrieving 17 records from CENTRAL.

#1 MeSH descriptor: [Rh-Hr Blood-Group System] this term only(62)

#2 (RhD or "rhesus D" or "Rh(D)" or "Rh-(D)" or "Rh D" or "Rh-D"):ti,ab,kw(94)

#3 (Rh-negative or Rh-positive):ti,ab,kw(20)

#4 ("Rhesus negative" or "Rhesus positive"):ti,ab,kw(16)

#5 (rh or rhesus) near/2 (factor or factors or antigen* or system or group):ti,ab,kw(106)

#6 #1 or #2 or #3 or #4 or #5(238)

#7 MeSH descriptor: [Rh Isoimmunization] this term only(40)

#8 (isoimmuni* or iso-immuni* or isoimmune or iso-immune) near/6 (rh or rhesus or maternal or pregnan*):ti,ab,kw(68)

#9 (alloimmuni* or allo-immuni* or alloimmune or allo-immune) near/6 (rh or rhesus or maternal or pregnan*):ti,ab,kw(22)

#10 (unsensitised or unsensitized or un-sensitised or un-sensitized or non-sensitised or non-sensitized) near/6 (rh or rhesus or maternal or pregnan*):ti,ab,kw(3)

#11 (sensitisation* or sensitization* or sensitised or sensitized) near/6 (rh or rhesus or maternal or pregnan*):ti,ab,kw(32)

#12 (fetomaternal or feto-maternal or foetomaternal or foeto-maternal) near/2 (immunisation or immunization):ti,ab,kw(1)

#13 (rh or rhesus) near/2 (immunisation or immunization or autoimmunisation or autoimmunization): ti,ab,kw(29)

#14 #7 or #8 or #9 or #10 or #11 or #12 or #13(123)

#15 MeSH descriptor: [Erythroblastosis, Fetal] explode all trees(72)

#16 (hemolytic or haemolytic) near/2 (disease* or disorder*):ti,ab,kw(99)

#17 HDFN:ti,ab,kw(3)

#18 (rhesus or rh) near/2 (disease* or disorder*):ti,ab,kw(628)

#19 (rhesus or rh or RhD) near/2 (incompatib* or antagonism):ti,ab,kw(22)

#20 (erythroblastoses or erythroblastosis) near/2 (fetal* or foetal*):ti,ab,kw(72)

#21 #15 or #16 or #17 or #18 or #19 or #20(732)

#22 #6 or #14 or #21(978)

#23 MeSH descriptor: [Prenatal Diagnosis] this term only(363)

#24 MeSH descriptor: [Maternal Serum Screening Tests] this term only(5)

#25 MeSH descriptor: [Hematologic Tests] this term only(196)

#26 (prenatal or pre-natal or antenatal or ante-natal) near/3 (test* or screen* or diagnos* or determin* or detect*):ti,ab,kw(868)

#27 (fetal or foetal or fetus* or foetus*) near/3 (test* or screen* or diagnos* or determin* or detect*):ti,ab,kw(571)

#28 (NIPD or NIPT):ti,ab,kw(10)

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#29 #23 or #24 or #25 or #26 or #27 or #28(1480)

#30 MeSH descriptor: [Genotyping Techniques] this term only(18)

#31 (genotype* or genotyping) near/2 (fetal or foetal or fetus* or foetus* or prenatal or pre-natal or antenatal or ante-natal):ti,ab,kw(5)

#32 ((genotype* or genotyping) near/2 (maternal or pregnan*)):ti,ab,kw(15)

#33 ((genotype* or genotyping) near/2 (noninvasive or non-invasive)):ti,ab,kw(0)

#34 ("cell-free foetal DNA" or "cell-free fetal DNA"):ti,ab,kw(7)

#35 cffDNA:ti,ab,kw(1)

#36 #30 or #31 or #32 or #33 or #34 or #35(42)

#37 #22 and #29(33)

#38 #22 and #36(4)

#39 #37 or #38(34)

Note: The strategy above was used to search CENTRAL and CDSR. The 34 results at line #39 include Cochrane reviews, DARE, HTA and NHS EED records as well as trials from CENTRAL.

Key

MeSH descriptor = indexing term (MeSH heading)

* = truncation

:ti,ab,kw = terms in either title or abstract or keyword fields

near/2 = terms within two words of each other (any order)

next = terms are next to each other

" " = phrase search

Cochrane Database of Systematic Reviews (via Wiley Online Library, http://onlinelibrary.wiley.com/)

Issue 11 of 12, November 2015.

Date searched: 6 November 2015.

Records retrieved: 8.

See *Cochrane Central Register of Controlled Trials* for search strategy used.

The search was updated on 26 February 2016, retrieving nine records from CDSR.

Database of Abstracts of Reviews of Effects (via Centre for Reviews and Dissemination, www.crd.york.ac.uk/CRDWeb)

Date range searched: inception to 31 March 2015.

Date searched: 6 November 2015.

Records retrieved: 9.

The strategy below was used to search DARE, NHS EED and the HTA database. The hits column shows the total number of records found in all three databases.

Line	Search	Hits
1	MeSH DESCRIPTOR Rh-Hr Blood-Group System EXPLODE ALL TREES	16
2	(RhD or "rhesus D" or Rh-D)	24
3	(Rh-negative or Rh-positive)	7
4	("Rhesus negative" or "Rhesus positive")	9
5	((rh or rhesus) NEAR2 (factor or factors or antigen* or system or group))	18
6	((factor or factors or antigen* or system or group) NEAR2 (rh or rhesus))	1
7	#1 OR #2 OR #3 OR #4 OR #5 OR #6	35
8	MeSH DESCRIPTOR Rh Isoimmunization	15
9	((isoimmuni* or iso-immuni* or isoimmune or iso-immune) NEAR6 (rh or rhesus or maternal or pregnan*))	10
10	((rh or rhesus or maternal or pregnan*) NEAR6 (isoimmuni* or iso-immuni* or isoimmune or iso-immune))	17
11	((alloimmuni* or allo-immuni* or alloimmune or allo-immune) NEAR6 (rh or rhesus or maternal or pregnan*))	12
12	((rh or rhesus or maternal or pregnan*) NEAR6 (alloimmuni* or allo-immuni* or alloimmune or allo-immune))	8
13	((unsensitised or unsensitized or un-sensitised or un-sensitized or non-sensitised or non-sensitized) NEAR6 (rh or rhesus or maternal or pregnan*))	3
14	((rh or rhesus or maternal or pregnan*) NEAR6 (unsensitised or unsensitized or un-sensitised or un-sensitized or non-sensitised or non-sensitized))	0
15	((sensitisation* or sensitization* or sensitised or sensitized)NEAR6 (rh or rhesus or maternal or pregnan*))	6
16	((rh or rhesus or maternal or pregnan*) NEAR6 (sensitisation* or sensitization* or sensitised or sensitized))	5
17	((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) NEAR2 (immunisation or immunization))	0
18	((immunisation or immunization) NEAR2 (fetomaternal or feto-maternal or foetomaternal or foeto-maternal))	0
19	((rh or rhesus) NEAR2 (immunisation or immunization or autoimmunisation or autoimmunization))	4
20	((immunisation or immunization or autoimmunisation or autoimmunization) NEAR2 (rh or rhesus))	0
21	#8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20	29
22	MeSH DESCRIPTOR Erythroblastosis, Fetal EXPLODE ALL TREES	18
23	((hemolytic or haemolytic) NEAR2 (disease* or disorder*))	16
24	((disease* or disorder*) NEAR2 (hemolytic or haemolytic))	1
25	(HDFN)	1
26	((rhesus or rh) NEAR2 (disease* or disorder*))	3
27	((disease* or disorder*) NEAR2 (rhesus or rh))	1
28	((rhesus or rh or RhD) NEAR2 (incompatib* or antagonism))	3

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Line	Search	Hits
29	((incompatib* or antagonism) NEAR2 (rhesus or rh or RhD))	0
30	((erythroblastoses or erythroblastosis) NEAR2 (fetal* or foetal*))	14
31	#22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30	28
32	#7 OR #21 OR #31	56
33	MeSH DESCRIPTOR Prenatal Diagnosis	216
34	MeSH DESCRIPTOR Maternal Serum Screening Tests	5
35	MeSH DESCRIPTOR Hematologic Tests	30
36	((prenatal or pre-natal or antenatal or ante-natal) NEAR3 (test* or screen* or diagnos* or determin* or detect*))	380
37	((test* or screen* or diagnos* or determin* or detect*) NEAR3 (prenatal or pre-natal or antenatal or ante-natal))	171
38	((test* or screen* or diagnos* or determin* or detect*) NEAR3 (fetal or foetal or fetus* or foetus*))	124
39	((fetal or foetal or fetus* or foetus*) NEAR3 (test* or screen* or diagnos* or determin* or detect*))	130
40	(NIPD or NIPT)	6
41	#33 OR #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40	534
42	MeSH DESCRIPTOR Genotyping Techniques	6
43	((genotype* or genotyping) NEAR2 (fetal or foetal or fetus* or foetus* or prenatal or pre-natal or antenatal or ante-natal))	3
44	((fetal or foetal or fetus* or foetus* or prenatal or pre-natal or antenatal or ante-natal) NEAR2 (genotype* or genotyping))	3
45	((genotype* or genotyping) NEAR2 (maternal or pregnan*))	2
46	((maternal or pregnan*) NEAR2 (genotype* or genotyping))	2
47	((genotype* or genotyping) NEAR2 (noninvasive or non-invasive))	1
48	((noninvasive or non-invasive) NEAR2 (genotype* or genotyping))	4
49	("cell-free foetal DNA" or "cell-free fetal DNA")	7
50	(cffDNA)	2
51	#42 OR #43 OR #44 OR #45 OR #46 OR #47 OR #48 OR #49 OR #50	18
52	#32 AND #41	16
53	#32 AND #51	6
54	#52 OR #53	18

Key

MeSH DESCRIPTOR = indexing term (MeSH heading)

* = truncation

NEAR2 = terms within two words of each other (order specified)

" " = phrase search

EMBASE (via Ovid, http://ovidsp.ovid.com/)

Date range searched: 1974 to 2015 November 04.

Date searched: 5 November 2015.

Records retrieved: 3092.

The search was updated on 26 February 2016, retrieving 221 records.

1. blood group rhesus system/ (8133)
2. rhesus D antigen/ (785)
3. (RhD or "rhesus D" or "Rh(D)" or "Rh-(D)" or Rh D).ti,ab. (5254)
4. (Rh-negative or Rh-positive).ti,ab. (1197)
5. (Rhesus negative or Rhesus positive).ti,ab. (320)
6. ((rh or rhesus) adj2 (factor or factors or antigen$ or system or group)).ti,ab. (4401)
7. or/1-6 (15,398)
8. rhesus isoimmunization/ (1536)
9. ((isoimmuni$ or iso-immuni$ or isoimmune or iso-immune) adj6 (rh or rhesus or maternal or pregnan$)).ti,ab. (1313)
10. ((alloimmuni$ or allo-immuni$ or alloimmune or allo-immune) adj6 (rh or rhesus or maternal or pregnan$)).ti,ab. (1319)
11. ((unsensiti#ed or un-sensiti#ed or non-sensiti#ed) adj6 (rh or rhesus or maternal or pregnan$)).ti,ab. (37)
12. ((sensiti#ation$ or sensiti#ed) adj6 (rh or rhesus or maternal or pregnan$)).ti,ab. (1306)
13. ((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) adj2 immuni#ation).ti,ab. (90)
14. ((rh or rhesus) adj2 (immuni#ation or autoimmuni#ation)).ti,ab. (772)
15. or/8-14 (5218)
16. exp newborn hemolytic disease/ (11,867)
17. ((hemolytic or haemolytic) adj2 (disease$ or disorder$)).ti,ab. (5302)
18. HDFN.ti,ab. (294)
19. ((rhesus or rh) adj2 (disease$ or disorder$)).ti,ab. (838)
20. ((rhesus or rh or RhD) adj2 (incompatib$ or antagonism)).ti,ab. (913)
21. ((erythroblastoses or erythroblastosis) adj2 f?etal$).ti,ab. (739)
22. rhesus incompatibility/ (1131)
23. or/16-22 (16,217)
24. 7 or 15 or 23 (30,562)
25. prenatal diagnosis/ (50,220)
26. prenatal screening/ (6356)
27. maternal serum screening test/ (145)
28. blood examination/ (10,293)
29. non invasive procedure/ (17,457)
30. diagnostic accuracy/ (195,290)
31. ((prenatal or pre-natal or antenatal or ante-natal) adj3 (test$ or screen$ or diagnos$ or determin$ or detect$)).ti,ab. (40,821)
32. ((fetal or foetal or fetus$ or foetus$) adj3 (test$ or screen$ or diagnos$ or determin$ or detect$)).ti,ab. (25,280)
33. (NIPD or NIPT).ti,ab. (561)
34. or/25-33 (301,546)
35. genotyping technique/ (4081)
36. ((genotype$ or genotyping) adj2 (fetal or foetal or fetus$ or foetus$ or prenatal or pre-natal or antenatal or ante-natal)).ti,ab. (800)
37. ((genotype$ or genotyping) adj2 (maternal or pregnan$)).ti,ab. (924)
38. ((genotype$ or genotyping) adj2 (noninvasive or non-invasive)).ti,ab. (90)

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39. cell-free f?etal DNA.ti,ab. (741)
40. cffDNA.ti,ab. (168)
41. or/35-40 (6300)
42. 24 and 34 (3084)
43. 24 and 41 (419)
44. 42 or 43 (3160)
45. (editorial or note).pt. (1,117,567)
46. 44 not 45 (3107)
47. animal/ (1,701,987)
48. exp animal experiment/ (1,895,782)
49. nonhuman/ (4,645,212)
50. (rat or rats or mouse or mice or hamster or hamsters or animal or animals or dog or dogs or cat or cats or bovine or sheep).ti,sh. (4,564,702)
51. 47 or 48 or 49 or 50 (7,266,921)
52. exp human/ (16,514,549)
53. human experiment/ (344,858)
54. 52 or 53 (16,515,997)
55. 51 not (51 and 54) (5,693,442)
56. 46 not 55 (3092)

Key

/ = indexing term (Emtree heading)

exp = exploded indexing term (Emtree heading)

$ = truncation

= mandated wildcard – stands for one character

? = optional wildcard – stands for zero or one character

.ti,ab. = terms in either title or abstract fields

.pt. = publication type

sh. = subject heading field

adj = terms next to each other (order specified)

adj2 = terms within two words of each other (any order)

Health Technology Assessment database (via www.crd.york.ac.uk/CRDWeb)

Date range searched: inception to 31 March 2015.

Date searched: 6 November 2015.

Records retrieved: 3.

See above under *Database of Abstracts of Reviews of Effects* for search strategy used.

Maternity and infant care (via Ovid, http://ovidsp.ovid.com/)

Date range searched: 1971 to September 2015.

Date searched: 5 November 2015.

Records retrieved: 238.

The search was updated on 26 February 2016, retrieving 11 records.

1. Rh-Hr blood-group system.de. (26)
2. (RhD or "rhesus D" or "Rh(D)" or "Rh-(D)" or Rh D).ti,ab. (285)
3. (Rh-negative or Rh-positive).ti,ab. (81)
4. (Rhesus negative or Rhesus positive).ti,ab. (76)
5. ((rh or rhesus) adj2 (factor or factors or antigen$ or system or group)).ti,ab. (57)
6. 1 or 2 or 3 or 4 or 5 (439)
7. (Rh isoimmunisation or Rh isoimmunisation - therapy or "Rh isoimmunisation - prevention and control").de. (317)
8. Alloimmunisation.de. (29)
9. ((isoimmuni$ or iso-immuni$ or isoimmune or iso-immune) adj6 (rh or rhesus or maternal or pregnan$)).ti,ab. (148)
10. ((alloimmuni$ or allo-immuni$ or alloimmune or allo-immune) adj6 (rh or rhesus or maternal or pregnan$)).ti,ab. (201)
11. ((unsensiti#ed or un-sensiti#ed or non-sensiti#ed) adj6 (rh or rhesus or maternal or pregnan$)).ti,ab. (9)
12. ((sensiti#ation$ or sensiti#ed) adj6 (rh or rhesus or maternal or pregnan$)).ti,ab. (96)
13. ((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) adj2 immuni#ation).ti,ab. (3)
14. ((rh or rhesus) adj2 (immuni#ation or autoimmuni#ation)).ti,ab. (61)
15. 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 (616)
16. Erythroblastosis - fetal.de. (118)
17. ((hemolytic or haemolytic) adj2 (disease$ or disorder$)).ti,ab. (281)
18. HDFN.ti,ab. (24)
19. ((rhesus or rh) adj2 (disease$ or disorder$)).ti,ab. (96)
20. rhesus.sx. (435)
21. ((rhesus or rh or RhD) adj2 (incompatib$ or antagonism)).ti,ab. (42)
22. ((erythroblastoses or erythroblastosis) adj2 f?etal$).ti,ab. (27)
23. 16 or 17 or 18 or 19 or 20 or 21 or 22 (669)
24. 6 or 15 or 23 (1005)
25. Prenatal diagnosis.de. (4460)
26. ((prenatal or pre-natal or antenatal or ante-natal) adj3 (test$ or screen$ or diagnos$ or determin$ or detect$)).ti,ab. (7133)
27. ((fetal or foetal or fetus$ or foetus$) adj3 (test$ or screen$ or diagnos$ or determin$ or detect$)).ti,ab. (4763)
28. (NIPD or NIPT).ti,ab. (89)
29. 25 or 26 or 27 or 28 (12,193)
30. ((genotype$ or genotyping) adj2 (fetal or foetal or fetus$ or foetus$ or prenatal or pre-natal or antenatal or ante-natal)).ti,ab. (96)
31. ((genotype$ or genotyping) adj2 (maternal or pregnan$)).ti,ab. (89)
32. ((genotype$ or genotyping) adj2 (noninvasive or non-invasive)).ti,ab. (6)
33. cell-free f?etal DNA.ti,ab. (148)
34. cffDNA.ti,ab. (31)
35. (genotype$ or genotyping).ti,ab. (881)
36. 30 or 31 or 32 or 33 or 34 (291)
37. 24 and 29 (237)

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38. 24 and 36 (67)
39. 37 or 38 (245)
40. (editorial or commentary).pt. (14,906)
41. 39 not 40 (238)

Key

.de. = subject heading search

$ = truncation

= mandated wildcard – stands for one character

? = optional wildcard – stands for zero or one character

.ti,ab. = terms in either title or abstract fields

.pt. = publication type

adj = terms next to each other (order specified)

adj2 = terms within two words of each other (any order)

NHS Economic Evaluations Database (via Centre for Reviews and Dissemination, www.crd.york.ac.uk/CRDWeb)

Date range searched: inception to 31 March 2015.

Date searched: 6 November 2015.

Records retrieved: 6.

See above under *Database of Abstracts of Reviews of Effects* for search strategy used.

PubMed (www.ncbi.nlm.nih.gov/pubmed)

Date searched: 26 February 2016.

Records retrieved: 112.

((((((((((“Prenatal Diagnosis”[Mesh:NoExp]) OR “Maternal Serum Screening Tests”[Mesh:NoExp]) OR “Hematologic Tests”[Mesh:NoExp]) OR ((((test[Title/Abstract] OR tests[Title/Abstract] OR testing[Title/Abstract] OR tested[Title/Abstract] OR screen*[Title/Abstract] OR diagnos*[Title/Abstract] OR determin*[Title/Abstract] OR detect*[Title/Abstract])))) AND ((prenatal[Title/Abstract] OR pre-natal[Title/Abstract] OR antenatal[Title/Abstract] OR ante-natal[Title/Abstract] OR fetal[Title/Abstract] OR foetal[Title/Abstract] OR fetus*[Title/Abstract] OR foetus*[Title/Abstract]))))) OR ((NIPD[Title/Abstract] OR NIPT[Title/Abstract])))) OR (((“Genotyping Techniques”[Mesh:NoExp]) OR ((((genotype*[Title/Abstract] OR genotyping[Title/Abstract]))) AND ((((fetal[Title/Abstract] OR foetal[Title/Abstract] OR fetus*[Title/Abstract] OR foetus*[Title/Abstract] OR prenatal[Title/Abstract] OR pre-natal[Title/Abstract] OR antenatal[Title/Abstract] OR ante-natal[Title/Abstract])) OR (maternal[Title/Abstract] OR pregnan*[Title/Abstract])) OR (noninvasive[Title/Abstract] OR non-invasive[Title/Abstract])))) OR ((“cell-free fetal DNA”[Title/Abstract] OR “cell-free foetal DNA”[Title/Abstract] OR cffDNA[Title/Abstract])))))) AND ((((((((((“Erythroblastosis, Fetal”[Mesh]) OR ((“hemolytic

disease"[Title/Abstract] OR "hemolytic diseases"[Title/Abstract] OR "hemolytic disorder"[Title/Abstract] OR "hemolytic disorders"[Title/Abstract]))) OR (("haemolytic disease" OR "haemolytic diseases" OR "haemolytic disorder" OR "haemolytic disorders"))) OR HDFN[Title/Abstract]) OR (("rhesus disease" [Title/Abstract] OR "rhesus diseases"[Title/Abstract] OR "rhesus disorder"[Title/Abstract] OR "rhesus disorders"[Title/Abstract] OR "rh disease"[Title/Abstract] OR "rh diseases"[Title/Abstract] OR "rh disorder"[Title/Abstract] OR "rh disorders"[Title/Abstract]))) OR (((rhesus[Title/Abstract] OR rh[Title/Abstract] OR RhD[Title/Abstract])) AND (incompatib*[Title/Abstract] OR antagonism[Title/Abstract]))) OR (((erythroblastoses[Title/Abstract] OR erythroblastosis[Title/Abstract])) AND (fetal*[Title/Abstract] OR foetal* [Title/Abstract])))) OR (((((“Rh Isoimmunization"[Mesh:noexp]) OR (((((((isoimmuni*[Title/Abstract] OR iso-immuni*[Title/Abstract] OR isoimmune[Title/Abstract] OR iso-immune[Title/Abstract]))) OR ((alloimmuni* [Title/Abstract] OR allo-immuni*[Title/Abstract] OR alloimmune[Title/Abstract] OR allo-immune[Title/ Abstract]))) OR ((unsensitised[Title/Abstract] OR unsensitized[Title/Abstract] OR un-sensitised[Title/Abstract] OR un-sensitized[Title/Abstract] OR non-sensitised[Title/Abstract] OR non-sensitized[Title/Abstract]))) OR ((sensitisation*[Title/Abstract] OR sensitization*[Title/Abstract] OR sensitised[Title/Abstract] OR sensitized [Title/Abstract])))) AND ((rh[Title/Abstract] OR rhesus[Title/Abstract] OR maternal[Title/Abstract] OR pregnan* [Title/Abstract])))) OR (((fetomaternal[Title/Abstract] OR feto-maternal[Title/Abstract] OR foetomaternal[Title/ Abstract] OR foeto-maternal[Title/Abstract])) AND (immunisation[Title/Abstract] OR immunization[Title/ Abstract]))) OR (((rh[Title/Abstract] OR rhesus[Title/Abstract])) AND (immunisation[Title/Abstract] OR autoimmunisation[Title/Abstract] OR immunization[Title/Abstract] OR autoimmunization[Title/Abstract])))) OR (((((“Rh-Hr Blood-Group System"[Mesh:noexp]) OR (((RhD[Title/Abstract] OR "rhesus D"[Title/Abstract] OR "Rh(D)"[Title/Abstract] OR "Rh-(D)"[Title/Abstract] OR "Rh D"[Title/Abstract]))) OR ((Rh-negative[Title/ Abstract] OR Rh-positive[Title/Abstract]))) OR (("Rhesus negative"[Title/Abstract] OR "Rhesus positive"[Title/ Abstract]))) OR (("rh factor"[Title/Abstract] OR "rh factors"[Title/Abstract] OR "rh antigen"[Title/Abstract] OR "rh antigens"[Title/Abstract] OR "rh system"[Title/Abstract] OR "rh group"[Title/Abstract]))) OR (("rhesus factor"[Title/Abstract] OR "rhesus factors"[Title/Abstract] OR "rhesus antigen"[Title/Abstract] OR "rhesus antigens"[Title/Abstract] OR "rhesus system"[Title/Abstract] OR "rhesus group"[Title/Abstract]))))) AND ((((pubstatusaheadofprint OR publisher[sb] OR pubmednotmedline[sb]))) OR (((inprocess[sb] or medline [sb])) AND ("2016/02/20"[Date - Entrez] : "3000"[Date - Entrez]))))

Science Citation Index (via Web of Science, Thomson Reuters, http://thomsonreuters.com/thomson-reuters-web-of-science)

Date range searched: 1900 to 4 November 2015.

Date searched: 6 November 2015.

Records retrieved: 801.

The strategy below was used to search Science Citation Index and the Conference Proceedings Citation Index: Science. As both databases were searched together the records retrieved refer to results from both databases.

The searches for Science Citation Index and the Conference Proceedings Citation Index: Science were updated on 26 February 2016, retrieving 811 records.

# 34	801	#32 NOT #33
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 33	20	#31 OR #30
		Refined by:DOCUMENT TYPES: (EDITORIAL MATERIAL)
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years

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# 32	821	#31 OR #30
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 31	287	#29 AND #19
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 30	744	#23 AND #19
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 29	2378	#28 OR #27 OR #26 OR #25 OR #24
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 28	79	TS=cffDNA
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 27	543	TS=("cell-free foetal DNA" or "cell-free fetal DNA")
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 26	204	TS=((genotype* or genotyping) NEAR/2 (noninvasive or non-invasive))
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 25	1222	TS=((genotype* or genotyping) NEAR/2 (maternal or pregnan*))
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 24	779	TS=((genotype* or genotyping) NEAR/2 (fetal or foetal or fetus* or foetus* or prenatal or pre-natal or antenatal or ante-natal))
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 23	51,060	#22 OR #21 OR #20
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 22	632	TS=(NIPD or NIPT)
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 21	21,197	TS=((fetal or foetal or fetus* or foetus*) NEAR/3 (test* or screen* or diagnos* or determin* or detect*))
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 20	36,396	TS = ((prenatal or pre-natal or antenatal or ante-natal) NEAR/3 (test* or screen* or diagnos* or determin* or detect*))
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 19	15,143	#18 OR #12 OR #5
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 18	5220	#17 OR #16 OR #15 OR #14 OR #13
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 17	581	TS = ((erythroblastoses or erythroblastosis) NEAR/2 f$etal*)
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 16	413	TS = ((rhesus or rh or RhD) NEAR/2 (incompatib* or antagonism))
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 15	1248	TS = ((rhesus or rh) NEAR/2 (disease* or disorder*))
		Indexes = SCI-EXPANDED, CPCI-S Timespan = All years

# 14	102	TS = HDFN Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 13	3593	TS = ((hemolytic or haemolytic) NEAR/2 (disease* or disorder*)) Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 12	2937	#11 OR #10 OR #9 OR #8 OR #7 OR #6 Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 11	565	TS = ((rh or rhesus) NEAR/2 (immuni?ation or autoimmuni?ation)) Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 10	32	TS = ((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) NEAR/2 immuni?ation) Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 9	899	TS = ((sensiti?ation* or sensiti?ed) NEAR/6 (rh or rhesus or maternal or pregnan*)) Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 8	15	TS = ((unsensiti?ed or un-sensiti?ed or non-sensiti?ed) NEAR/6 (rh or rhesus or maternal or pregnan*)) Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 7	981	TS = ((alloimmuni* or allo-immuni* or alloimmune or allo-immune) NEAR/6 (rh or rhesus or maternal or pregnan*)) Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 6	736	TS = ((isoimmuni* or iso-immuni* or isoimmune or iso-immune) NEAR/6 (rh or rhesus or maternal or pregnan*)) Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 5	8522	#4 OR #3 OR #2 OR #1 Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 4	5198	TS = ((rh or rhesus) NEAR/2 (factor or factors or antigen* or system or group)) Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 3	121	TS = ("Rhesus negative" or "Rhesus positive") Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 2	479	TS = (Rh-negative or Rh-positive) Indexes = SCI-EXPANDED, CPCI-S Timespan = All years
# 1	3491	TS = (RhD or "rhesus D" or "Rh(D)" or "Rh-(D)" or "Rh D" or "Rh-D") Indexes = SCI-EXPANDED, CPCI-S Timespan = All years

Ongoing, unpublished or grey literature search strategies

ClinicalTrials.gov (https://clinicaltrials.gov/)

Date searched: 10 November 2015.

Records retrieved: 44.

RhD OR "rhesus D" OR "Rh(D)" OR "Rh-(D)" OR "Rh D" OR "Rh-negative" OR "Rh-positive" OR "Rhesus negative" OR "Rhesus positive"

The search was updated on 26 February 2016, retrieving two new records.

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Conference Proceedings Citation Index: Science (via Web of Science, Thomson Reuters, http://thomsonreuters.com/thomson-reuters-web-of-science)

Date range searched: 1990 to 4 November 2015.

Date searched: 6 November 2015.

Records retrieved: 801.

See *Science Citation Index* for search strategy used. As both databases were searched together, the records retrieved refers to results from both databases.

The searches for Science Citation Index and the Conference Proceedings Citation Index: Science were updated on 26 February 2016, retrieving 811 records.

EU Clinical Trials Register (www.clinicaltrialsregister.eu/ctr-search/search)

Date searched: 10 November 2015.

Records retrieved: 4.

"RhD" OR "rhesus D" OR "Rh(D)" OR "Rh-(D)" OR "Rh D" OR "Rh-negative" OR "Rh-positive" OR "Rh negative" OR "Rh positive" OR "Rhesus negative" OR "Rhesus positive"

The search was updated on 26 February 2016 but no new records were retrieved.

PROSPERO (www.crd.york.ac.uk/PROSPERO)

Date searched: 10 November 2015.

Records retrieved: 4.

RhD or Rh-D or Rh-negative or Rh-positive in all fields.

The search was updated on 26 February 2016, retrieving one new record.

World Health Organization's International Clinical Trials Registry Platform (www.who.int/ictrp/search/en)

Date searched: 10 November 2015.

Records retrieved: 29.

RhD OR rhesus OR Rh-negative OR Rh-positive.

The search was updated on 26 February 2016 but no new records were retrieved.

Guideline searches

The following websites were searched for relevant guidelines.

All guideline website searches were updated on 4 March 2016; however, no new guidelines were retrieved.

National Guidelines Clearinghouse (www.guideline.gov)

Date searched: 17 November 2015.

(rhd or rhesus or 'rh negative" or "rh positive")' and '(pregnan* or maternal or antenatal or ante-natal or prenatal or pre-natal or intrapartum)

A total of 23 results were retrieved and browsed for relevance; 18 relevant guidelines were found.

National Institute for Health and Care Excellence (www.nice.org.uk)

Date searched: 13 November 2015.

1. Browsed for relevant guidance in the fertility, pregnancy and childbirth section: www.nice.org.uk/guidance/conditions-and-diseases/fertility–pregnancy-and-childbirth.
2. Searched NICE website using general search box with keyword RhD.
3. Searched NICE website using general search box with keyword Rhesus.
4. Relevant guidelines found.

NHS Evidence (www.evidence.nhs.uk)

Searched on: 17 November 2015.

(rhd OR rhesus OR "rh negative" OR "rh positive") AND (pregnan* OR maternal OR antenatal OR ante-natal OR prenatal OR pre-natal OR intrapartum) limited to guidelines.

A total of 81 results were retrieved and browsed for relevance. Seven relevant guidelines were found.

Royal College of Obstetricians and Gynaecologists (www.rcog.org.uk/en)

Date searched: 13 November 2015.

1. Browsed all guidelines.
2. Searched all guidelines by keyword – RhD or rhesus.

Four relevant guidelines were found.

Turning Research into Practice database (www.tripdatabase.com)

Date searched: 17 November 2015.

(rhd OR rhesus OR "rh negative" or "rh positive") AND title:(pregnan* OR maternal OR antenatal OR ante-natal OR prenatal OR pre-natal OR intrapartum)

A total of 37 results were retrieved and browsed for relevance; 17 relevant guidelines were found.

UK National Screening Committee (www.gov.uk/government/groups/uk-national-screening-committee-uk-nsc)

Date searched: 13 November 2015.

Recommendations list was filtered by antenatal and the resulting list browsed.

One relevant report was found.

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Search strategies: systematic reviews of antenatal anti-D prophylaxis

MEDLINE and MEDLINE In-Process & Other Non-Indexed Citations (via Ovid, http://ovidsp.ovid.com/)

Date range searched: 1946 to October Week 5 2015.

Date searched: 18 January 2016.

Records retrieved: 45.

The search was updated on 4 March 2016, retrieving 45 records.

1. systematic$ review$.ti,ab. (75,835)
2. meta-analysis as topic/ (14,365)
3. meta-analytic$.ti,ab. (4298)
4. meta-analysis.ti,ab,pt. (89,180)
5. metanalysis.ti,ab. (140)
6. metaanalysis.ti,ab. (1210)
7. meta analysis.ti,ab. (70,616)
8. meta-synthesis.ti,ab. (331)
9. metasynthesis.ti,ab. (166)
10. meta synthesis.ti,ab. (331)
11. meta-regression.ti,ab. (3249)
12. metaregression.ti,ab. (344)
13. meta regression.ti,ab. (3249)
14. (synthes$ adj3 literature).ti,ab. (1689)
15. (synthes$ adj3 evidence).ti,ab. (4926)
16. integrative review.ti,ab. (1177)
17. data synthesis.ti,ab. (7985)
18. (research synthesis or narrative synthesis).ti,ab. (1041)
19. (systematic study or systematic studies).ti,ab. (8551)
20. (systematic comparison$ or systematic overview$).ti,ab. (2200)
21. evidence based review.ti,ab. (1467)
22. comprehensive review.ti,ab. (8251)
23. critical review.ti,ab. (11,964)
24. quantitative review.ti,ab. (517)
25. structured review.ti,ab. (542)
26. realist review.ti,ab. (102)
27. realist synthesis.ti,ab. (73)
28. or/1-27 (187,703)
29. review.pt. (2,049,547)
30. medline.ab. (68,680)
31. pubmed.ab. (46,181)
32. cochrane.ab. (39,786)
33. embase.ab. (40,092)
34. cinahl.ab. (12,936)
35. psyc?lit.ab. (879)
36. psyc?info.ab. (10,559)
37. (literature adj3 search$).ab. (32,390)
38. (database$ adj3 search$).ab. (30,393)
39. (bibliographic adj3 search$).ab. (1461)
40. (electronic adj3 search$).ab. (11,252)
41. (electronic adj3 database$).ab. (13,910)

42. (computeri?ed adj3 search$).ab. (2857)
43. (internet adj3 search$).ab. (2045)
44. included studies.ab. (9670)
45. (inclusion adj3 studies).ab. (8188)
46. inclusion criteria.ab. (44,510)
47. selection criteria.ab. (22,215)
48. predefined criteria.ab. (1258)
49. predetermined criteria.ab. (787)
50. (assess$ adj3 (quality or validity)).ab. (48,127)
51. (select$ adj3 (study or studies)).ab. (43,640)
52. (data adj3 extract$).ab. (34,903)
53. extracted data.ab. (8161)
54. (data adj2 abstracted).ab. (3617)
55. (data adj3 abstraction).ab. (1017)
56. published intervention$.ab. (121)
57. ((study or studies) adj2 evaluat$).ab. (121,595)
58. (intervention$ adj2 evaluat$).ab. (7046)
59. confidence interval$.ab. (258,288)
60. heterogeneity.ab. (106,141)
61. pooled.ab. (53,158)
62. pooling.ab. (8496)
63. odds ratio$.ab. (171,463)
64. (Jadad or coding).ab. (133,119)
65. or/30-64 (923,716)
66. 29 and 65 (141,974)
67. review.ti. (299,976)
68. 67 and 65 (62,549)
69. (review$ adj4 (papers or trials or studies or evidence or intervention$ or evaluation$)).ti,ab. (119,221)
70. 28 or 66 or 68 or 69 (340,645)
71. letter.pt. (897,674)
72. editorial.pt. (391,059)
73. comment.pt. (647,299)
74. 71 or 72 or 73 (1,445,828)
75. 70 not 74 (331856)
76. exp animals/ not humans/ (4,171,020)
77. 75 not 76 (321762)
78. "Rho(D) Immune Globulin"/ (1190)
79. (immune adj2 globulin adj2 rh$).ti,ab. (257)
80. anti-D.ti,ab. (2610)
81. (D-Gam or Partobulin or Rhophylac or WinRho).ti,ab. (47)
82. or/78-81 (3165)
83. 77 and 82 (45)

Key

/ = indexing term (MeSH heading)

$ = truncation

? = optional wildcard – stands for zero or one character

.ti,ab. = terms in either title or abstract fields

.pt. = publication type

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adj = terms next to each other (order specified)

adj2 = terms within two words of each other (any order)

Cochrane Database of Systematic Reviews (via Wiley Online Library, http://onlinelibrary.wiley.com/)

Issue 1 of 12, January 2016.

Date searched: 18 January 2016.

Records retrieved: 6.

The search was updated on 4 March 2016, retrieving six records from CDSR.

#1 MeSH descriptor: [Rho(D) Immune Globulin] this term only(51)

#2 (immune near/2 globulin near/2 rh*):ti,ab,kw(5)

#3 anti-D:ti,ab,kw(110)

#4 (D-Gam or Partobulin or Rhophylac or WinRho):ti,ab,kw(10)

#5 #1 or #2 or #3 or #4(119)

#6 #1 or #2 or #3 or #4 in Cochrane Reviews (Reviews and Protocols)(6)

Key

MeSH descriptor = indexing term (MeSH heading)

* = truncation

:ti,ab,kw = terms in either title or abstract or keyword fields

near/2 = terms within two words of each other (any order)

Database of Abstracts of Reviews of Effects (via Centre for Reviews and Dissemination, www.crd.york.ac.uk/CRDWeb)

Date range searched: inception to 31 March 2015.

Date searched on: 20 January 2016.

Records retrieved: 8.

1. (anti-D) IN DARE, HTA (15)
2. ((D-Gam or Partobulin or Rhophylac or WinRho)) IN DARE, HTA (1)
3. ((immune NEAR globulin NEAR rh*)) IN DARE, HTA (0)
4. ((immune NEAR rh* NEAR globulin)) IN DARE, HTA (0)
5. ((rh* NEAR immune NEAR globulin)) IN DARE, HTA (5)
6. ((rh* NEAR globulin NEAR immune)) IN DARE, HTA (0)
7. ((globulin NEAR rh* NEAR immune)) IN DARE, HTA (0)
8. ((globulin NEAR immune NEAR rh*)) IN DARE, HTA (0)
9. MeSH DESCRIPTOR Rho(D) Immune Globulin IN DARE,HTA (5)
10. #1 OR #2 OR #5 OR #9 (15)
11. (#1 or #2 or #5 or #9) IN DARE (8)
12. (#1 or #2 or #5 or #9) IN HTA (7)

Health Technology Assessment database (via Centre for Reviews and Dissemination, www.crd.york.ac.uk/CRDWeb)

Date range searched: inception to 31 March 2015.

Date searched: 20 January 2016.

Records retrieved: 7.

See above under *Database of Abstracts of Reviews of Effects* for search strategy used.

PubMed (www.ncbi.nlm.nih.gov/pubmed)

Date searched on: 20 January 2016.

Records retrieved: 57.

The search was updated on 4 March 2016, retrieving 58 records.

(((“Rho(D) Immune Globulin”[Mesh:noexp] OR “rh* immune globulin”[Title/Abstract]) OR (“RHO(D) antibody”[Supplementary Concept] OR “RHO(D) antibody”[All Fields] OR “anti d”[All Fields])) OR (Partobulin[Title/Abstract] OR Rhophylac[Title/Abstract] OR WinRho[Title/Abstract])) AND systematic[sb]

Search strategies: cost-effectiveness

EconLit (via Ovid, http://ovidsp.ovid.com/)

Date range searched: 1886 to November 2015.

Date searched: 4 December 2015.

Records retrieved: 4.

1. (RhD or “rhesus D” or “Rh(D)” or “Rh-(D)” or Rh D).ti,ab. (3)
2. (Rh-negative or Rh-positive).ti,ab. (0)
3. (Rhesus negative or Rhesus positive).ti,ab. (0)
4. ((rh or rhesus) adj2 (factor or factors or antigen$ or system or group)).ti,ab. (1)
5. ((isoimmuni$ or iso-immuni$ or isoimmune or iso-immune) adj6 (rh or rhesus or maternal or pregnan$)).ti,ab. (0)
6. ((alloimmuni$ or allo-immuni$ or alloimmune or allo-immune) adj6 (rh or rhesus or maternal or pregnan$)).ti,ab. (0)
7. ((unsensiti#ed or un-sensiti#ed or non-sensiti#ed) adj6 (rh or rhesus or maternal or pregnan$)).ti,ab. (0)
8. ((sensiti#ation$ or sensiti#ed) adj6 (rh or rhesus or maternal or pregnan$)).ti,ab. (0)
9. ((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) adj2 immuni#ation).ti,ab. (0)
10. ((rh or rhesus) adj2 (immuni#ation or autoimmuni#ation)).ti,ab. (0)
11. ((hemolytic or haemolytic) adj2 (disease$ or disorder$)).ti,ab. (0)
12. HDFN.ti,ab. (0)
13. ((rhesus or rh) adj2 (disease$ or disorder$)).ti,ab. (0)
14. ((rhesus or rh or RhD) adj2 (incompatib$ or antagonism)).ti,ab. (0)
15. ((erythroblastoses or erythroblastosis) adj2 f?etal$).ti,ab. (0)
16. or/1-15 (4)

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Key
$ = truncation

= mandated wildcard – stands for one character

? = optional wildcard – stands for zero or one character

.ti,ab. = terms in either title or abstract fields

adj2 = terms within two words of each other (any order)

NHS Economic Evaluations Database (via Centre for Reviews and Dissemination, www.crd.york.ac.uk/CRDWeb)

Date range searched: inception to 31 March 2015.

Date searched on: 4 December 2015.

Records retrieved: 6.

1	MeSH DESCRIPTOR Rh-Hr Blood-Group System EXPLODE ALL TREES	16
2	(RhD or "rhesus D" or Rh-D)	24
3	(Rh-negative or Rh-positive)	7
4	("Rhesus negative" or "Rhesus positive")	9
5	((rh or rhesus) NEAR2 (factor or factors or antigen* or system or group))	18
6	((factor or factors or antigen* or system or group) NEAR2 (rh or rhesus))	1
7	#1 OR #2 OR #3 OR #4 OR #5 OR #6	35
8	MeSH DESCRIPTOR Rh Isoimmunization	15
9	((isoimmuni* or iso-immuni* or isoimmune or iso-immune) NEAR6 (rh or rhesus or maternal or pregnan*))	10
10	((rh or rhesus or maternal or pregnan*) NEAR6 (isoimmuni* or iso-immuni* or isoimmune or iso-immune))	17
11	((alloimmuni* or allo-immuni* or alloimmune or allo-immune) NEAR6 (rh or rhesus or maternal or pregnan*))	12
12	((rh or rhesus or maternal or pregnan*) NEAR6 (alloimmuni* or allo-immuni* or alloimmune or allo-immune))	8
13	((unsensitised or unsensitized or un-sensitised or un-sensitized or non-sensitised or non-sensitized) NEAR6 (rh or rhesus or maternal or pregnan*))	3
14	((rh or rhesus or maternal or pregnan*) NEAR6 (unsensitised or unsensitized or un-sensitised or un-sensitized or non-sensitised or non-sensitized))	0
15	((sensitisation* or sensitization* or sensitised or sensitized)NEAR6 (rh or rhesus or maternal or pregnan*))	6
16	((rh or rhesus or maternal or pregnan*) NEAR6 (sensitisation* or sensitization* or sensitised or sensitized))	5
17	((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) NEAR2 (immunisation or immunization))	0
18	((immunisation or immunization) NEAR2 (fetomaternal or feto-maternal or foetomaternal or foeto-maternal))	0
19	((rh or rhesus) NEAR2 (immunisation or immunization or autoimmunisation or autoimmunization))	4
20	((immunisation or immunization or autoimmunisation or autoimmunization) NEAR2 (rh or rhesus))	0
21	#8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20	29
22	MeSH DESCRIPTOR Erythroblastosis, Fetal EXPLODE ALL TREES	18
23	((hemolytic or haemolytic) NEAR2 (disease* or disorder*))	16
24	((disease* or disorder*) NEAR2 (hemolytic or haemolytic))	1
25	(HDFN)	1

26	((rhesus or rh) NEAR2 (disease* or disorder*))	3
27	((disease* or disorder*) NEAR2 (rhesus or rh))	1
28	((rhesus or rh or RhD) NEAR2 (incompatib* or antagonism))	3
29	((incompatib* or antagonism) NEAR2 (rhesus or rh or RhD))	0
30	((erythroblastoses or erythroblastosis) NEAR2 (fetal* or foetal*))	14
31	#22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30	28
32	#7 OR #21 OR #31	56
33	MeSH DESCRIPTOR Prenatal Diagnosis	216
34	MeSH DESCRIPTOR Maternal Serum Screening Tests	5
35	MeSH DESCRIPTOR Hematologic Tests	30
36	((prenatal or pre-natal or antenatal or ante-natal) NEAR3 (test* or screen* or diagnos* or determin* or detect*))	380
37	((test* or screen* or diagnos* or determin* or detect*) NEAR3 (prenatal or pre-natal or antenatal or ante-natal))	171
38	((test* or screen* or diagnos* or determin* or detect*) NEAR3 (fetal or foetal or fetus* or foetus*))	124
39	((fetal or foetal or fetus* or foetus*) NEAR3 (test* or screen* or diagnos* or determin* or detect*))	130
40	(NIPD or NIPT)	6
41	#33 OR #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40	534
42	MeSH DESCRIPTOR Genotyping Techniques	6
43	((genotype* or genotyping) NEAR2 (fetal or foetal or fetus* or foetus* or prenatal or pre-natal or antenatal or ante-natal))	3
44	((fetal or foetal or fetus* or foetus* or prenatal or pre-natal or antenatal or ante-natal) NEAR2 (genotype* or genotyping))	3
45	((genotype* or genotyping) NEAR2 (maternal or pregnan*))	2
46	((maternal or pregnan*) NEAR2 (genotype* or genotyping))	2
47	((genotype* or genotyping) NEAR2 (noninvasive or non-invasive))	1
48	((noninvasive or non-invasive) NEAR2 (genotype* or genotyping))	4
49	("cell-free foetal DNA" or "cell-free fetal DNA")	7
50	(cffDNA)	2
51	#42 OR #43 OR #44 OR #45 OR #46 OR #47 OR #48 OR #49 OR #50	18
52	#32 AND #41	16
53	#32 AND #51	6
54	#52 OR #53	18

Please note that the total number of hits at line 54 refers to the total number of results from DARE, HTA database and NHS EED.

Key

MeSH DESCRIPTOR = indexing term (MeSH heading)

* = truncation

NEAR2 = terms within two words of each other (order specified)

" " = phrase search

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Research Papers in Economics (http://repec.org/)

Date searched: 4 December 2015.

Records retrieved: 0.

"RhD" | rhesus | "hemolytic disease" | "haemolytic disease" | HDFN | erythroblastoses | erythroblastosis | "fetomaternal immunisation" | "fetomaternal immunization" | "foetomaternal immunisation" | "foetomaternal immunization"

Key

" " = phrase search

| = OR

Appendix 2 Included studies

TABLE 35 List of included studies

Study (author, date)	Full title	Country	Linked publications
Included studies: diagnostic accuracy			
Akolekar *et al.*, 2011[19]	Fetal *RHD* genotyping in maternal plasma at 11–13 weeks of gestation. *Fetal Diagn Ther* **29**:301–6	UK (London)	None
Banch Clausen *et al.*, 2014[20]	Routine non-invasive prenatal screening for fetal *RHD* in plasma of RhD-negative pregnant women – 2 years of screening experience from Denmark. *Prenat Diagn* **34**:1000–5	Denmark	Full-text papers: Damkjaer *et al.*, 2012;[27] and Banch Clausen *et al.*, 2012[24] Abstracts: Banch Clausen;[35] and Dziegiel *et al.*, 2012[37]
Chitty *et al.*, 2014[12]	Diagnostic accuracy of routine antenatal determination of fetal *RHD* status across gestation: population based cohort study. *BMJ* **349**:g5243	UK (Bristol)	Full-text paper: none Abstracts: Chitty *et al.*, 2011,[29] 2012;[30] and Daniels *et al.*, 2012[31]
Finning *et al.*, 2008[17]	Effect of high throughput *RHD* typing of fetal DNA in maternal plasma on use of anti-RhD immunoglobulin in RhD negative pregnant women: prospective feasibility study. *BMJ* **336**:816–18	UK (Bristol)	None
Grande *et al.*, 2013[22]	Clinical application of midtrimester non-invasive fetal *RHD* genotyping and identification of *RHD* variants in a mixed-ethnic population	Spain	None
Soothill *et al.*, 2015[18]	Use of cffDNA to avoid administration of anti-D to pregnant women when the fetus is RhD-negative: implementation in the NHS. *BJOG* **122**:1682–6	UK (Bristol)	None
Thurik *et al.*, 2015[21]	Analysis of false-positive results of fetal *RHD* typing in a national screening program reveals vanishing twins as potential cause for discrepancy	The Netherlands	Full-text paper: de Haas *et al.*, 2012[25] Abstracts: de Haas *et al.*, 2012;[46,47] Scheffer *et al.*, 2013;[44] Thurik *et al.*, 2014;[42,43] van der Schoot *et al.*, 2005;[45] and Veldhuisen *et al.*, 2013,[41] 2014[40]
Wikman *et al.*, 2012[23]	Non-invasive single-exon fetal *RHD* determination in a routine screening program in early pregnancy. *Obstet Gynecol* **120**:227–34	Sweden	Full-text papers: none Abstracts: Tiblad *et al.*, 2010;[54] Wikman *et al.*, 2010,[53] 2011,[51] 2012;[50] and Wikman 2013[52]
Included studies: clinical effectiveness			
Banch Clausen *et al.*, 2014[20]	Routine non-invasive prenatal screening for fetal *RHD* in plasma of RhD-negative pregnant women – 2 years of screening experience from Denmark. *Prenat Diagn* **34**:1000–5	Denmark	Full-text papers: Banch Clausen *et al.*, 2012;[24] and Damkjaer *et al.*, 2012[27] Abstracts: Banch Clausen *et al.*, 2011;[38] Banch Clausen 2012;[35,36] Dziegiel *et al.*, 2012;[37] and Steffensen *et al.*, 2012[39]

continued

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TABLE 35 List of included studies (*continued*)

Study (author, date)	Full title	Country	Linked publications
Banch Clausen *et al.*, 2012[24]	Report of the first nationally implemented clinical routine screening for fetal *RHD* in D- pregnant women to ascertain the requirement for antenatal RhD prophylaxis. *Transfusion* **52**:752–8	Denmark	Full-text papers: Banch Clausen *et al.*, 2014;[20] and Damkjaer *et al.*, 2012[27] Abstracts: Banch Clausen *et al.*, 2011;[38] Banch Clausen 2012;[35,36] Dziegiel *et al.*, 2012;[37] and Steffensen *et al.*, 2012[39]
Damkjaer *et al.*, 2012[27]	Study of compliance with a new, targeted antenatal D immunisation prevention programme in Denmark. *Vox Sang* **103**:145–9	Denmark	Full-text papers: Banch Clausen *et al.*, 2012,[24] 2014[20] Abstracts: Banch Clausen *et al.*, 2011;[38] Banch Clausen 2012;[35,36] Dziegiel *et al.*, 2012;[37] and Steffensen *et al.*, 2012[39]
de Haas *et al.*, 2012[25]	A nation-wide fetal *RHD* screening programme for targeted antenatal and postnatal anti-D. *ISBT Sci Ser* **7**:164–7	The Netherlands	Full-text paper: Thurik *et al.*, 2015[21] Abstracts: de Haas *et al.*, 2012,[46] 2013;[47] Thurik *et al.*, 2014;[42,43] Scheffer *et al.*, 2013;[44] van der Schoot *et al.*, 2005;[40–45] and Veldhuisen *et al.*, 2013,[41] 2014[40]
Grande *et al.*, 2013[22]	Clinical application of midtrimester non-invasive fetal *RHD* genotyping and identification of *RHD* variants in a mixed-ethnic population	Spain	None
Soothill *et al.*, 2015[18]	Use of cffDNA to avoid administration of anti-D to pregnant women when the fetus is RhD-negative: implementation in the NHS. *BJOG* **122**:1682–6	UK (Bristol)	Full-text paper: none Abstracts: Finning *et al.*, 2014,[33] 2015[32]
Tiblad *et al.*, 2013[26]	Targeted routine antenatal anti-D prophylaxis in the prevention of RhD immunisation–outcome of a new antenatal screening and prevention program. *PLOS ONE* **8**(8)	Sweden	Full-text paper: none Abstracts: Tiblad 2012;[57] and Tiblad *et al.*, 2012,[55] 2014[56]
Included studies: implementation			
Banch Clausen *et al.*, 2014[20]	Routine non-invasive prenatal screening for fetal *RHD* in plasma of RhD-negative pregnant women – 2 years of screening experience from Denmark. *Prenat Diagn* **34**:1000–5	Denmark	Report: Banch Clausen *et al.*, 2012[24] Full-text papers: Banch Clausen *et al.*, 2013;[13] and Damkjaer *et al.*, 2012[27] Abstract: Banch Clausen *et al.*, 2011[38]
Banch Clausen *et al.*, 2012[24]	Report of the first nationally implemented clinical routine screening for fetal *RHD* in D- pregnant women to ascertain the requirement for antenatal RhD prophylaxis. *Transfusion* **52**:752–8	Denmark	Linked to above
Clausen *et al.*, 2013[13]	Pre-analytical conditions in non-invasive prenatal testing of cell-free fetal *RHD*. *PLOS ONE* **8**:e76990	Denmark	Linked to above
Damkjaer *et al.*, 2012[27]	Study of compliance with a new, targeted antenatal D immunisation prevention programme in Denmark. *Vox Sang* **103**:145–9	Denmark	Linked to above
Brojer *et al.*, 2005[94]	Non-invasive determination of fetal *RHD* status by examination of cell-free DNA in maternal plasma.' *Transfusion* **45**:1473–80	Poland	None

TABLE 35 List of included studies (*continued*)

Study (author, date)	Full title	Country	Linked publications
Finning *et al.*, 2008[17]	Effect of high throughput *RHD* typing of fetal DNA in maternal plasma on use of anti-RhD immunoglobulin in RhD negative pregnant women: prospective feasibility study. *BMJ* **336**:816–18	UK (Bristol)	Full-text paper: none Abstract: Finning *et al.*, 2014,[33] 2015[32]
Grande *et al.*, 2013[22]	Clinical application of midtrimester non-invasive fetal *RHD* genotyping and identification of *RHD* variants in a mixed-ethnic population	Spain	None
Thurik *et al.*, 2015[21]	Analysis of false-positive results of fetal *RHD* typing in a national screening program reveals vanishing twins as potential cause for discrepancy	The Netherlands	Full-text paper: none Abstracts: Veldhuisen *et al.*, 2014[40]
de Hass *et al.*, 2012[25]	A nation-wide fetal *RHD* screening programme for targeted antenatal and postnatal anti-D. *ISBT Sci Ser* **7**:164–7	The Netherlands	Linked to Thurik *et al.*[21]
Oxenford *et al.*, 2013[28]	Routine testing of fetal Rhesus D status in Rhesus D negative women using cell-free fetal DNA: an investigation into the preferences and information needs of women. *Prenat Diagn* **33**:688–94	UK (London)	None
Soothill *et al.*, 2015[18]	Use of cffDNA to avoid administration of anti-D to pregnant women when the fetus is RhD-negative: implementation in the NHS. *BJOG* **122**:1682–6	UK (Bristol)	None
Wikman *et al.*, 2012[23]	Non-invasive single-exon fetal *RHD* determination in a routine screening program in early pregnancy. *Obstet Gynecol* **120**:227–34	Sweden	Full-text paper: Tiblad *et al.*, 2013[26] Abstract: Wikman *et al.*, 2011,[51] 2012[50]
Tiblad *et al.*, 2013[26]	Targeted routine antenatal anti-D prophylaxis in the prevention of RhD immunisation–outcome of a new antenatal screening and prevention program. *PLOS ONE* **8**:e70984	Sweden	Linked to Wikman *et al.*[23]

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Appendix 3 List of excluded studies

Not high-throughput non-invasive prenatal testing (123 references)

Abildinova G, Baynova M, Kamalieva B, Kostina A. Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma with real-time PCR assay. Clinical Chemistry and Laboratory Medicine Conference, IFCC-WorldLab-EuroMedLab, Berlin, 2011, pp. S691.

Achargui S, Benchemsi N. Fetal rhesus D genotyping by PCR using plasma from RhD negative pregnant women. 19th Regional Congress of the International Society of Blood Transfusion, Eastern, 2009, pp. 144.

Achargui S, Tijane M, Benchemsi N. [Fetal *RHD* genotyping by PCR using plasma from D negative pregnant women.] *Transfus Clin Biol* 2011;**18**:13–19. http://dx.doi.org/10.1016/j.tracli.2010.10.002

Ahangari G, Zeinali S, Ebrahimi M, Mohsani F, Saremi AT. Analysis of fetal sex and RhD gene in fetal cells DNA from maternal blood by polymerase chain reaction. *Middle East Fertil Soc J* 2003;**8**:263–8.

Ahmadi MH, Amirizadeh N, Azarkeyvan A, Valikhani A, Sayyadipoor F, Navidrouyan M. Fetal *RHD* genotyping in plasma of Rh negative pregnant women by real time PCR. 25th Regional Congress of the International Society of Blood Transfusion, 2015, pp. 302.

Allen RW, Ward S, Harris R. Prenatal genotyping for the RhD blood group antigen: considerations in developing an accurate test. *Genet Test* 2000;**4**:377–81. http://dx.doi.org/10.1089/109065700750065126

Al-Yatama MK, Mustafa AS, Al-Kandari FM, Khaja N, Zohra K, Monem RA, Abraham S. Polymerase-chain-reaction-based detection of fetal rhesus D and Y-chromosome-specific DNA in the whole blood of pregnant women during different trimesters of pregnancy. *Med Princ Pract* 2007;**16**:327–32.

Amaral DR, Credidio DC, Ribeiro K, Cobianchi Costa D, Castilho L. Complexities on *RHD* genotyping in pregnant women from a multi-ethnic population. AABB Annual Meeting and TXPO, New Orleans, LA, 2009, pp. 121A.

Amaral DR, Castilho L. Fetal *RHD* genotyping by analysis of maternal plasma in a mixed population. *Vox Sang* 2010. 31st International Congress of the Society of Blood Transfusion, pp. 25.

Amaral DR, Castilho L. Evaluation of non-invasive fetal *RHD* genotyping in a multi-ethnic population. *Transfusion* 2010. AABB Annual Meeting and CTTXPO, Baltimore, MD, pp. 149A.

Amaral DRT, Credidio DC, Pellegrino J Jr, Castilho L. Fetal *RHD* genotyping by analysis of maternal plasma in a mixed population. *J Clin Lab Anal* 2011;**25**:100–4.

Arntfield S, Ainsworth P, Mackay J, Gagnon R. Prenatal diagnosis of fetal RhD type using free fetal DNA (ffDNA) in maternal plasma: a pilot study. *Am J Obstet Gynecol* 2008;**199**:S119.

Atamaniuk J, Stuhlmeier KM, Karimi A, Mueller MM. Comparison of PCR methods for detecting fetal RhDin maternal plasma. *J Clin Lab Anal* 2009;**23**:24–8. http://dx.doi.org/10.1002/jcla.20282

Aubin JT, Le Van KC, Mouro I, Colin Y, Bignozzi C, Brossard Y, Cartron JP. Specificity and sensitivity of *RHD* genotyping methods by PCR-based DNA amplification. *Br J Haematol* 1997;**98**:356–64.

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Aykut A, Onay H, Sagol S, Gunduz C, Ozkinay F, Cogulu O. Determination of fetal rhesus d status by maternal plasma DNA analysis. *Balkan J Med Genet* 2013;**16**:33–8. http://dx.doi.org/10.2478/bjmg-2013-0029

Banzola I, Kaufmann I, Lapaire O, Hahn S, Holzgreve W, Rusterholz C. Isolation of serum nucleic acids for fetal DNA analysis: comparison of manual and automated extraction methods. *Prenat Diagn* 2008;**28**:1227–31. http://dx.doi.org/10.1002/pd.2154

Benachi A, Delahaye S, Leticee N, Jouannic JM, Ville Y, Costa JM. Impact of non-invasive fetal RhD genotyping on management costs of rhesus-D negative patients: results of a French pilot study. *Eur J Obstet Gynecolo Reprod Biol* 2012;**162**:28–32.

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Bombard AT, Akolekar R, Farkas DH, VanAgtmael AL, Aquino F, Oeth P, Nicolaides KH. Fetal *RHD* genotype detection from circulating cell-free fetal DNA in maternal plasma in non-sensitized RhD negative women. *Prenat Diagn* 2011;**31**:802–8. http://dx.doi.org/10.1002/pd.2770

Cardo L, Garcia BP, Alvarez FV. Non-invasive fetal *RHD* genotyping in the first trimester of pregnancy. *Clin Chem Lab Med* 2010;**48**:1121–6.

Chan FY, Cowley NM, Wolter L, Stone M, Carmody F, Saul A, Hyland CA. Prenatal *RHD* gene determination and dosage analysis by PCR: clinical evaluation. *Prenat Diagn* 2001;**21**:321–6. http://dx.doi.org/10.1002/pd.60

Chinen PA, Nardozzaa LMM, Camano L, Moron AF, Pares DBS, Martinhago CD, Daher S. Non-invasive fetal *RHD* genotyping by real-time polymerase chain reaction using plasma from D-negative Brazilian pregnant women. *J Reprod Immunol* 2007;**75**:A8–A9.

Chinen P, Lopes C, Nardozza L, Camano L, Martinhago C, Moron A. Determination of fetal *RHD* genotype in maternal blood, using the real-time polymerase chain reaction technique. *Int J Gynecol Obstet* 2009;**107**:S523.

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Clausen FB, Krog GR, Rieneck K, Nielsen LK, Lundquist R, Finning K, *et al.* Reliable test for prenatal prediction of fetal RhD type using maternal plasma from RhD negative women. *Prenat Diagn* 2005;**25**:1040–4. http://dx.doi.org/10.1002/pd.1248

Clausen FB, Krog GR, Rieneck K, Råsmark EE, Dziegiel MH. Evaluation of two real-time multiplex PCR screening assays detecting fetal *RHD* in plasma from RhD negative women to ascertain the requirement for antenatal RhD prophylaxis. *Fetal Diagn Ther* 2011;**29**:155–63. http://dx.doi.org/10.1159/000321347

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Cotorruelo C, Biondi C, Borrás SG, Galizzi S, Di Mónaco R, Racca A. Molecular determination of RhD phenotype by DNA typing: clinical applications. *Ann Clin Biochem* 2000;**37**:781–9. http://dx.doi.org/10.1258/0004563001900101

Cozac AC, Miyashiro K, Silva CG, Pinto GN, Rizzatti EG. Non-invasive fetal *RHD* genotyping by maternal plasma in a racially mixed population. *Transfusion* 2011;**51**:39A.

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Sequenom Inc. *Evaluation of a Noninvasive Fetal* RHD *Genotyping Test*. Clinical trial NCT01054716. URL: https://ClinicalTrials.gov/show/NCT01054716

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Appendix 4 Characteristics of diagnostic accuracy studies

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TABLE 36 Characteristics of diagnostic accuracy studies

Study	Country	Study dates	Number tested	Number analysed	Gestational age (weeks), median (range)	Ethnicity (%)	Multiple pregnancies included?	DNA extraction tool	PCR technology	Multiple testing performed?
Akolekar *et al.*, 2011[19]	England	NR	591	586	12.4 (11–14)	White European 77.3, Asian 1.2, African 19.3, mixed 2.2	No	MDx BioRobot (Qiagen, Crawley, UK)	ABI 7900 detection system (ABI, Applied Biosystems, Foster City, CA, USA)	Yes (for *RHD* variants)
Banch Clausen *et al.*, 2014[20]	Denmark	2010–11	14,547	12,668	25 (73% between 23 and 28)	NR	NR	QIAsymphony SP; MagNA Pure LC; MagNA Pure Compact Instrument (Roche Ltd, Rotkreuz, Switzerland)	ABI 7900 detection system (Applied Biosystems) LightCycler 480 (Roche) PCR ABI 7500 (Applied BioSystems)	NR
Chitty *et al.*, 2014[12]	England	2009–12	4913	4913	19 (5–35) (18% under 11 weeks)	White European 78, Asian 6, Black or mixed race 4, unknown 12	No	MDx BioRobot (Qiagen, Crawley, UK)	ABI Prism 7900HT (Applied Biosystems)	Up to four samples per woman
Finning *et al.*, 2008[17]	England	NR	1997	1869	28 (8–38) (92% at 26–32)	White European 55, Asian 8, African 2, other 2, unknown 33	Yes ($n = 13$ pregnancies)	MDx BioRobot (Qiagen, Crawley, UK)	ABI Prism 7900HT (Applied Biosystems)	NR
Grande *et al.*, 2013[22]	Spain	February 2010–October 2011	284	282	24–26	White European 84, Asian 1.5, African 1.8, Latin American 12, other 0.7	Yes ($n = 16$ pregnancies)	COBAS AmpliPrep (Roche Ltd, Rotkreuz, Switzerland)	7300 Real-Time PCR System (Applied Biosystems)	Yes, two independent assays performed in triplicate for all
Soothill *et al.*, 2015[18]	England	April–September 2013	526	499	15–26	NR	No	MDx BioRobot (Qiagen, Crawley, UK)	NR	NR
Thurik *et al.*, 2015[21]	The Netherlands	July 2011–October 2012	24,986	18,383	26	NR	No	MagNa Pure 96 (Roche Ltd, Rotkreuz, Switzerland)	StepOnePlus Real-Time PCR System (Applied Biosystems)	Yes, PCR in triplicate
Wikman *et al.*, 2012[23]	Sweden	September 2009–May 2011	4118	3291	10 (3–40) (75.5% first trimester, 10% tested before 8 weeks)	NR	Yes ($n = 61$ pregnancies)	MagNA Pure LC (Roche Ltd, Rotkreuz, Switzerland)	PCR ABI 7500 (Applied BioSystems)	Yes, PCR on all samples in triplicate 211 samples reanalysed because of uninterpretable results

NR, not reported.

Appendix 5 Risk of bias and applicability of findings of diagnostic accuracy studies

TABLE 37 Risk of bias: patient selection

Study	Was a consecutive sample of patients enrolled?	Did the study avoid inappropriate exclusions?	Were key study population characteristics reported? (including ethnicity, GA, multiple pregnancies)	Risk of bias	Applicability: are there concerns that the included patients do not match the target population?
Akolekar *et al.*, 2011[19]	Unclear	No, excluded multiple pregnancies	Yes	High, reporting of selection process limited, much higher proportion of African than general population (19.3%)	Yes, much higher proportion of people of African ethnicity than general population (19.3%)
Banch Clausen *et al.*, 2014[20]	Unclear, not stated but seems likely	Unclear, appears fine	No, population characteristics (including ethnicity) NR	Low	Unclear, population characteristics (including ethnicity) NR
Chitty *et al.*, 2014[12]	Unclear, not stated but seems likely	No, excluded multiple pregnancies	Yes	Low	No
Finning *et al.*, 2008[17]	Unclear, not stated but seems likely	Yes	Yes	Low	No
Grande *et al.*, 2013[22]	Unclear	Yes	Yes	Low	Yes, ethnic distribution differs from general UK population (12% Latin American)
Soothill *et al.*, 2015[18]	Unclear, not stated but seems likely	Yes	No, ethnicity and multiple pregnancy NR. Gestational range could be inferred but was not clearly reported	Low	No
Thurik *et al.*, 2015[21]	Unclear, not stated but seems likely	No, multiple pregnancies excluded and treated as positive	No, ethnicity and number of multiple pregnancies NR	Low	Yes, exclusion of multiple pregnancies
Wikman *et al.*, 2012[23]	Unclear, not stated but seems likely	Unclear, exclusion criteria not reported	No, ethnicity NR	Low	Unclear, ethnicity unknown

NR, not reported.

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TABLE 38 Risk of bias: index test

Study	Were the index test results interpreted without knowledge of the results of the reference standard?	If a threshold was used, was it prespecified?	Were results from replicate samples dealt with appropriately?	Were results from multiple pregnancies dealt with appropriately	Risk of bias: could the conduct or interpretation of the index test have introduced bias?	Applicability: are there concerns that the index test, its conduct or interpretation differ from the review question?	Reporting: did the study report any adverse effect of the index test?
Akolekar *et al.*, 2011[19]	Unclear, likely not	Unclear, thresholds were reported, but unclear if prespecified	Yes	N/A, only singleton pregnancies	High, inconclusive results were not included in the main analysis. This may have inflated the accuracy estimates	Low	No
Banch Clausen *et al.*, 2014[20]	Yes	Yes	Unclear, NR	Unclear, NR	Low	Low	No
Chitty *et al.*, 2014[12]	Yes	Yes	Unclear, NR	N/A, only singleton pregnancies	Low	Low	No
Finning *et al.*, 2008[17]	Yes	Unclear, unclear if prespecified	Unclear, NR	Yes	Low	Low	No
Grande *et al.*, 2013[22]	Unclear	Unclear, unclear if prespecified	Yes	Yes	Low	Low	No
Soothill *et al.*, 2015[18]	Unclear, presumably as in Chitty *et al.*[12]	Unclear, presumably as in Chitty *et al.*[12]	Unclear, NR	Unclear, NR	Unclear, Presumably as in Chitty *et al.*[12]	Low	No
Thurik *et al.*, 2015[21]	Unclear, unclear for back-up plasma analysis, yes for samples not reanalysed	No, prediction algorithm is judged daily and adjusted as needed. *If we would have strictly followed the computed algorithm, the repeat rate would have been almost halved, with the expense of one false-negative and 20 more false-positive results*	Yes	No, all treated as positive and prescribed anti-D	High, change of diagnostic algorithm after start of study may have introduced bias	Low	No
Wikman *et al.*, 2012[23]	Unclear, likely not	Unclear	Yes	Yes	Low	High, only exon 4 was targeted	No

NR, not reported.

TABLE 39 Risk of bias: reference standard

Study	Is the reference standard likely to correctly classify the target condition?	Were the reference standard results interpreted without knowledge of the results of the index test?	Risk of bias: could the reference standard, its conduct or its interpretation have introduced bias?	Applicability: are there concerns that the study used a non-standard reference standard?	Reporting: did the study report any adverse effect of the reference standard?
Akolekar *et al.*, 2011[19]	Unclear, method NR	Unclear, NR	Unclear, method NR	Unclear, method NR	No
Banch Clausen *et al.*, 2014[20]	Yes	Unclear, NR	Low	Low	No
Chitty *et al.*, 2014[12]	Yes	Unclear, NR	Low	Low	No
Finning *et al.*, 2008[17]	Yes	Yes	Low	Low	No
Grande *et al.*, 2013[22]	Yes	Unclear, NR	Low	Low	No
Soothill *et al.*, 2015[18]	Yes	Unclear, NR	Low	Low	No
Thurik *et al.*, 2015[21]	Yes	Unclear, NR	Low	Low	No
Wikman *et al.*, 2012[23]	Yes	Unclear, NR	Low	Low, author contacted: appropriate except 5% of samples processed in citrate tubes	No

NR, not reported.

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TABLE 40 Risk of bias: flow and timing

Study	Was there an appropriate interval between index test(s) and reference standard?	Did all patients (who provided data) receive a reference standard?	Did all patients receive the same reference standard?	Were all patients included in the analysis?	Risk of bias: could the patient flow have introduced bias?
Akolekar *et al.*, 2011[19]	Yes	No, only those with reference standard result and live birth were included in the study	Unclear	No, only those with reference standard result and live birth were included in the study	Low
Banch Clausen *et al.*, 2014[20]	Yes	No	Yes	Yes	Low
Chitty *et al.*, 2014[12]	Yes	No, 185 without cord blood result, but unlikely significant bias	Yes	No, 13% excluded for various reasons (all reported)	Low
Finning *et al.*, 2008[17]	Yes	No, four did not because of fetal death	Yes	No, 128 fetal phenotypes were not available for paired analysis because 124 cord samples were untraceable and there were four fetal deaths	Low
Grande *et al.*, 2013[22]	Yes	Yes, appears so	Yes	No, only two RhD-positive mothers who underwent NIPT were excluded	Low
Soothill *et al.*, 2015[18]	Yes	No, 5% did not have cord blood serology results	Yes	Yes	Low
Thurik *et al.*, 2015[21]	Yes	No, 80% did. No reason provided for 20% not providing cord blood serology	Yes	No, 20% samples received NIPT but not cord serology	High, 20% samples received NIPT but not cord serology. No reasons provided
Wikman *et al.*, 2012[23]	Yes	No, 11% pregnancies with no reference standard measurement	No, 5% citrate samples (author contacted)	No, 11% pregnancies with no reference standard measurement	Low, despite limitations, risk of diagnostic accuracy results being significantly affected was not considered high

Appendix 6 Additional figures and tables for diagnostic accuracy analyses

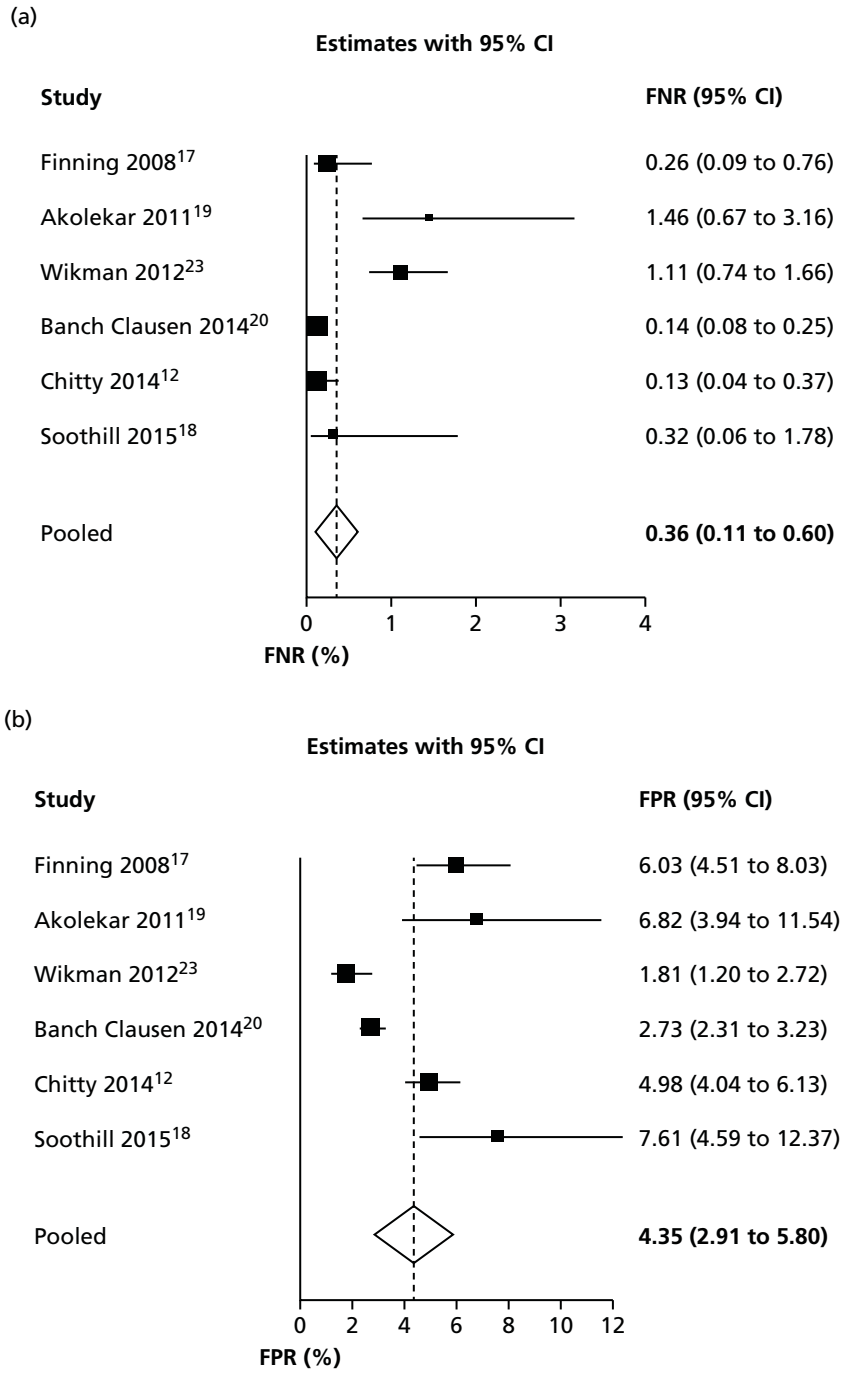

FIGURE 19 Forest plots for analysis case 2.

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Receiver operating characteristic plot for analysis case 3

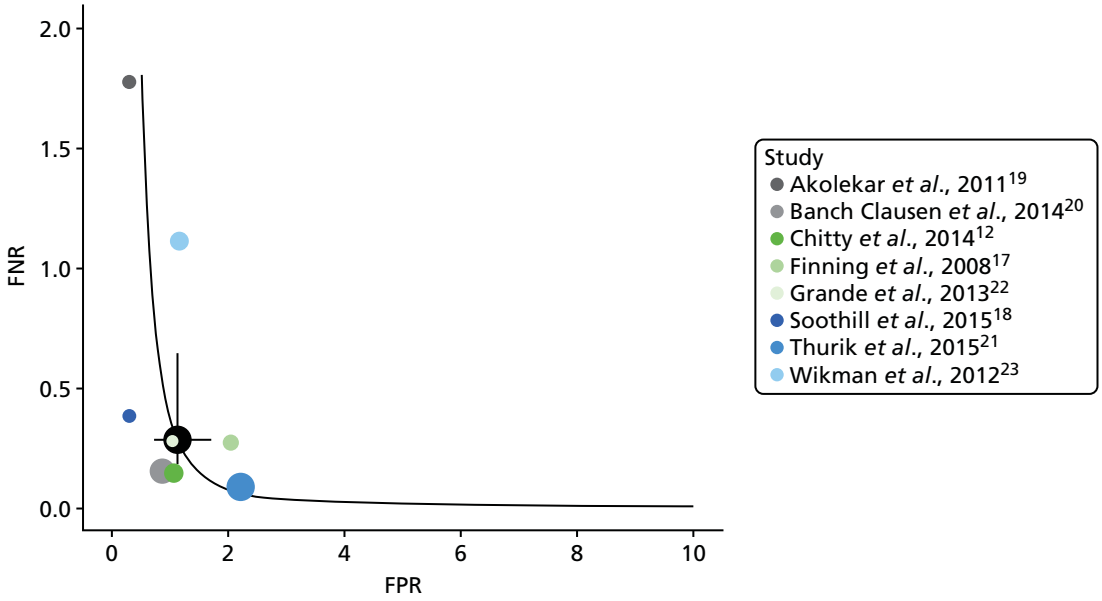

FIGURE 20 Receiver operating characteristic plot for analysis case 3.

TABLE 41 Results of diagnostic SAs

Analysis	FNR, % (95% CI)	FPR, % (95% CI)
Excluding Akelokar *et al.*, 2011[19] and Thurik *et al.*, 2015[21]		
Inconclusives treated as positive (with Grande *et al.*, 2013[22])	0.315 (0.14 to 0.70)	3.837 (2.36 to 6.19)
Inconclusives treated as positive (without Grande *et al.*, 2013[22])	0.260 (0.10 to 0.65)	4.004 (2.40 to 6.60)
Excluding inconclusives	0.349 (0.16 to 0.77)	1.205 (0.87 to 1.67)
Excluding Wikman *et al.*, 2012[23]		
Inconclusives treated as positive (with Thurik *et al.*, 2015[21] and Grande *et al.*, 2013[22])	0.292 (0.13 to 0.65)	4.478 (2.92 to 6.81)
Inconclusives treated as positive (without Thurik *et al.*, 2015[21] and Grande *et al.*, 2013[22])	0.334 (0.13 to 0.84)	5.245 (3.54 to 7.71)
Excluding inconclusives	0.279 (1.12 to 0.67)	1.142 (0.69 to 1.90)

Appendix 7 Quality assessment of clinical effectiveness studies

This appendix presents quality assessment tables performed for the two comparative studies included in the review of effectiveness studies.

When multiple outcomes were assessed within the same study, risk-of-bias judgements did not differ across outcomes unless otherwise specified. Further details of the quality assessment, including prespecified target randomised trials, target comparisons and specified confounding domains are available on request.

For full guidance, see Sterne JAC, Higgins JPT, Reeves BC on behalf of the development group for ACROBAT-NRSI. *A Cochrane Risk Of Bias Assessment Tool: for Non-Randomized Studies of Interventions (ACROBAT-NRSI)*. Version 1.0.0. 2014. URL: www.riskofbias.info (accessed 1 March 2016).

Risk-of-bias assessment: Banch Clausen *et al.*[20]

Outcomes and results assessed

Outcomes assessed	Compliance with prenatal anti-D
	Compliance with postnatal anti-D
	Compliance with *RHD* screening
Specific results being assessed	Compliance with antenatal anti-D: 93.2% vs. not applicable (not recommended in patients not receiving *RHD* screening)
	Compliance with postnatal anti-D: 99.7% vs. 95.7%
	Compliance with *RHD* screening: 84.2%

Risk-of-bias table for Banch Clausen et al.[20]

Bias domain	Signalling question	Judgement	Comment
Bias attributable to confounding	Is confounding of the effect of intervention unlikely in this study?	PN	Unadjusted analyses
	Were participants analysed according to their initial intervention group throughout follow-up?	PY	
	Did the authors use an appropriate analysis method that adjusted for all the critically important confounding domains?	No	Unadjusted analyses
	Risk-of-bias judgement	Critical	Analyses not adjusted for several potential confounders (including potential sensitising event, anti-D prophylaxis compliance, gestational age)
	What is the predicted direction of bias due to confounding?	Unpredictable	

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Bias domain	Signalling question	Judgement	Comment
Bias in selection of participants into the study	Was selection into the study unrelated to intervention or unrelated to outcome?	No	
	Do start of follow-up and start of intervention coincide for most subjects?	Yes	
	Were adjustment techniques used that are likely to correct for the presence of selection biases?	No	
	Risk-of-bias judgement	NI	Only participants from one of the five regions over 1 year (690/12,668) were included. Reasons were not provided
	What is the predicted direction of bias due to selection of participants into the study?	Unpredictable	
Bias in measurement of interventions	Is intervention status well defined?	Yes	*RHD* screening
	Was information on intervention status recorded at the time of intervention?	PY	
	Was information on intervention status unaffected by knowledge of the outcome or risk of the outcome?	Yes	
	Risk-of-bias judgement	Low	
	What is the predicted direction of bias due to measurement of outcomes or interventions?	Towards null	Low risk of bias
Bias due to departures from intended interventions	Were the critical cointerventions balanced across intervention groups?	NI	No information on non-routine anti-D and whether or not it was measured as separate from routine administration
	Were numbers of switches to other interventions low?	Yes	N/A
	Was implementation failure minor?	PY	No information but unlikely
	Were adjustment techniques used that are likely to correct for these issues?	No	
	Risk-of-bias judgement	Low	
	What is the predicted direction of bias due to departures from the intended interventions?	Towards null	Low risk of bias
Bias due to missing data	Are outcome data reasonably complete?	NI	No information on missing data
	Was intervention status reasonably complete for those in whom it was sought?	NI	
	Are data reasonably complete for other variables in the analysis?	No	Lack of reported data on confounders
	Are the proportion of participants and reasons for missing data similar across interventions?	NI	No information on missing data
	Were appropriate statistical methods used to account for missing data?	N/A	
	Risk-of-bias judgement	NI	
	What is the predicted direction of bias due to missing data?	Unpredictable	

Bias domain	Signalling question	Judgement	Comment
Bias in measurement of outcomes	Was the outcome measure objective?	Yes	
	Were outcome assessors unaware of the intervention received by study participants?	NI	
	Were the methods of outcome assessment comparable across intervention groups?	PY	
	Were any systematic errors in measurement of the outcome unrelated to intervention received?	NI	
	Risk-of-bias judgement	Low	No information to suggest otherwise
	What is the predicted direction of bias due to measurement of outcomes?	Towards null	
Bias in selection of the reported result	Is the reported effect estimate unlikely to be selected, on the basis of the results, from:		
	Multiple outcome measurements within the outcome domain?	PY	
	Multiple analyses of the intervention–outcome relationship?	PY	
	Different subgroups?	NI	Only participants from one of the five regions over 1 year (690/12,668) were included. Reasons were not provided
	Risk-of-bias judgement	NI	
	What is the predicted direction of bias due to selection of the reported result?	Unpredictable	
Overall bias	Risk-of-bias judgement	Critical	Only participants from one of the five regions over 1 year (690/12,668) were included. Analyses were not adjusted for any potential confounders
	What is the overall predicted direction of bias for this outcome?	Unpredictable	Unpredictable because of insufficient information, although may be more likely to favour the intervention

N/A, not applicable; NI, no information; PN, probably no; PY, probably yes.

Risk-of-bias assessment: Tiblad *et al.*[26]

Outcomes and results assessed

Outcomes assessed	Sensitisation (measured as development of anti-D antibodies after the first trimester of pregnancy or post partum)
Results	Adjusted odds ratio 0.41 (95% CI 0.22 to 0.78), 0.19% vs. 0.46% (favours intervention)

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Risk-of-bias table for Tiblad et al.[26]

Bias domain	Signalling question	Judgement	Comment
Bias due to confounding	Is confounding of the effect of intervention unlikely in this study?	No	Study with historical control and insufficiently adjusted analysis
	Were participants analysed according to their initial intervention group throughout follow-up?	No	In the reference group, no routine postpartum antibody testing was performed. The outcome was measured in the first trimester of the subsequent pregnancy
	Were intervention discontinuations or switches unlikely to be related to factors that are prognostic for the outcome?	PN	
	Did the authors use an appropriate analysis method that adjusted for all the critically important confounding domains and for time-varying confounding?	PN	Analyses adjusted for NIPT sensitivity. No significant differences in gestational age and preterm births. Compliance with RAADP not adjusted for
	Were confounding domains that were adjusted for measured validly and reliably by the variables available in this study?	N/A	
	Risk-of-bias judgement	Serious	Study with historical control. No adjustment for RAADP compliance or sensitising event
	What is the predicted direction of bias due to confounding?	Unpredictable	
Bias in selection of participants into the study	Was selection into the study unrelated to intervention or unrelated to outcome?	No	The control group was historical, pretargeted routine anti-D prophylaxis. In the reference group, immunisation after delivery was defined as presence of anti-D antibodies in the first trimester in the subsequent pregnancy. Routine antibody testing at 25 weeks in nulliparous women in routine management group was not performed
	Do start of follow-up and start of intervention coincide for most subjects?	PN	Only clear for intervention group, probably not for routine management group
	Were adjustment techniques used that are likely to correct for the presence of selection biases?	No	
	Risk-of-bias judgement	Serious	The control group was historical, pre-targeted routine anti-D prophylaxis. In the reference group, immunisation was defined as presence of anti-D antibodies in the first trimester in a subsequent pregnancy. This means that any pregnant woman with no recorded subsequent pregnancy was excluded

Bias domain	Signalling question	Judgement	Comment
	What is the predicted direction of bias due to selection of participants into the study?	Unpredictable	Insufficient information to assess, although it is possible events were underestimated in the reference group as sensitisation was not measured post partum in this group. On the other hand, it is plausible, as the authors stated, that not all women in the reference cohort had a subsequent pregnancy when antibodies from sensitisation late in the third trimester or at delivery in the previous pregnancy would be found, leading to rates of new RhD immunisations being somewhat underestimated
Bias in measurement of interventions	Is intervention status well defined?	Yes	
	Was information on intervention status recorded at the time of intervention?	Yes	
	Was information on intervention status unaffected by knowledge of the outcome or risk of the outcome?	Yes	'Hard' outcome
	Risk-of-bias judgement	Low	
	What is the predicted direction of bias due to measurement of outcomes or interventions?	Towards null	
Bias due to departures from intended interventions	Were the critical cointerventions balanced across intervention groups?	NI	
	Were numbers of switches to other interventions low?	PY	
	Was implementation failure minor?	NI	
	Were adjustment techniques used that are likely to correct for these issues?	No	
	Risk-of-bias judgement	Low	
	What is the predicted direction of bias due to departures from the intended interventions?	Unpredictable	
Bias due to missing data	Are outcome data reasonably complete?	NI	In the control group, it appears that any pregnant woman with no recorded subsequent pregnancy was excluded
	Was intervention status reasonably complete for those in whom it was sought?	NI	Insufficient information
	Are data reasonably complete for other variables in the analysis?	No	Limited data on participants excluded from the analyses as there was no recorded subsequent pregnancy in the reference group
	Are the proportion of participants and reasons for missing data similar across interventions?	NI	
	Were appropriate statistical methods used to account for missing data?	No	

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Bias domain	Signalling question	Judgement	Comment
	Risk-of-bias judgement	NI	In the control group, it appears that any pregnant woman with no recorded subsequent pregnancy was excluded (based on Tiblad *et al.*[26])
	What is the predicted direction of bias due to missing data?	Unpredictable	
Bias in measurement of outcomes	Was the outcome measure objective?	Yes	
	Were outcome assessors unaware of the intervention received by study participants?	NI	No mention of blinding
	Were the methods of outcome assessment comparable across intervention groups?	Yes	
	Were any systematic errors in measurement of the outcome unrelated to intervention received?	PN	
	Risk-of-bias judgement	Low	
	What is the predicted direction of bias due to measurement of outcomes?	Towards null	
Bias in selection of the reported result	Is the reported effect estimate unlikely to be selected, on the basis of the results, from:		
	multiple outcome measurements within the outcome domain?	PN	
	multiple analyses of the intervention–outcome relationship?	PN	
	different subgroups?	PN	
	Risk-of-bias judgement	Low	
	What is the predicted direction of bias due to selection of the reported result?	Towards null	
Overall bias	Risk-of-bias judgement	Serious	Primarily because of risk of selection bias, confounding and missing data
	What is the overall predicted direction of bias for this outcome?	Unpredictable	Unpredictable because of insufficient information. Note: the generalisability of the study findings to the UK is limited given that RAADP is recommended as part of routine care

N, no; NI, no information; PN, probably no; PY, probably yes; Y, yes.

Appendix 8 Summary of anti-D reviews

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TABLE 42 Summary of anti-D reviews

	Review details			Results					
Review	Studies	Anti-D group	Control	Outcome	Anti-D group	Control group	RR	Lower CI	Upper CI
McBain *et al.*, 2015[64]	2	Anti-D after 28 weeks	No treatment (standard care)	Alloimmunisation in pregnancy or post partum	5	13	0.42	0.15	1.17
	2			Alloimmunisation within one year	6	16	0.39	0.10	1.62
	1			Positive Kleihauer at birth	73	119	0.60	0.46	0.79
	1			Jaundice	1	4	0.26	0.03	2.30
Turner *et al.*, 2012[63]	10	Anti-D (500 IU) 28–34 weeks	Standard postpartum or at sensitisation	Postpartum sensitisation			0.31	0.17	0.56
Pilgrim *et al.*, 2009[62]	8 (total)	Anti-D (various doses) 28–34 weeks	No antenatal anti-D	Sensitisation					
	4			500 IU	0.30%	0.89%	0.33	0.20	0.55
	3			1500 IU	0.34%	1.60%	0.20	0.13	0.29
	2			500 IU community	0.35%	0.95%	0.37	0.21	0.65
	1			Compliance			90% dose 1, 79% dose 2		
Fyfe *et al.*, 2014[61]	8	Not described	None	Compliance			80–90%		

Appendix 9 Existing cost-effectiveness evidence: list of excluded papers

1. Bernhofen DM. The empirics of comparative advantage: overcoming the tyranny of nonrefutability. *Rev Int Econ* 2005;**13**:1017–23.

2. Druzic G. Bankarski sustav u RH. [Banking System in the Republic of Croatia. With English summary.] Zbornik Radova Ekonomskog Fakulteta u Rijeci: Casopis za Ekonomsku Teoriju i Praksu. *J Econ Bus* 2002;**20**:67–90.

3. Du Laney T, Dibner M, Moise K. Pharmacoeconomic analysis of prenatal determination of fetal *RHD* genotype through non-invasive maternal serum testing. *Am J Obst Gynecol* 2006;**195**:S119.

4. Duan Q, Liao TW. Optimization of blood supply chain with shortened shelf lives and ABO compatibility. *Int J Prod Econ* 2014;**153**:113–29.

5. Leistikow EA, Collin MF, Savastano GD, de Sierra TM, Leistikow BN. Wasted health care dollars. Routine cord blood type and Coombs' testing. *Arch Pediatr Adolesc Med* 1995;**149**:1147–51.

6. Ma KK, Rodriguez MI, Cheng YW, Norton ME, Caughey AB. Should cell-free DNA testing be used to target antenatal rhesus immune globulin administration? *J Matern Fetal Neonatal Med* 2015;**29**:1866–70.

7. Moise KJ. Costs and clinical outcomes of noninvasive fetal RhD typing for targeted prophylaxis. *Obstet Gynecol* 2013;**122**:1306. http://dx.doi.org/10.1097/AOG.0000000000000036

8. Roque H. Fetal RhD genotyping by maternal serum analysis: a two-year experience. *Am J Obstet Gynecol* 2006;**194**:905–6.

9. Szczepura A, Bonsel G, Krauth C, Osipenko L, Haverkamp A. Fetal *RHD* typing: Is fetal *RHD* typing in all RhD negative women cost effective? *BMJ* 2008;**336**:906. http://dx.doi.org/10.1136/bmj.39556.499549.80

10. van der Schoot CE, Soussan AA, Bonsel GJ, de Haas M. Non invasive screening for fetal *RHD*-genotype in all D-negative women is reliable and cost-effective. *Blood* 2005;**106**:165A.

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Appendix 10 Previous National Institute for Health and Care Excellence technology appraisals

Two previous TAs were carried out on RAADP. The more recent appraisal (NICE TA156) concluded that, compared with having no RAADP, RAADP reduces the incidence of sensitisation and, consequently, of haemolytic disease of the newborn infant. The economic analysis undertaken suggested that RAADP given to all RhD-negative pregnant women was likely to be cost-effective at a threshold of around £30,000 per QALY gained (*Table 43*). The total cost of providing RAADP to RhD-negative multigravidae in England and Wales was estimated to be around £2M–2.6M per year (2008 values). *Table 43* considers only results relating to the multigravidae option as, in the current work, we assume that anti-D immunoglobulin and high-throughput NIPT would be provided in all eligible pregnancy (women RhD-negative and not previously sensitised) and not restricted based on whether or not it was the woman's first pregnancy.

An updated assessment of RAADP was done under the current assessment. The following amendments and updating were performed:

- We made amendments to discount the total QALYs according to the timing of subsequent pregnancies and to retain a constant probability of RhD-positive fetus per pregnancy across the whole cohort of RhD-negative pregnant women.
- We updated the model to the current price year and more recent NHS reference costs.
- We updated the model to more recent population values, estimates of birth rates and sensitisation.

The previous model compared RAADP plus postpartum anti-D immunoglobulin with postpartum anti-D immunoglobulin only. Many elements that were common to both arms were omitted from the model but we are required to introduce them as they may be affected by the introduction of high-throughput NIPT. The following alterations to address the current decision problem were performed:

- We included the costs relating to potentially sensitising events (including phlebotomy, FMH test and anti-D immunoglobulin treatment).
- We included the costs relating to postpartum treatment (including cord serology, phlebotomy, FMH test and anti-D immunoglobulin treatment).

The routine anti-D immunoglobulin characterised in our model is determined by the results of the audit. We used actual rates of single- and two-dose regimen implementation to determine a weighted cost that is based on the lowest BNF price available. As a result of the amendments, the update and, most significantly, the introduction of additional doses of anti-D immunoglobulin for potentially sensitising events and post partum, the total costs in our updated model are significantly higher for every strategy but the QALYs are not markedly different (*Table 44*). The total cost of RAADP is estimated to be £16.7M and the total QALYs 2.4 million. The updated results are in line with the previous HTA showing that, under a probabilistic set up, RAADP has an ICER of £14,444 compared with no RAADP. This is lower than the previous estimate of £20,108, largely a result of the reduced unit cost of anti-D immunoglobulin based on updated BNF prices and the increased birth rate.

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TABLE 43 Incremental cost-effectiveness outcomes associated with RAADP vs. no RAADP (multigravidae) – NICE TA156[62]

Strategies	Incremental cost (£)	Number of sensitisations avoided	Number of affected pregnancies avoided	Number of fetal losses avoided	Life-years gained	Incremental QALYs	Cost per sensitisation avoided (£)	Cost per affected pregnancy avoided (£)	Cost per fetal loss avoided (£)	Cost per life-year gained (£)	ICER, cost per QALY gained (£)
No RAADP[a]	1,796,546	630.5	353.4	14.1	2,878,648	2,533,240	–	–	–	–	–
2 × 500 IU RAADP (multi)	2,645,120	232.9	72.1	2.9	120.4	100.0	11,358	36,679	916,982	21,977	26,455
1 × 1500 IU RAADP (multi)	2,010,568	232.9	72.1	2.9	120.4	100.0	8634	27,880	697,002	16,705	20,108

a No RAADP is an absolute amount.

TABLE 44 Incremental cost-effectiveness outcomes associated with RAADP vs. no RAADP in the current diagnostic assessment (2016): deterministic and probabilistic results

Strategies[a]	Incremental cost (£)	Number of sensitisations avoided	Number of affected pregnancies avoided	Number of fetal losses avoided	Life-years gained	Incremental QALYs	Cost per sensitisation avoided (£)	Cost per affected pregnancy avoided (£)	Cost per fetal loss avoided (£)	Cost per life-year gained (£)	ICER, cost per QALY gained (£)
Deterministic results											
No RAADP[b]	12,412,184	356.8	202.8	10.14	2,764,972	2,433,227	–	–	–	–	–
RAADP	3,576,953	218.69	124.38	6.22	257.46	195.13	16,356	28,758	575,167	13,893	18,331
Probabilistic results											
No RAADP[b]	13,203,011	406.29	249.07	12.47	2,764,874	2,432,875	–	–	–	–	–
RAADP	3,476,596	249.07	152.84	7.66	317.40	240.69	£13,959	22,747	454,043	10,953	14,444

a For both strategies prophylactic anti-D immunoglobulin after a potentially sensitising event is considered together with further postpartum anti-D immunoglobulin administration to any RhD-negative women whose baby's RhD status is confirmed to be positive after cord serology. For the RAADP strategy, treatment is delivered to all RhD-negative pregnant women, under either single- or two-dose regimens.
b No RAADP is an absolute amount.

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