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# Assessment Group's Report

**Title of the project**

**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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**Note**: This report contains some information which is currently commercial in confidence. Sections with confidential information are [redacted].

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# List of abbreviations

CI Confidence interval
HSROC Hierarchical summary receiver operating characteristic
ICER Incremental cost-effectiveness ratio
FNR False negative rate
FPR False positive rate
NHSBT NHS Blood and Transplant
NICE National Institute for Health and Care Excellence
NIPT Non-invasive prenatal testing
QALY Quality-adjusted life-year
QoL Quality of life
QUADAS Quality Assessment of Diagnostic Accuracy Studies
RhD Rhesus blood group (D antigen)
RR Relative risk

# 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:** Incorrect negative test result – number of diseased persons with a negative test result.

**False positive:** Incorrect positive test result – 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 whose 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.

**Meta-regression:** 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 which 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:** Proportion of people with the target disorder who have a positive test result.

**Specificity:** Proportion of people without the target disorder who have a negative test result.

**True negative:** Correct negative test result – number of non-diseased persons with a negative test result.

**True positive:** Correct positive test result – number of diseased persons with a positive test result.

# Abstract

*Introduction*

High-throughput non-invasive prenatal testing (NIPT) for fetal Rhesus D (RhD) status could avoid unnecessary treatment with routine anti-D immunoglobulin for RhD negative women found to be carrying an RhD negative fetus. We investigated the clinical and cost-effectiveness of high-throughput NIPT for fetal RhD status in RhD negative women not known to be sensitised to the RhD antigen for the NHS.

*Objectives*

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

*Methods*

We searched MEDLINE and other databases to February 2016. Two reviewers screened titles and abstracts. We undertook quality assessment. Bivariate models were fitted to calculate summary estimates of false-positive and false-negative rates with 95% confidence intervals (CIs). A narrative synthesis was employed for clinical effectiveness and implementation reviews. Clinical effectiveness evidence was used to conduct a simulation study.

To address limitations in existing studies we developed a *de-novo* probabilistic decision tree based cohort model to characterise the decision problem from a NHS and PSS perspective. The model was informed by evidence from the clinical effectiveness and cost effectiveness reviews and the NICE technology appraisal of routine anti-D immunoglobulin. The model considered four alternative ways by which the results of an NIPT test could guide the use of anti-D immunoglobulin antenatally and within post-partum management. A range of sensitivity analyses were conducted to address key uncertainties and model assumptions.

*Results*

3921 references records were identified through electronic searches. Eight studies were included in the diagnostic accuracy review, seven studies in the clinical effectiveness review, and twelves studies in the review of implementation. The majority of included studies were judged to be at low risk of bias. In the primary analysis for diagnostic accuracy, women with an inconclusive test result were treated as having tested positive. Meta-analyses showed that the pooled false negative rate (women at risk of sensitisation) was 0.34% (95% CI 0.15 to 0.76) and the pooled false positive rate (women needlessly receiving anti-D) was 3.86% (95% CI 2.54 to 5.82). Sensitivity analyses did not materially

alter the overall result. There was limited, poor quality evidence for the effectiveness of high-throughput NIPT testing on clinical outcomes including sensitisation rates. Our simulation suggests that use of NIPT testing could substantially reduce unnecessary use of antenatal anti-D in women with an RhD negative fetus with only a small increase in risk of sensitisation.

Seven cost-effectiveness studies were included in the review, and broadly the conclusions indicated that the potential for the use of the NIPT to produce cost savings was highly dependent on the cost of the test itself. Our *de-novo* economic model suggested that the use of high-throughput NIPT to guide the prenatal and post-partum provision of anti-D immunoglobulin prophylaxis is likely to be cost saving compared to current practice of providing RAADP to all women who are RhD-negative. The extent of the cost-saving appeared sufficient to outweigh the small increase in sensitisations and the associated small QALY loss through using high-throughput NIPT compared to current practice. However, the magnitude of the cost saving is highly sensitive to the cost of the 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.

*Conclusions*

High-throughput NIPT testing is sufficiently accurate to detect fetal RhD status in RhD negative women and would considerably reduce unnecessary treatment with routine anti-D immunoglobulin, potentially resulting in cost savings of between £296,000 and £409,000 per 100,000 pregancies.

## Plain English Summary

About three in twenty women in the UK have a blood type called RhD-negative. If they become pregnant around six in ten will have babies 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, potentially with severe consequences such as a need for blood transfusions or even 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 an RhD negative baby do not need this injection. A non-invasive prenatal blood test (NIPT) can 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 using this NIPT blood test was a reliable, effective, safe way to manage RhD negative pregnant women and whether 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 three and 27 additional sensitisations and a small loss in health per 100,000 pregnancies. However, the test is inconclusive in around 7% of women who could still be offered the anti-D injection. The evidence suggests that using the NIPT would reduce the number of women receiving anti-D unnecessarily but that this may or may not be cost saving depending on the additional cost of the NIPT.

# 1 Executive Summary

## 1.1 Background

Approximately 17% of women giving birth in England and Wales are RhD negative. Pregnant women who have RhD negative blood type may carry an 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 immune system response process, called sensitisation, can happen at any time during pregnancy, although it is most common in the third trimester and during childbirth. The process of sensitisation itself has no adverse effects to the mother and usually does not affect the pregnancy during which it occurs.

However, in a subsequent pregnancy with an 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, which may result in haemolytic disease of the fetus and newborn. 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.

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 an 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 serologic cord testing at birth. However, the clinical 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.

## 1.2 Objectives

This assessment aims to evaluate the clinical 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 with any consequent changes in treatment management.

## 1.3 Methods

### 1.3.1 Assessment of clinical effectiveness

A range of bibliographic databases including MEDLINE, MEDLINE In-Process, EMBASE, CINAHL, Maternity and Infant Care, Science Citation Index, CDSR, DARE, Cochrane Central Register of Controlled Trials (CENTRAL) were searched from inception to November 2015. An updated search was performed in February 2016. Both published and unpublished literature were

identified from systematic searches of electronic sources, consultation with experts in the field, and reference checking of relevant systematic reviews and included studies.

For diagnostic accuracy outcomes, we included prospective cohort studies reporting absolute numbers of true positive, false positive, true negative, and false negative, allowing the calculation of diagnostic accuracy. If reported, we extracted data on the number of inconclusive or undetermined results. For clinical effectiveness outcomes, we included any experimental or observational study in which high-throughput NIPT testing was used to determine fetal RhD status, where anti-D prophylaxis was given as required, and that reported relevant clinical outcomes for this appraisal. For implementation outcomes, we considered all publications reporting issues related to implementation of, or practical advice relating to, high-throughput NIPT testing as a screening tool to guide use of anti-D prophylaxis. We also sought to identify systematic reviews reporting any aspect of the process of using routine antenatal anti-D prophylaxis to prevent sensitisation.

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 DNA tests of maternal plasma used to determine fetal RhD status. The reference standard considered was serologic cord blood testing at birth, or any other suitable post-natal blood test of the infant.

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 (QUADAS-2) checklist.

For diagnostic accuracy outcomes, estimates of sensitivity, specificity, false-positive and false-negative rates were calculated and presented on forest plots and in receiver-operating characteristic (ROC) space to assess the heterogeneity in test accuracy within and between studies. The hierarchical bivariate model was fitted to calculate summary estimates of sensitivity, specificity, false-positive and false-negative rates and the associated 95% confidence intervals (CIs). The hierarchical summary ROC (HSROC) model was fitted to produce summary ROC curves. Sensitivity analyses were performed to explore the robustness of the results by including and excluding inconclusive test results (treated as being test positive, in accordance with current practice in the UK), as well as by investigating the test accuracy in UK (Bristol)-based studies only.

For clinical effectiveness outcomes, mean differences, relative risks or odds ratios (with 95% confidence intervals) were extracted from comparative studies, where reported. A narrative synthesis was employed due to the heterogeneity in reported outcomes and study design. For the review of implementation studies 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. Additionally, because we found very little evidence on the likely clinical efficacy of high-throughput NIPT and its impact on future sensitisation rates and adverse events, 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 reviews of antenatal anti-D prophylaxis.

### 1.3.2 **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 impact on the care pathway. Evidence in support of NIPT implementation was mixed. The main determinant for the negative outcome for NIPT was the cost of the test itself and the associated royalty fee. However, none of the existing studies reviewed were considered to be generalisable to the current decision problem.

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 of the relevant testing and treatment strategies. The results of high-throughput NIPT could impact on pre and post-partum care, potentially enabling prophylactic anti-D immunoglobulin and further testing to be withheld. To address this, four alternative scenarios were designed and explored in which the use of high-throughput NIPT may impact on the existing post-partum care pathway were explored. These represent separate and distinct testing and management strategies for the different sub-populations and evaluate how the introduction of NIPT could impact on the use of cord serology, fetal-maternal haemorrage tests and anti-D immunoglobulin following delivery. First and subsequent pregnancies together with 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 are the total lifetime costs and total lifetime 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 developed for NICE TA156 on 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 and two way sensitivity analysis.

## 1.4 Results

### 1.4.1 Diagnostic accuracy

Eight studies [1-8] were included in the diagnostic review of high-throughput NIPT testing. All the studies were prospective cohort studies, and were conducted in the UK, Denmark, Spain, Netherlands and Sweden. There were three high-quality studies [1-3] where NIPT testing was performed by the International Blood Group Reference Laboratory, NHS Blood and Transplant (NHSBT), Bristol (UK). The reference standard used in all studies was cord blood serology at birth. The majority of included studies were judged to be at low risk of bias, but two studies [4, 6] were judged to be at high risk of bias.

Meta-analyses showed very high diagnostic accuracy of high-throughput NIPT testing. In the primary analyses, where women with inconclusive test results were treated as being testing positive, the pooled false negative rate (i.e. women at risk of sensitisation) was 0.34% (95% CI 0.15 to 0.76) and the pooled false positive rate (i.e. women receiving anti-D unnecessarily) was 3.86% (95% CI 2.54 to 5.82). Sensitivity analyses did not materially alter the overall result.

A subgroup analysis based on studies of high-throughput NIPT testing at Bristol (UK) only, showed a pooled false negative rate of 0.21% (95% CI 0.09 to 0.48) and a pooled false positive rate of 5.73% (95% CI 4.58 to 7.16). It showed that the three Bristol studies had a slightly lower false-negative rate and a higher false positive rate than the remaining studies. This may be a chance finding, or it may be that a different threshold for the detection algorithm may be used in Bristol, which further reduces the false negative rate, consequently increasing the false positive rate.

The diagnostic accuracy performance of high-throughput NIPT varied by gestational age. The data suggest that high-throughput NIPT testing was less accurate before around 11 week's gestation (i.e. in first trimester), but diagnostic accuracy was consistent at any time after 11 week's gestation. This was most obvious in the Chitty study[1] that investigated test performance at multiple time points by gestational age. We were unable to conduct a subgroup analysis based on ethnicity due to lack of relevant data from included studies.

### 1.4.2 Clinical effectiveness

Seven studies [3, 5, 7, 9-12] were included in the clinical effectiveness review. A narrative synthesis was employed due to the considerable heterogeneity in outcomes and study designs. Only two studies had a control group, and all studies were judged to be at high risk of bias. One large prospective cohort study reported that implementation 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).[11]

Three non-comparative studies reported on reduction in administration of anti-D. All suggested that anti-D administration was largely avoided in women with an RhD negative fetus. A pilot study [3] in England found that around 35% of women who received NIPT avoided unnecessary anti-D administration.

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 testing uptake rates ranged from 70% to over 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 testing 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 3 sensitisations per 100,000 women if cord blood testing is continued (at least in women with a negative NIPT test result) as the basis for administering postpartum anti-D. If cord blood testing is withdrawn (except for women who did not receive an NIPT test, or who had an inconclusive test result) and the NIPT test 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 to the underlying rate of sensitisation with antenatal anti-D (280 per 100,000 women). Sensitisation rates could be higher if women who do not receive an NIPT test are also less likely to receive antenatal anti-D. These results suggest that cord blood testing could potentially be withdrawn, and NIPT test results (if available and conclusive) may be used to prescribe postpartum anti-D. This conclusion will partly depend on whether the extra 10 sensitisations per 100,000 RhD negative women caused by withdrawing cord blood testing can be considered an ethically acceptable increase.

### 1.4.3 **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 with diagnostic accuracy data, while one study was a survey based in the UK (London). All the cohort studies suggested that high throughput RhD genotyping of foetuses in all RhD negative women was feasible. A number of studies reported potential issues of implementation such as those relating to programme anti-D prophylaxis compliance. The UK survey study[13] revealed that women's current knowledge of Rhesus blood groups and anti-D administration was found to be limited, which could be an issue to implementation.

### 1.4.4 **Cost-effectiveness**

The *de-novo* health economic model suggests that high-throughput NIPT appears cost saving but also less effective than current practice, irrespective of the post-partum scenario evaluated. However, the

magnitude of the potential cost-savings appears sufficient to outweigh the small increase in sensitisations and the associated small QALY loss through using NIPT compared to current practice. Based on a cross section of 100,000 pregnancies, the likely magnitude of cost savings ranges between £296,000 and £409,000 across the separate post-partum 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 fetal-maternal haemorrhage and post-partum anti-D immunoglobulin) had the highest probability of being cost-effective.

The magnitude of the cost saving appears highly sensitive to the cost of the 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. A small increase in the cost assumed of [redacted] 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 consideratioon is the rate of high-throughput NIPT inconclusive results. Our findings demonstrate that even with a high-throughput NIPT inconclusive result rate close to 15%, the introduction of NIPT appears to compare favourably to current practice.

## 1.5 Discussion

### 1.5.1 Strengths, limitations and uncertainties

A comprehensive literature search was undertaken to identify both published and unpublished studies. Appropriate synthesis methods were employed by taking into account the heterogeneity of study characteristics. The bivariate and HSROC models were used for diagnostic accuracy data, which take into account the trade-off between true/false-positives and models between-study heterogeneity.

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

Due to the limited data available on the evaluation of clinical effectiveness, the potential clinical impact of high-throughput NIPT testing on the care pathway remains unclear. No studies compared NIPT testing to universal administration of antenatal anti-D. No studies were identified reporting comparative data relating to patient-related outcomes such as quality of life or anxiety. Whether the diagnostic performance of high-throughput NIPT testing differs between different ethnic groups remains unclear.

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. However, 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.

### 1.5.2 Generalisability of the findings

Diagnostic data from the three UK (Bristol) studies are mostly generalisable to the UK setting. Due to differences in high-throughput NIPT testing devices and in antenatal care within different countries, 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. In terms of implementing high-throughput NIPT testing in healthcare settings, no studies were identified reporting compliance rates to antenatal anti-D treatment in the UK settings. Although a few non-UK studies reported compliance rates to anti-D prophylaxis treatment, the generalisability of their findings to the UK setting remains uncertain due to variations in national guidelines and health policies between different countries. Because most participants in included studies were European white, the generalisability of their findings to non-white population also remains uncertain.

## 1.6 Conclusions

### 1.6.1 Implications for service provision

The evidence from this assessment suggests that high throughput NIPT testing is highly accurate for the detection of fetal Rhesus D status in RhD negative women, if performed after 11 week's gestation. Only 1% of women will have an incorrect test result (nearly all false positives) and around 7% will have an inconclusive result. Sensitivity analyses did not materially alter the results.

The use of high-throughput NIPT testing 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 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 ensuring high compliance to NIPT and continuing to offer antenatal anti-D to RhD negative women who refuse, or miss, the NIPT test.

### 1.6.2 Cost-effectiveness

Targeted provision of anti-D immunoglobulin prophylaxis through the use of high-throughput NIPT prophylaxis is estimated to be cost saving compared to current practice of providing prophylactic pre-natal anti-D immunoglobulin to all women who are RhD-negative. Four alternative scenarios were compared in which the results of the high-throughput NIPT are used to guide post-partum testing and administration of anti-D immunoglobulin. A post-partum strategy that distinguishes between inconlusive results and positive results offers the greatest cost-savings. The potential savings appear highly sensitive to the cost of the NIPT.

### 1.6.3 **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. For example, in people with African ethnicity, because of the high prevalence of RHD-pseudogenes,[14] prenatal detection of fetal Rh type from maternal blood would reveal an RhD-positive type but confirmed as RhD-negative by cord blood serology, thus leading to higher rates of false positives results in this particular population.

Further research to improve the test itself would be useful, particularly focusing on reducing the number of inconclusive test results.

In this assessment we identified very limited evidence on the clinical impact of NIPT testing. Appropriate auditing of the NIPT testing and anti-D administration process should be considered, if it is implemented, recording clinical outcomes, such as sensitisation rates, NIPT test and anti-D compliance, and quality of life.

Currently there is large uncertainty over the cost of introducing the high-throughput NIPT in the clinical pathway of RhD-negative women. Unit cost varies with the level of throughput, and, in addition, may be subject to a royalty fee which is in itself uncertain. Further clarifications over the potential additional costs for blood drawing, transporting of samples, and antenatal care visits to administer the test and deliver counselling and results, is needed.

Little evidence exists as to the impact of sensitisations in terms of their long term health and cost consequences. Further reseach to comprehensively appreciate the full impact of sensitisations over mothers and children is warranted.

# 2 Background

## 2.1 Description of health problem.

Pregnant women who have RhD negative blood type may carry an 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 feto-maternal haemorrhage. 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 usually does not affect the pregnancy during which it occurs. However, in a subsequent pregnancy with an 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. This can cause severe fetal anaemia, leading 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.[15] 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 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% to 0.28%. This has led to a decrease in mortality associated with haemolytic disease of the fetus and newborn, from 46 in 100,000 births before 1969 to 1.6 in 100,000 births by 1991.[16]

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.[17] Approximately 40% of these women carry an RhD negative fetus (around 39,000 per year) and therefore do not need administration of anti-D immunoglobulin. White populations of European descent have approximately a 15% incidence of RhD negativity, while it is 3% to 5% in African American and is very rare in those of Eastern Asian

origin.[18] Despite mixing of the genes, the majority of RhD negative white people are a result of gene deletion and RHD gene variants are relatively rare in white people, with only less than 1% of all RhD negative people. However, in black Africans, an inactive RHD gene (named as the RHD pseudogene *RHDψ*), which is mostly the result of genes that contain RhD sequences but do not produce D antigen, is present in 66% of RhD negative people. The distributions of this gene varied between black Africans and other African origins,[14] with 24% in African Americans and 17% in black South Africans.[19]

## 2.2 Current service provision and care pathway

The NICE guideline on antenatal care (2008) recommends that women should be offered testing for blood group and rhesus D status in early pregnancy.[20] All women identified as RhD negative will be tested for the presence of RhD antibodies, regardless of whether they are known to be sensitised or not. 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.[20] Following potentially sensitising events, anti-D immunoglobulin should be administered within 72 hours of the event.[16]

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 reports that of the women eligible for anti-D immunoglobulin, 99.0% received anti-D immunoglobulin.[21]

For pregnant women who are RhD negative and are sensitised to RhD antigen, the Royal College of Obstetricians and Gynaecologists have published guidance on the management of women with red cell antibodies during pregnancy.[22] This guideline recommends that all RhD negative women who are sensitised to RhD antigen should attend for pre-pregnancy counselling with a clinician with 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; be offered non-invasive fetal RhD genotyping using maternal blood if maternal RhD antibodies are present. Once an RhD positive fetus is identified, additional monitoring and treatment are required during the pregnancy.

## 2.3 Description of technology under assessment

### 2.3.1 Summary of technologies (index tests)

The technology under this assessment is high-throughput non-invasive prenatal testing (NIPT) for fetal Rhesus D status (International Blood Group Reference Laboratory, Bristol).

High-throughput NIPT of fetal RhD status uses a real time quantitative polymerase chain reaction (PCR) method for predicting fetal RhD genotype from fetal DNA in the plasma of RhD negative women. The test principle is based on analysis of cell-free fetal DNA - small fragments of fetal extracellular DNA shed from the placenta 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 an RHD gene in an RhD negative pregnant woman suggests an RhD positive fetus.

High-throughput NIPT is performed using samples of maternal anti-coagulated 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 to 6 hours.

High-throughput NIPT for fetal RhD status may enable anti-D immunoglobulin to be withheld from RhD negative women who are carrying an 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. Additionally, these women may not need the provision of anti-D immunoglobulin following potentially sensitising events, and there may no longer be a need for serologic cord testing at birth.

### 2.3.2 Identification of important sub-groups

There are potential challenges for the detection of fetal Rhesus D status when performing non-invasive prenatal testing in pregnant women. Dealing with the presence of RHD-pseudogene poses a challenge. In people of European ethnicity, the majority of RhD-negative individuals are a result of gene deletion; however, in people with African ethnicity, Rh-negative phenotype is mostly the result of genes that contain RhD sequences but do not produce D antigen (RHD-pseudogene).[14] In the presence of RHD-pseudogene, prenatal determination of fetal Rh type from maternal blood would reveal an RhD-positive type but 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 positives results when performing NIPT tests 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 exon 7 (or exon 10) only would not detect presence of RHD-pseudogene and other variants; targeting exon 10 only would not detect presence of RHD-pseudogene or the hybrid RHD-CE-D(s) gene, which is commonly present in people with African ethnicity.

Evidence suggests that the diagnostic accuracy of NIPT testing may vary according to different gestational ages at time of sampling. Two meta-analyses found that diagnostic accuracy of NIPT was higher in the first trimester compared with the second and third trimester.[23, 24] 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 11 weeks' gestation than before this time.[1]

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

### 2.3.3 **Current usage in the NHS**

Currently, all high-throughput NIPT for fetal RhD status determination in the UK is performed by the 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 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.[25] There would also need to be reporting systems in place to ensure accurate transmission of test results back to the women and their physicians and midwives.

### 2.3.4 **Anticipated costs associated with technology**

The potential costs associated with high-throughput NIPT to the NHS are made of two components. First, the unit cost of the diagnostic test itseflf, which varies with the level of throughput and to which a royalty may be added. An estimated unit cost for high-throughput NIPT of [redacted] and a royalty payment of [redacted] were considered. It should be noted that these estimates were provided in confidence

by the company with the underlying assumption that Bristol will be the sole provider of the test nationally. Second, the potential costs of incorporating the test into routine antenatal care, which may bring additional costs relating to the time for antenatal care appointments to provide information about the test, counselling and deliver test results, and also relating to blood drawing and blood sample transportation.

# 3 Definition of decision problem

## 3.1 Decision problem

The clinical 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. High-throughput NIPT for fetal RhD status may enable anti-D immunoglobulin to be withheld from RhD negative women who are carrying an RhD negative fetus. This subgroup of women could therefore avoid unnecessary prophylaxis with anti-D immunoglobulin during pregnancy, along with 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 test results. It is also unclear whether the cost of instituting the NIPT screening will outweigh the savings from reduced use of anti-D treatment.

This report, undertaken for the NICE Diagnostics Assessment Programme, examines the clinical and cost effectiveness of high-throughput, non-invasive prenatal testing. 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 the benefits are worth the cost of the tests for use in the NHS.

## 3.2 Overall aims and objectives of assessment

The purpose of this project was to assess the clinical 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 testing for fetal RhD status.
B. To perform a systematic review of the clinical impacts of high-throughput NIPT testing, 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 testing and its impact on 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 post-delivery testing.

This report is considered in two sections: Clinical effectiveness (covering objectives A and B) is discussed in Section 4. Cost effectiveness (objectives C, D and E) is discussed in Section 5.

# 4 Assessment of Clinical Effectiveness

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

1. A review of the diagnostic accuracy of high-throughput NIPT for detecting RhD positive foetuses.
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 where it has been used, examining feasibility, guidance or recommendations for practice, and need for further research
4. A review of existing systematic reviews of antenatal anti-D prophylaxis, identifying numbers of sensitisations, compliance and incidence of adverse events. This review facilitated modelling of the likely clinical impact of high-throughput NIPT, and supported the subsequent cost-effectiveness analyses.

The methodology of these reviews is described below.

## 4.1 Methodology of the clinical effectiveness reviews

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

### 4.1.1 Searches

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

The search strategy was developed in MEDLINE (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 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 or cost effectiveness studies from inception to November 2015: MEDLINE, MEDLINE In-Process, 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 on-going, unpublished or grey literature: ClinicalTrials.gov, Conference Proceedings Citation Index: Science, EU Clinical Trials Register, PROSPERO and the WHO International Clinical Trials Registry Platform portal.

The following websites were searched to identify any relevant guidelines: National Guidelines Clearinghouse, National Institute for Health and Care Excellence, NHS Evidence, Royal College of Obstetricians and Gynaecologists website, TRIP database, and the UK National Screening Committee. Reference lists of relevant 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 10.1.

### 4.1.2 Selection criteria

#### 4.1.2.1 Types of studies

***Diagnostic accuracy***

Prospective cohort studies in which the index test (high-throughput NIPT testing) and reference standard test (cord blood sampling) were done independently in the same group of women to assess fetal RHD status, and that reported sufficient data to construct a two-by-two 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) in which high-throughput NIPT testing was used to determine fetal RhD status, where anti-D prophylaxis was given as required, and that reported relevant clinical outcomes as listed below.

***Implementation***

Any publications discussing existing or experimental high-throughput NIPT screening programmes. Papers had to report issues related to implementation of, or practical advice relating to, high-throughput NIPT testing as a screening tool to guide use of anti-D prophylaxis. This included publications with no numerical data, but which 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.

The following types of report were excluded: editorials and opinions; case reports; 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 or less 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.

#### 4.1.2.2 **Population**

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

#### 4.1.2.3 **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 tests which were conducted using an automated robotic platform (including automated DNA extraction and liquid handling) and were able to process large numbers of samples rapidly for large scale screening purposes. Studies where 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 allow targeted anti-D prophylaxis.

#### 4.1.2.4 **Reference standard**

For diagnostic accuracy studies, the reference standard considered was serologic cord blood testing at birth, or any other suitable post-natal blood test of the infant.

#### 4.1.2.5 **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 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. Only reports published in English were sought. 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.

#### 4.1.3 **Data extraction**

We selected the most recent or most complete report in cases of multiple reports for a given study or when we cannot 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 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 x 2 tables. If such data were not provided by the study authors, we attempted to contact them to construct the 2 x 2 table for the study population or the pre-specified 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 for 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 on these as numbers of women or fetuses experiencing the specified outcome. Mean differences, relative risks or odds ratios (with 95% confidence intervals) were extracted from comparative studies, where 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, relative risks or odds ratios (with 95% confidence intervals) were extracted, where reported.

### 4.1.4 **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 (QUADAS-2) checklist.[26] 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 (1) the participants and setting; (2) the index test, its conduct or interpretation; and (3) the target condition as defined by the reference standard were applicable to the UK context.

The Cochrane ROBINS tool for non-randomised studies was used for comparative studies reporting other eligible clinical outcomes. The quality assessment was checked by another reviewer. Any disagreements were resolved by consensus or by a third party.

Quality of studies in the implementation review was not assessed due to a lack of validated tool for assessing the quality of studies on the implementation of health interventions.

### 4.1.5 **Methods of data synthesis**

Using extracted diagnostic accuracy data from the 2 x 2 tables, estimates of sensitivity, specificity, false-positive and false-negative rates 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.[27] was fitted, which calculates summary estimates of sensitivity, specificity, false-positive and false-negative rates and the associated 95% confidence intervals (CIs). The hierarchical summary ROC (HSROC) model[28] 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. Where meta-analyses were performed, data were pooled using standard

random-effects DerSimonian-Laird meta-analyses. Analyses were conducted in R and/or Stata software, as appropriate.

#### 4.1.5.1 **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, where 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. Bristol test vs. other)
- Ethnicity (e.g. European vs. African)

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

#### 4.1.5.2 **Sensitivity analyses**

We conducted sensitivity analyses to explore:

- The impact of including and excluding undetermined or uninterpretable NIPT test results on the pooled test accuracy estimates.
- Test accuracy in UK (Bristol)-based studies only

Where 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.

#### 4.1.5.3 **Narrative synthesis**

Where 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.

### 4.1.6 **Simulation study of clinical effectiveness**

During the course of this report we found very little evidence on the likely 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 review of systematic reviews of antenatal anti-D prophylaxis.

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

- Rates of women with 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, the review of antenatal anti-D prophylaxis, and other primary sources, where necessary.

We considered the following clinical scenarios:

- No antenatal anti-D; 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; postpartum anti-D based on cord blood test for all RhD negative women.[1]
- Antenatal and postpartum anti-D offered based on NIPT only. No cord blood testing.[2]

*1: This 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.*
*2 This 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.*

A Monte Carlo simulation of 10 million women was performed in R. For each scenario 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.

## 4.2 Clinical Effectiveness Results

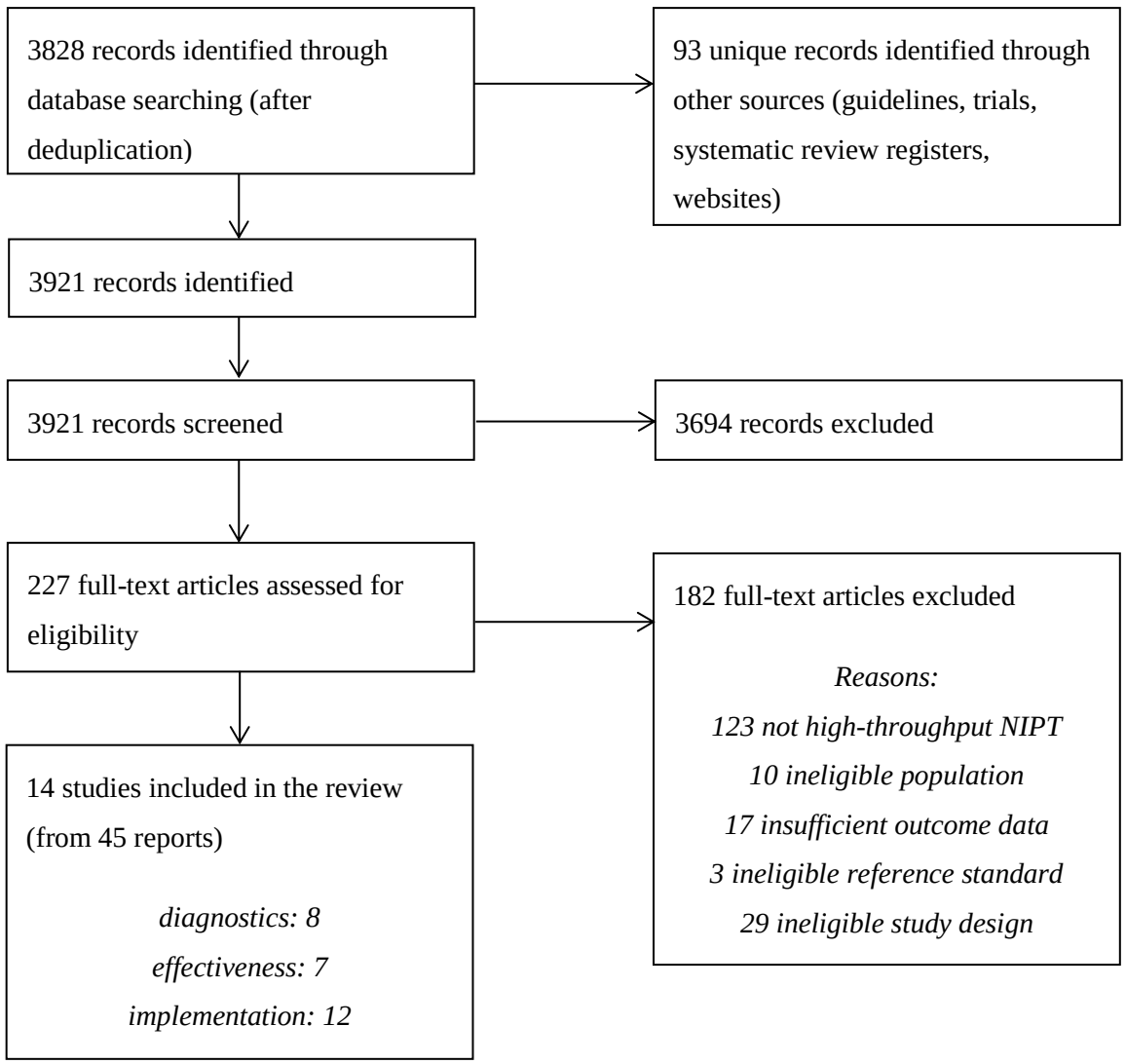
This chapter is structured as follows. The next section provides information on the quantity of research available, including characteristics and risk of bias of the included studies. This is then followed by the results sections with diagnostic accuracy, clinical effectiveness and implementation of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

### 4.2.1 Quantity and quality of research available

#### 4.2.1.1 Number of studies included

The literature searches of bibliographic databases identified 3921 references. After initial screening of titles and abstracts, 226 were considered to be potentially relevant and were ordered for full paper screening. In total eight studies[1-8] were included in the diagnostic review of high-throughput NIPT testing, seven studies [3, 5, 7, 9-12] were included in the clinical effectiveness review, and twelve studies [2, 3, 5-13, 25]were included in the review of implementation of high-throughput NIPT testing (with some overlap between studies). Figure 1 shows a flow diagram outlining the screening process with reasons for exclusion of full-text papers.

**Figure 1 Flow diagram: Study selection process**



All studies except two [13, 21] 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 included in any analysis. Table 1 presents an overview of these cohorts; their included studies and how papers were included in each review. Appendix 10.2 presents a list of all included references.

**Table 1 Overview of included cohorts and studies**

| **Cohort (country)** | **Number of full papers** | **Number of conference abstracts** | **Papers included in review:** | | | |
|---|---|---|---|---|---|---|
| | | | **Diagnostic accuracy (full paper)** | **Clinical effectiveness (full paper)** | **Implementation (full paper)** | **Linked conference abstracts** |
| **UK (Bristol)** | 3 | 5 | Chitty (2014)[1] Finning (2008) [2] Soothill (2015) [3] | Soothill (2015)[3] | Finning (2008) [2] Soothill (2015) [3] | Chitty 2011[29]; Chitty 2012 [30]; Daniels 2012[31]; Finning 2015[32]; Finning 2014[33]; Ford 2016[34] |
| **UK (London/other)** | 2 | 0 | Akolekar (2011) [4] | None | Oxenford (2013) [13] | None |
| **Denmark** | 4 | 5 | Banch Clausen (2014)[5] | Banch Clausen (2014)[5] Banch Clausen 2012 [9] Damkjaer 2012 [12] | Banch Clausen (2014) [5] Banch Clausen 2012 [9] Banch Clausen 2013 [25] Damkjaer 2012 [12] | Banch Clausen [35]; Dziegiel 2012 [36] ; Banch Clausen 2012[37]; Banch Clausen 2011[38]; Steffensen 2012[39] |
| **Netherlands** | 2 | 9 | Thurik (2015) [6] | De Haas (2012)[10] | De Haas (2012)[10] Thurik (2015) [6] | Veldhuisen 2014[40]; Veldhuisen 2013[41]; Thurik 2014[42]; Thurik 2014[43]; Scheffer 2013[44]; Van der Schoot 2005[45]; De Haas 2012[46]; De Haas 2012[47]; Grootkerk-Tax 2006[48]; Van der Ploeg 2015[49] |
| **Spain** | 1 | 0 | Grande (2013) [7] | Grande (2013) [7] | Grande (2013) [7] | None |
| **Sweden** | 2 | 8 | Wikman (2012) [8] | Tiblad (2013) [11] | Wikman (2012) [8] Tiblad 2013 [11] | Wikman 2012[50]; Wikman 2011[51]; Wiman 2013[52]; Wikman 2010[53]; Tiblad 2010[54]; Tiblad 2012[55]; Tiblad 2014[56]; Tiblad 2012[57]; Neovius 2014[56]; Neovius 2015[58] |
| **Total** | | | 8 | 7 | 12 | 31 |

#### 4.2.1.2 **Excluded studies**

A list of full-text papers that were excluded along with the reasons for their exclusions is given in Appendix 10.3. 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.

### 4.2.2 **Results: assessment of diagnostic accuracy**

#### 4.2.2.1 **Characteristics of the included studies**

Table 2 presents the summary information of characteristics of the included diagnostic accuracy studies. There were eight studies[1-8] for the diagnostic review. All the studies were prospective studies and conducted in European countries. Four studies were conducted in England,[1-4] three of which were based at Bristol (UK).[1-3]

**Table 2 Characteristics of the diagnostic accuracy studies**

| Study (First author / year) | Location | DNA extraction tool | Gestational age at time of NIPT (median/range) | Sample size[a] | RhD positive fetuses | RhD negative fetuses | Inconclusive test results |
|---|---|---|---|---|---|---|---|
| Akolekar 2011[4] | UK (London) | MDx BioRobot (Qiagen) | 12.4 (11 – 14) | 586 | 410 | 176 | 84 |
| Banch Clausen 2014[5] | Denmark | QIAsymphony SP; MagNA Pure LC; MagNA Pure Compact Instrument (Roche) | 25 (23 – 28) | 12668 | 7830 | 4838 | 274 |
| Chitty 2014[1] | UK (Bristol) | MDx BioRobot (Qiagen) | 19 (5 – 35) | 4913 | 2890 | 2023 | 393 |
| Finning 2008[2] | UK (Bristol) | MDx BioRobot (Qiagen) | 28 (8 – 38) | 1869 | 1156 | 713 | 64 |
| Grande 2013[7] | Spain | COBAS AmpliPrep (Roche) | 24 - 26 | 282 | 186 | 96 | NR |
| Soothill 2015[3] | UK (Bristol) | MDx BioRobot (Qiagen) | 15 – 17 (mostly) | 499* | 315 | 184 | 61 |
| Thurik 2015[6] | Netherlands | MagNa Pure 96 (Roche) | 26 | 18383* | 11283 | 7100 | NR |
| Wikman 2012[8] | Sweden | MagNA Pure LC (Roche) | 8 - 40 | 3291[#] | 2073 | 1218 | 13 |

*[a] Number of blood samples unless otherwise specified; * number of participants; [#] excludes pre-8 weeks gestation pregnancies*

*NR: not reported*

The sample size (number of patients/samples analysed) of studies ranged from 282 to 18383. Most studies recruited pregnant women with gestational age of median 10 to 28 weeks. Most participants were European white. 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 including MDx BioRobot, MagNa Pure 96, MagNA Pure LC, and COBAS AmpliPrep. For PCR, all studies targeted at least two exons (generally exons 5 and 7) and at least two controls for RHD assay (RhD positive DNA and RhD negative DNA) except for the study by Wikman et al. which targeted exon 4 only and used *GAPDH* DNA as control. The reference standard used in all studies was cord blood serology, except for Akolekar et al. which did not describe its reference standard. Inconclusive

results were reported in all but two studies (Thurik and Grande). Appendix 10.4 presents further details of included studies.

#### 4.2.2.2 **Risk of bias of the included studies**

All the eight full-text papers were 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 10.5 presents results of quality assessment for the individual studies. Despite some gaps in reporting, most studies were considered at low risk of bias for these four domains. NIPT as an automated procedure was deemed at limited risk of human error, and multiple controls were used for RHD assays in all except one study (Wikman 2012). Cord blood serology was the reference standard in all studies. The index test of NIPT was conducted independent 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 (Finning 2008, Wikman 2012, Grande 2013). Multiple pregnancies can pose specific challenges for NIPT testing (for example, 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 pre-specified during the conduct of the screening programme (Chitty 2014, Finning 2008, Banch Clausen 2014). None of the studies reported whether there were any adverse events from the index test or reference standard.

Two studies (Akolekar 2011 and Thurik 2015) were judged to be at high risk of bias. The study by Akolekar et al. stated that the targeted RhD negative women were selected from a database; however, it was unclear whether this selection was performed on a random basis. The study recruited a large proportion of Africans (19.3%) which 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 performedand may have contributed to the larger than average proportion of inconclusive results (15%). Akolekar excluded inconclusive results from its analyses, thereby potentially inflating its diagnostic accuracy estimates. Characteristics of the reference standard were also poorly reported.

Thurik (2015) excluded multiple pregnancies from 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 this likely introduced bias in the diagnostic accuracy

estimates (the authors reported the estimated impact of these changes to 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.[4, 8] The test used by Wikman (2012) only targeted exon 4, unlike all other included studies which targeted at least two exons (5, 7 and/or10). It is generally advocated that a combination of exons 5 and 7 is targeted to discriminate the pseudogene *RHDφ*, particularly present in individuals of African origin.[19, 59] In addition, most participants in Wikman (2012) 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.

Overall, the majority of included studies were judged to be at low risk of bias, but two studies, Akolekar (2011) and Thurik (2015) were judged to be at high risk of bias.

**Table 3 Risk of Bias of included studies**

| | **Risk of bias** | | | | **Applicability concerns** | | |
|---|---|---|---|---|---|---|---|
| **Study** | **Patient selection** | **Index test** | **Reference standard** | **Flow and timing** | **Patient selection** | **Index test** | **Reference standard** |
| Akolekar (2011) [4] | High | High | Unclear | Unclear | High | Low | Unclear |
| Banch- Clausen (2014) [5] | Low | Low | Low | Low | Unclear | Low | Low |
| Chitty (2014) [1] | Low | Low | Low | Low | Low | Low | Low |
| Finning (2008) [2] | Low | Low | Low | Low | Low | Low | Low |
| Grande (2013) [7] | Low | Low | Low | Low | Low | Low | Low |
| Soothill (2015) [3] | Low | Unclear | Low | Low | Low | Low | Low |
| Thurik (2015) [6] | Low | High | Low | High | Low | Low | Low |
| Wikman (2012) [8] | Low | Low | Low | Low | Unclear | High | Low |

High: High risk of bias; Low: low risk of bias

#### 4.2.2.3 **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. While this was the policy in the three high-quality studies performed in Bristol, data on inconclusive tests were not reported in two studies (Thurik and Grande). Given these differences we considered four approaches to the diagnostic analysis

1. Women with inconclusive tests treated as test positive (***including*** Thurik and Grande studies)
2. Women with inconclusive tests treated as test positive (***excluding*** Thurik and Grande studies)
3. Excluding all women with inconclusive test results
4. Studies conducted in Bristol only

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 test was conducted at or before 11 week's gestation were excluded because of concerns that the diagnostic accuracy is poorer before 11 weeks, and the test should not be conducted before then (see also Section 4.2.2.5). In one study (Finning 2008) a small number tests may have been performed before 11 weeks, 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 testing 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 false positive rate (incorrectly testing positive, and so offered unnecessary anti-D) and false negative rate (incorrectly testing negative, and so at risk of sensitisation as do not receive anti-D treatment).

A summary of all the results of the bivariate meta-analyses of false positive and negative rates are presented in Table 4.

**Table 4 Bivariate meta-analyses of false positive and negative rates**

| Analysis case (see list above) | Number of studies | False negative rate (at risk of sensitisation) | | False positive rate (unnecessary anti-D) | |
|---|---|---|---|---|---|
| | | Estimate (%) | 95% Conf. Int. | Estimate (%) | 95% Conf. Int. |
| 1 | 8 | 0.34 | 0.15– 0.76 | 3.86 | 2.54 – 5.82 |
| 2 | 6 | 0.38 | 0.15 – 0.94 | 4.37 | 2.79 – 6.78 |
| 3 | 8 | 0.35 | 0.15 – 0.82 | 1.26 | 0.87 – 1.83 |
| 4 | 3 | 0.21 | 0.09 – 0.48 | 5.73 | 4.58 – 7.16 |

It can be seen that results are broadly consistent across the four scenarios. NIPT is very accurate among women with an RhD positive fetus; only 2 to 4 such women in 1000 will have a negative test result and so be at risk of sensitisation due to not being offered anti-D. NIPT is slightly less accurate among women with an 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 false positive rate was 1.3%; rising to 3.9% to 5.7% 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.

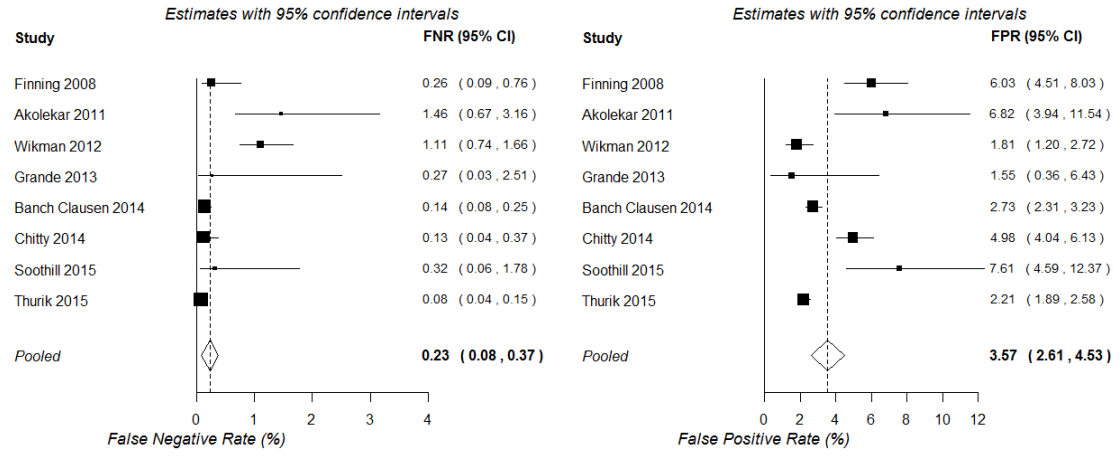
Assuming that 60% of RhD negative women have an RhD positive fetus; about 0.5% of women have a conclusive, but incorrect, positive test result. About 0.1% to 0.2% of women have a false negative test result.

We consider the results of each analysis in more detail below.

***Considering inconclusive results as test positive***

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

**Figure** 2 **Forest plots of FPR and FNR when counting an inconclusive test result as being test positive**

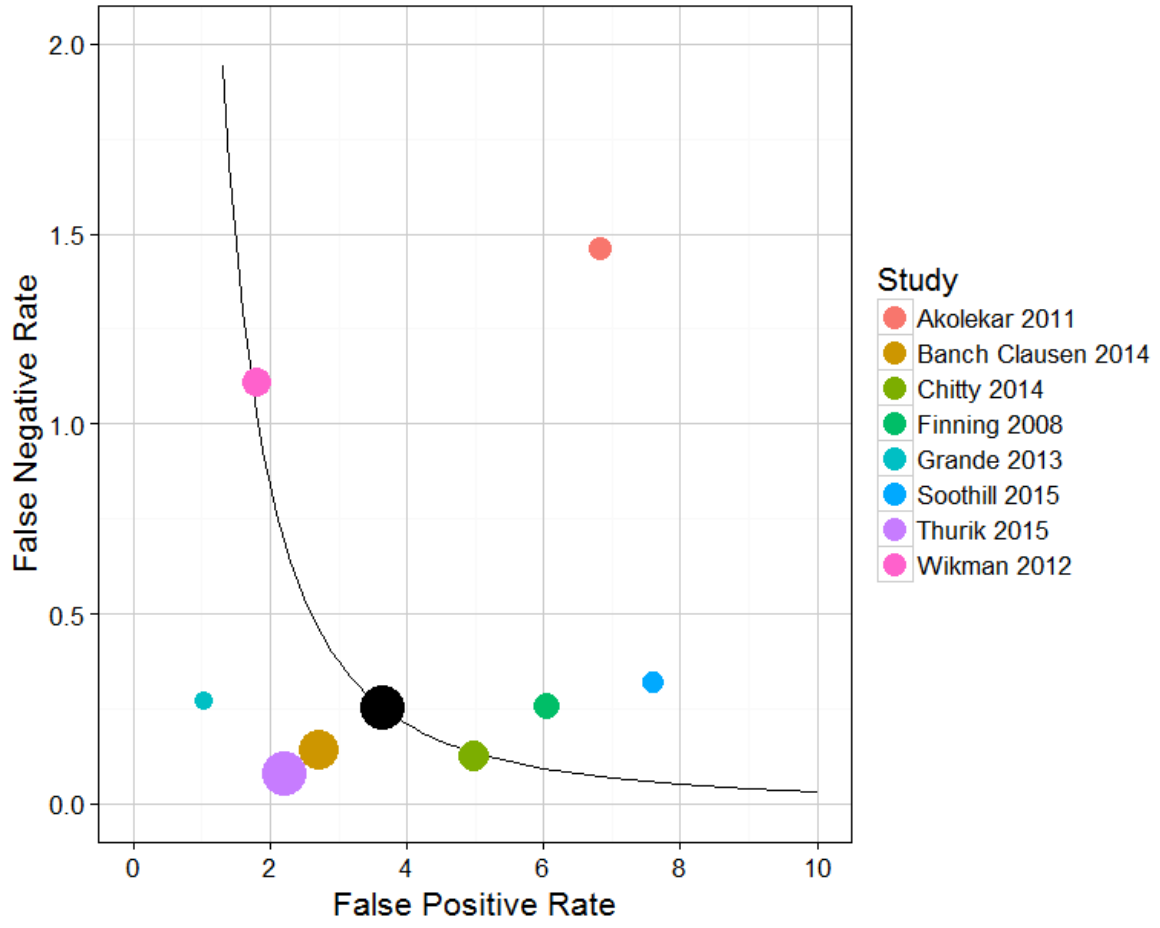



There was some evidence of inconsistency across studies. The $I^2$ statistic for heterogeneity was 75% for the FNR and 99% for the FPR. It should be noted that these high heterogeneities are, in part, a consequence of the high accuracy of the test and the large size of the studies (and consequent small within-study variance, because $I^2$ increases as the average within-study variance declines). They do

not necessarily indicate any clinically meaningful differences between studies. The heterogeneity in false positive rates 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 (Akolekar and Wikman). The Wikman study performed most NIPT tests in the first trimester, earlier than other studies. As discussed later (Section 4.2.2.5), the timing of the NIPT test may have an impact on the false-negative rate. The studies are less consistent in false positive rates. This is most likely because the studies have different numbers of inconclusive test results, and different methods of handling such results. Because women with an inconclusive result are treated as positive, women with an inconclusive result, but an RhD negative fetus, will be false positives. There may also be some heterogeneity due to differences in the threshold used and how different testing machines operated.

**Figure 3 HSROC and bivariate analysis when counting an inconclusive test result as being RhD positive**



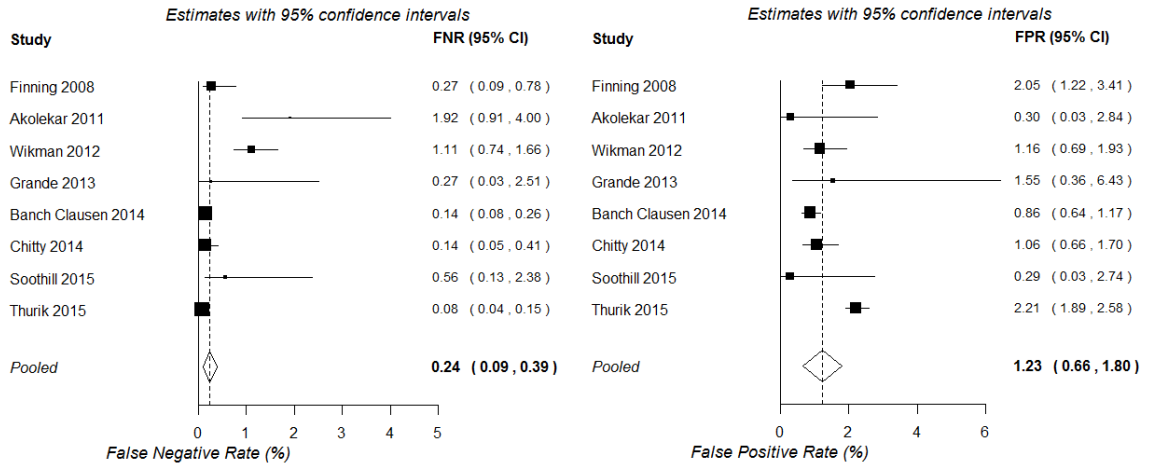
When excluding the two studies that did not report numbers of inconclusive tests (Thurik and Grande), the results were broadly similar, as seen in Table 4. The forest plots of false positive rate and false negative rate for this analysis are given in Appendix 10.6.

### *Excluding inconclusive results*

We considered the diagnostic accuracy of NIPT excluding all inconclusive test results to identify the "optimal" diagnostic accuracy where 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 false negative rate and false positive rate are shown in

Figure **4**.

**Figure** 4 **Forest plots of FPR and FNR excluding women with inconclusive test results**




Excluding women with inconclusive test results has no meaningful impact on false negative results (since those women are always assumed to have a positive result). It does, however, considerably reduce the false positive rate. The false positive rate, at 1.2%, is low, but still considerably higher than the false negative rate. This suggests that NIPT is more accurate in women with an RhD positive fetus than in those with an RhD negative fetus. There was some evidence of heterogeneity across studies. The $I^2$ statistic for heterogeneity was 75% for the false negative rate and 99% for the false positive rate. The ROC plot with bivariate and HSROC analyses is given in Appendix 10.7.

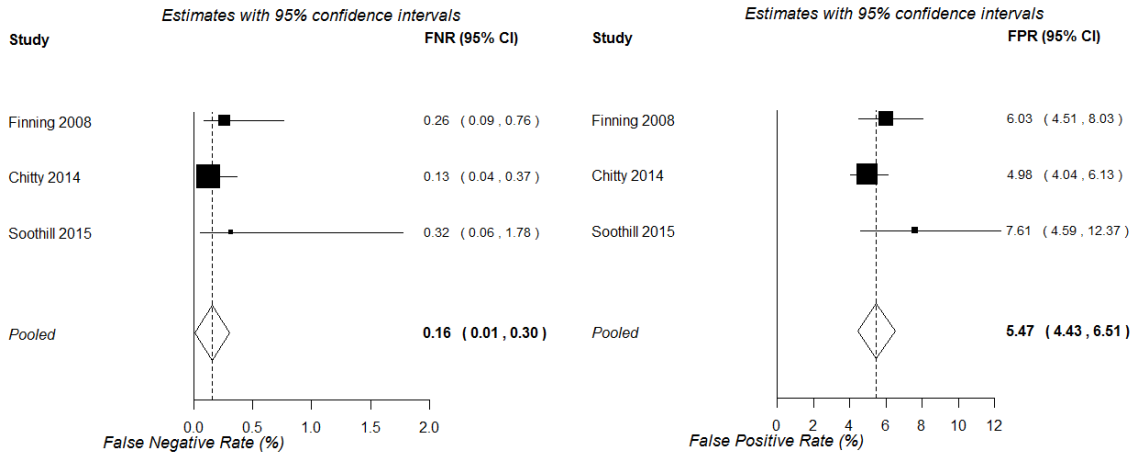
### *Bristol studies*

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

applicability of recruited participants; a higher proportion of Africans (19.3%) in this study 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.

**Figure** 5 **Forest plots of FPR and FNR for the Bristol studies**




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

#### 4.2.2.4 **Inconclusive test results**

As noted in the diagnostic analyses above, 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 inconclusive test results across included studies.

**Table 5 Inconclusive test results in the included studies**

| Study (First author / year) | Location | RhD positive fetuses (%) | Inconclusive test results (%) | RhD positive fetuses in women with inconclusive test results (%) |
|---|---|---|---|---|
| Akolekar 2011[4] | UK (London) | 70.0 | 14.3 | 85.7 |
| Banch Clausen 2014[5] | Denmark | 61.8 | 2.2 | 66.8 |
| Chitty 2014[1] | UK (Bristol) | 58.8 | 7.0 | 76.6 |
| Finning 2008[2] | UK (Bristol) | 61.9 | 3.4 | 54.7 |
| Grande 2013[7] | Spain | 66.0 | Not reported | |
| Soothill 2015[3] | UK (Bristol) | 63.1 | 12.2 | 77.0 |
| Thurik 2015[6] | Netherlands | 61.4 | Not reported | |
| Wikman 2012[8] | Sweden | 63.0 | 0.4 | 38.5 |

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

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 an 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 an RhD negative fetus who would receive anti-D unnecessarily.

**Table 6 Meta-analyses of inconclusive results**

| Studies included | Estimated inconclusive rate | 95% confidence interval |
|---|---|---|
| All reporting inconclusive tests | 4.0% | 1.5% to 10.3% |
| Bristol studies only | 6.7% | 3,.7% to 11.7% |

#### 4.2.2.5 **Subgroup analyses**

We considered the effect of the timing of the NIPT on its diagnostic accuracy. Figure 6 shows the false negative rates plotted by gestational age at time of high-throughput NIPT testing. It suggests that false negative rates after 11 weeks' gestation were consistent, irrespective of timing, but false negative rates were higher before 11 weeks' gestation. Figure 7 shows the false positive rates plotted by gestational age at time of high-throughput NIPT testing. There was no obvious pattern from this figure. Only one study (Chitty 2014) examined test performance at multiple time points. Figure 8 shows the false positive and false negative rates at different times for this study. It indicates that false negative rates were higher before 11 week's gestation, and were generally stable after 11 weeks' gestation, as reported in the original publication of this study. We did not perform any formal statistical analyses on the timing data (such as a meta-regression) because the relationship appears to be a step change in accuracy, rather than a linear trend over time. These results together suggest that NIPT testing is insufficiently accurate before around 11 week's gestation (i.e. in first trimester), but is accurate at any time after the end of the first trimester.

We also considered the impact of the timing of high-throughput NIPT testing on the number of inconclusive test results (Figure 9). Despite the data from Wikman (2012) 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 (2014) study which reported numbers of inconclusive tests at different times.

**Figure 6 False negative rate by gestational age at time of NIPT**

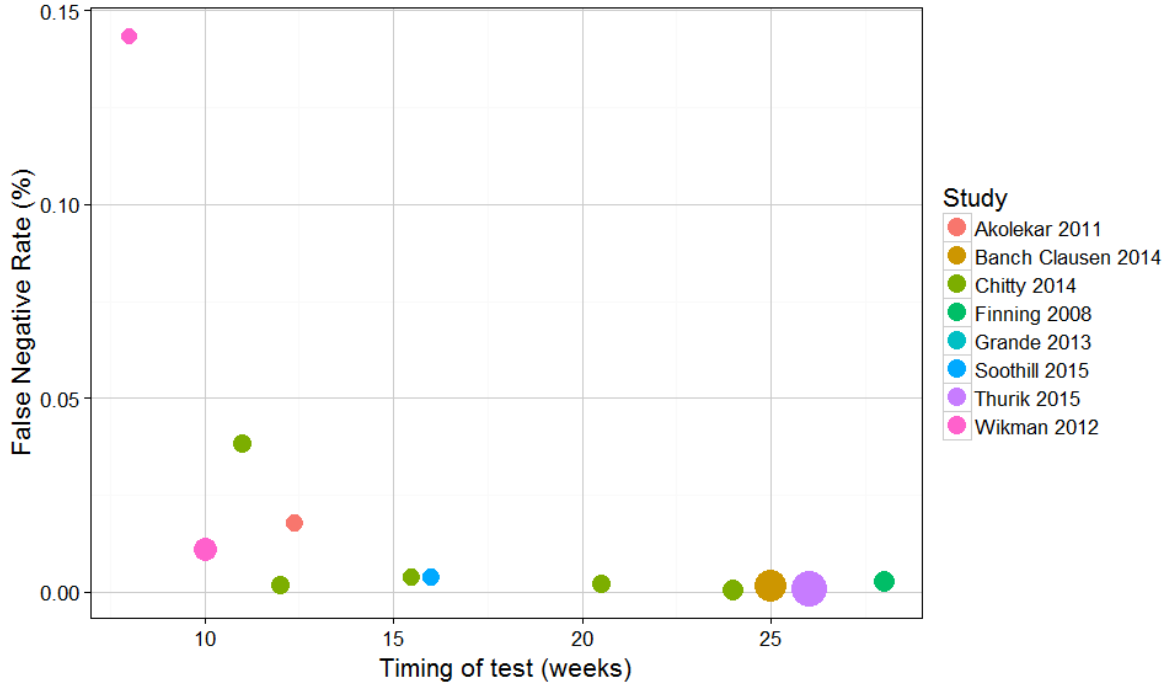



**Figure 7 False positive rate by gestational age at time of NIPT**

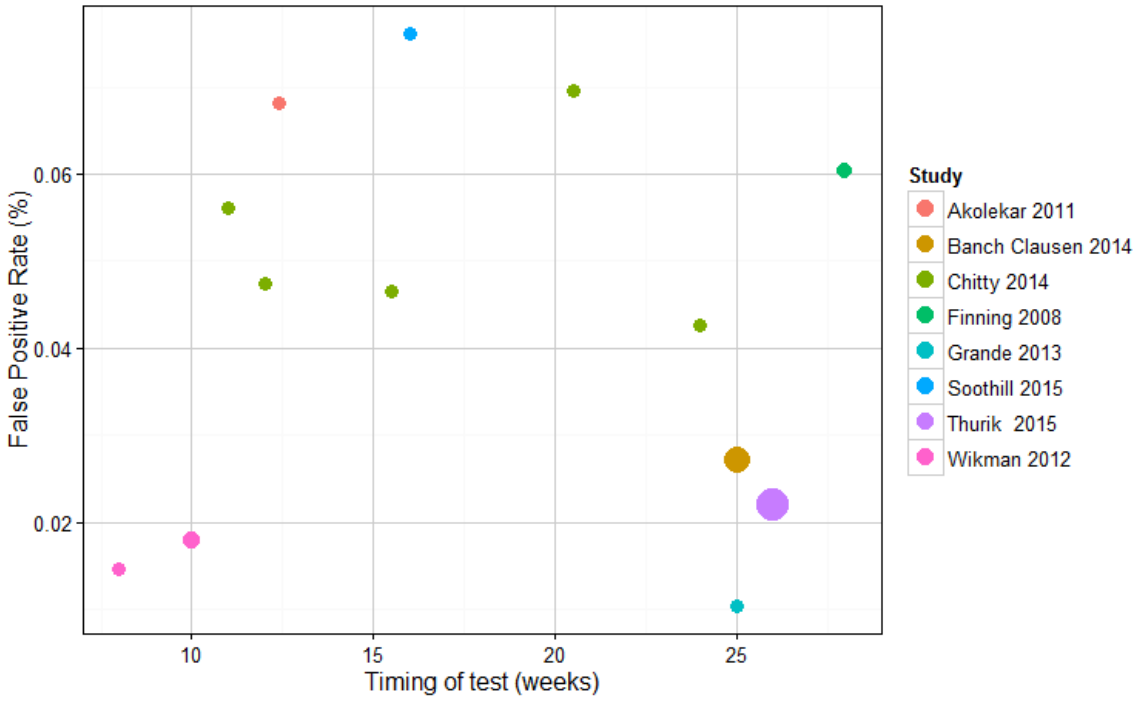


**Figure 8 FNR against FPR for Chitty study**

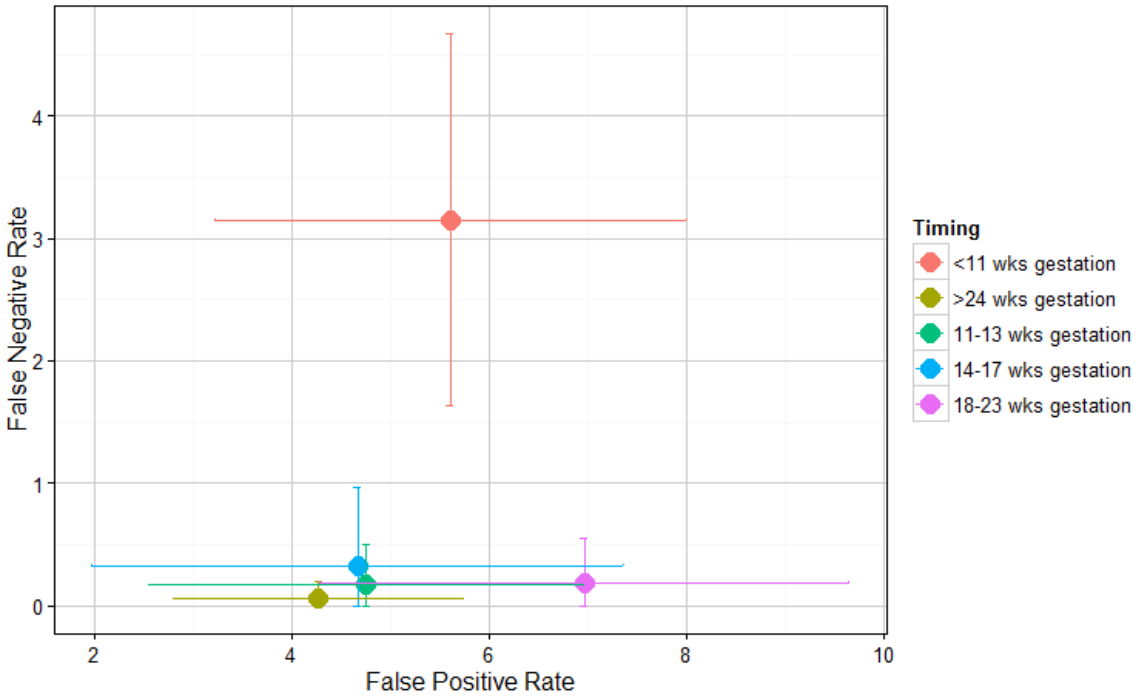
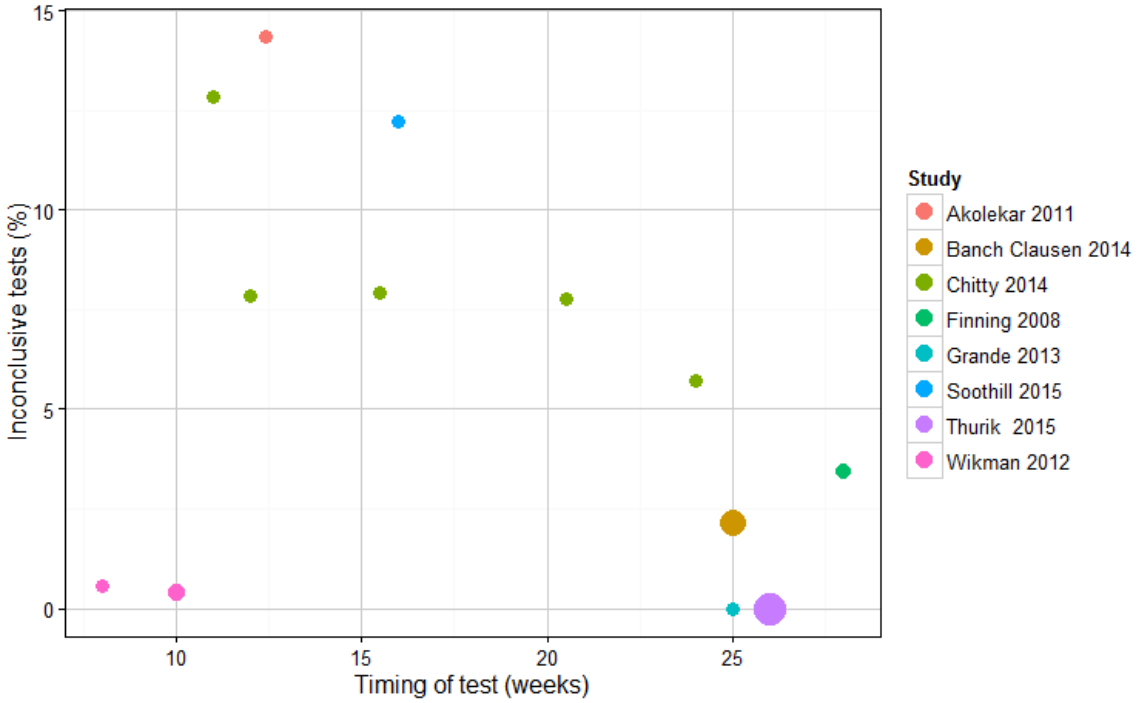



**Figure 9 Inconclusive results by test timing**



We were unable to conduct any subgroup analysis based on ethnicity as the relevant data was 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 Section 4.2.2.3 above.

### 4.2.3 **Results: assessment of clinical effectiveness**

#### 4.2.3.1 **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, 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 European white. Most studies recruited women with gestational age of median 10 to 26 weeks. Three studies reported using routine antenatal anti-D prophylaxis at between 28 and 30 weeks.

**Table 7 Characteristics of effectiveness studies**

| Study | Location | Study dates | Sample size[a] | Gestational age at time of NIPT testing (weeks) | Routine antenatal anti-D prophylaxis | Comparator |
|---|---|---|---|---|---|---|
| Banch-Clausen (2014)[5] | Denmark, 1 region | 01/2010-06/2010 | 591 | median 25 | 250 to 300 µg at 29 weeks | Postnatal anti-D only (n=109) |
| Banch Clausen 2012 [9] | Denmark, nationwide | 01/2010-06/2010 | 2312 | median 25 | 250 to 300 µg at 29 weeks | None |
| Damkjaer 2012 [12] | Denmark, 1 hospital | 06/2010-09/2010 | 239 | mean 27 | 250 to 300 µg at 29 weeks | None |
| de Haas (2012)[10] | Netherlands, nationwide | 07/2011-01/2012 | 15126* | mean 26 | 250 µg at 30 week & after birth | None |
| Grande (2013)[7] | Spain, Barcelona | 02/2010-10/2011 | 284 | range 24-26 | NR | None |
| Soothill (2015)[3] | England, 3 NHS Trusts in South West England | 04/2013 -09/2013 | 529 | range 15-26 | 500 or 1500 µg (timing NR) | None |
| Tiblad (2013)[11] | Sweden Stockholm area | 09/2009 - 03/2012 (reference cohort: 2004-2008) | 8347[#] | 10 (3-40) | 250-300 µg at 28-30 weeks | Postnatal anti-D only (historical control) (n=18,546) |

[a] Number of blood samples undergoing NIPT unless otherwise specified; * number of participants undergoing NIPT; [#] number of pregnancies undergoing NIPT

Only two studies compared women receiving NIPT to controls (Tiblad 2013, Banch Clausen 2014). One study (Tiblad 2013) compared patients undergoing NIPT with routine management with no NIPT and routine postnatal anti-D prophylaxis only (historical control). The other comparative study (Banch Clausen 2014) reported data on anti-D compliance in a small subgroup of participants from one region in Denmark, comparing participants receiving NIPT versus no NIPT.

#### 4.2.3.2 **Risk of bias of the included studies**

The results of the quality assessment of the two comparative studies are given in Appendix 10.8. In summary, both studies had significant limitations. Tiblad (2013) was considered at serious risk of bias, primarily due to concerns about patient selection, confounding and missing data. Banch Clausen (2014) was considered at critical risk of bias due to concerns about patient 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 routine antenatal anti-D prophylaxis.

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.

#### 4.2.3.3 **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 Ig use. We performed a narrative synthesis due to the considerable heterogeneity in outcomes and study designs.

***Sensitisations***

One study reported data on the incidence of sensitisation (defined as developed anti-D antibodies after the 1st trimester) and haemolytic disease of the newborn. Tiblad (2013) 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 testing was 0.26 % (95% CI 0.15 to 0.36%, n=8347) compared to 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 risk ratio (RR) 0.55, 95% CI 0.35 to 0.87) compared with historical controls. An updated analysis by Neovius 2015[58] found an adjusted odds ratio of 0.41 (95% CI 0.22 to 0.87). Additionally, this study reported one severe haemolytic disease case diagnosed soon after birth in a nulliparous mother who did not receive routine anti-D prophylaxis.

***NIPT uptake***

Rates of NIPT uptake are presented in Table 8. Seven studies reported on uptake rates of NIPT screening.[3, 5, 7, 10-12] Uptake rates ranged from 70% to more than 95% across the studies. In the pilot study conducted by Soothill et al. 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 (2014) reported that 88% of the 3069 participants consented to receive RHD genotyping. The only country which reported nationwide NIPT screening uptake data was the Netherlands, where more than 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 | Rates of NIPT uptake | Country |
|---|---|---|
| Banch Clausen 2014[5] | 84.2% (581/690) | Denmark |
| Chitty 2014[1] | 88% (372/3069 ) | England |
| Damkjaer 2012[12] | 90% (215/ 239) | Denmark |
| De Haas 2012 #3368 | >95% (15126/ approx. 15750) | Netherlands |
| Grande 2013[7] | 94% (284/302) | Spain |
| Soothill 2015[3] | 70% (approx.) | England |
| Tiblad 2013[11] | 89% (8374/9380) | Sweden |

***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 routine antenatal anti-D prophylaxis in women who accepted NIPT and received a positive result, ranging from 86% to 96.1%.[5, 11, 12, 49] One study reported nationwide data in women receiving RhD genotyping in the Netherlands, where 96.1% of approximately of 18,383 women received antenatal prophylaxis anti-D. Tiblad et al. reported a slightly lower rate, with 90% of 5104 women with a positive NIPT result receiving routine antenatal anti-D prophylaxis. Further data on uptake of routine antenatal anti-D prophylaxis in women who received a negative result (2 studies),[3, 7] those who received an inconclusive result (1 study),[3] and those who refused NIPT (2 studies),[3, 12] were limited. None of the included studies reported whether 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 due 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%.[5, 12, 49] One study reported nationwide data in women receiving RhD genotyping in the Netherlands, where 92% of approximately of 18,383 women received postnatal prophylaxis anti-D. A subgroup analysis by Banch Clausen (2014) (including a total of 690 pregnancies) found slightly higher uptake of postnatal anti-D among women who received NIPT (99.7%, 353/354) compared with 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).[12] None of the included studies reported whether all women who received postpartum anti-D prophylaxis received the intended dosage at the intended time.

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

| **Routine antenatal anti-D prophylaxis (RAADP)** | **% (n/N)** | **Source** | **Country** |
|---|---|---|---|
| (1) Uptake of RAADP with no NIPT (current practice) | 99% (N= 5276) receiving at least one injection<br>87.5% (N= 5276) receiving the correct dose at the correct time.<br>90%* (NR/5276) receiving all injections at correct doses | UK anti-D audit[21#] | UK |
| | 100% (10/10) | Soothill (2015)[3] | England |
| (2) Uptake of RAADP in those who refuse NIPT | 0 (0/23) | Damkjaer 2012[12] | Denmark |
| | 80% (4/5) | Soothill (2015)[3] | England |
| (3) Uptake of RAADP in those who accept NIPT and receive a positive result | 93.2% (330/354) | Banch Clausen 2014[5] | Denmark |
| | 86% (NR) | Damkjaer 2012[12] | Denmark |
| | 90% (4590/5104) | Tiblad 2013[11] | Sweden |
| | 96.1% (of approx. 18383) | Van der Ploeg 2015[49] | Netherlands |
| (4) Uptake of RAADP in those who accept NIPT and receive an inconclusive result | 100% (5/5) | Soothill (2015)[3] | England |
| (5) Uptake of RAADP in those who accept NIPT and receive a negative result | 6% (1/18) | Soothill (2015)[3] | England |
| | 5% (5/95) | Grande 2013[7] | Spain |
| **Postnatal routine anti-D uptake** | | | |
| (6) Uptake of postnatal anti-D with no testing | 98.4% (91.6% correct dose and time)(NR/3392) | UK anti-D audit[21#] | UK |
| | 95.7% (66/69) | Banch Clausen 2014[5] | Denmark |
| (7) Uptake of postnatal anti-D in those who refuse NIPT | >99% (NR) | Damkjaer 2012[12] | Denmark |
| (8) Uptake of postnatal anti-D in those who accept NIPT and receive a positive result | 99.7% (353/354) | Banch Clausen 2014[5] | Denmark |
| | 99.3% (151/152) | Damkjaer 2012[12] | Denmark |
| | 92% (of approx. 18383) | Van der Ploeg 2015[49] | Netherlands |
| (9) Uptake of postnatal anti-D in those who accept NIPT and receive an inconclusive result | No data | NA | NA |
| (10) Uptake of postnatal anti-D in those who accept NIPT and receive a negative result | 0 (0/227) | Banch Clausen 2014[5] | Denmark |
| | 0 (0/85) | Damkjaer 2012[12] | Denmark |

| | | | |
|---|---|---|---|
| | 0.087% (2/NR) | Banch Clausen 2012[9] | Denmark |
| | 0 (NR) | Soothill 2015[3] | England |

*Full compliance (correct dose, correct time) with single dose regime. 99% received at least one dose.

# Although this study did not meet the selection criteria for this review (no NIPT), it is included here for informative purposes

***Reduction in anti-D use***

Three non-comparative studies reported outcome measures relating to anti-D doses administered. Soothill (2014) reported a significant 6% reduction per month of anti-D administration (95% CI 4 to 8%, Poisson regression) within six 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 2014 study,{#58) which reported that, of 12668 pregnancies, 4706 (37.1%) women avoided unnecessary anti-D administration within two years of prenatal RHD screening programme. The study by Grande (2013) reported that, of 95 women carrying an RhD-negative fetus, five women requested anti-D administration; unnecessary anti-D administration was therefore avoided in 95% of women carrying an RhD-negative fetus.

***Adverse events***

None of the studies reported any data on adverse events of either NIPT testing 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 of social consequences of NIPT testing (such as revealing false paternity). No studies reported data on health related quality of life and patients' anxiety associated with NIPT testing.

### 4.2.4 Simulation study of clinical effectiveness

As seen in the review of clinical effectiveness (Section 4.2.3), very limited comparative evidence on the clinical outcomes of NIPT have been reported. In order to better understand the likely 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 discussed above. Prevalence and diagnostic accuracy parameters are derived from the three high-quality Bristol-based studies wherever 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 wherever available. Some important parameters, such as incidence of sensitization with and without anti-D, were not reported in any papers included in the diagnostic accuracy or clinical

effectivness reviews. To inform other parameter estimates for this simulation, we sought to identify relevant systematic reviews of antenatal anti-D prophylaxis. Four relevant reviews (McBain 2015; Turner 2012; Pilgrim 2009; Fyfe 2014) were identified. These reviews provided data on probability estimates of the events used in the simulation study, including sensitisation and compliance rates. These reviews are summarised in Appendix 10.9.

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.

**Table 10 Probability estimates derived from published data, used in the simulation study**

| **Probability** | **Estimate** | **Source** |
|---|---|---|
| | | |
| Rhesus positive fetus | 60.7% | Bristol-based diagnostic studies |
| Rhesus positive fetus (with inconclusive NIPT) | 70.7% | Bristol-based diagnostic studies |
| False negative NIPT test | 0.21% | Diagnostic meta-analysis (Bristol studies) |
| Inconclusive NIPT test | 6.7% | Bristol-based diagnostic studies |
| False-positive test (if conclusive) | 1.5% | Diagnostic meta-analysis (Bristol studies) * |
| | | |
| Compliance with antenatal anti-D (without NIPT) (received at least one dose of anti-D) | 99% | UK 2013 audit |
| Uptake of NIPT | 96% | De Haas 2012 (clinical effectiveness review) |
| Compliance with postpartum anti-D | 99% | UK 2013 audit |
| Compliance with antenatal anti-D (if NIPT test refused or missed) | 80% | Soothill (2015) (clinical effectiveness review) |
| Compliance with antenatal anti-D (if NIPT inconclusive) | 99% | Soothill (2015) |
| Uptake of antenatal anti-D in women with negative NIPT | 6% | Soothill (2015) |
| Compliance with postpartum anti-D after NIPT process | 99% | No data. Assumed same as without NIPT |
| | | |
| Sensitisation with antenatal anti-D and post-partum anti-D | 0.35% | Pilgrim et al 2009 HTA report |
| Sensitisation with only postpartum anti-D | 0.95% | Pilgrim et al 2009 HTA report |
| Sensitisation with no anti-D | 10.7% | From Pilgrim, and Crowther et al. 1997 |
| | | |
| Subsequent pregnancy in sensitised women | 62% | Used by Chitty (2014), no source given |
| Death of RhD negative fetus in sensitised women | 5% | Used by Chitty (2014), no source given |
| | | |

** This result is based on a diagnostic meta-analysis of the Bristol-based studies, excluding women with inconclusive test results. This was not reported in Section 4.2.2.3.*

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

**Table 11 Results of simulation study**

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

These results show that using the NIPT test 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 (2014). This is a consequence of the substantial drop in unnecessary anti-D administration in women with RhD negative foetuses, from 39% of women to 5.7%. Using the NIPT approach means about 1.2% of women miss out on potentially beneficial prophylaxis, mainly because of non-compliance, compared to 0.6% with universal anti-D administration.

Because sensitisation is rare very few additional women will be sensitised if NIPT testing is used. Assuming all women still receive a postnatal cord blood test and anti-D if required NIPT test in will result in about 3 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 to the total number of sensitisations due to failure of anti-D treatment (around 284 per 100,000 women) and compared to 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 test, only approximately 5 extra deaths with occur per million RhD negative women.

This simulation assumes that women who do not receive an NIPT test, for whatever reason, would still be offered, and generally receive, antenatal anti-D. As a sensitivity analysis we consider the impact of a strategy of requiring an NIPT test as a prerequisite to antenatal anti-D, or, equivalently,

assuming that women who do not comply with an NIPT test would not comply with the whole antenatal anti-D immunisation process. These results are shown in Table 12.

**Table 12 Results of simulation study assuming women who do not receive NIPT are not offered anti-D**

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

These results show that anti-D administration rates will be further reduced (to 62.7%) if women who do not receive an NIPT test do not receive antenatal anti-D. The number of women who miss out on potentially beneficial anti-D will rise to 4.5%. This means 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 testing 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 the cost of the test does not outweigh this saving. The use of NIPT testing 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 to a universal offering of antenatal anti-D is very small, provided care is taken to ensure women who do not receive an NIPT test are still offered and receive anti-D.

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 the NIPT test. This conclusion depends on whether the increase in sensitisations (approximately 13 per 100,000 RhD negative women) is considered ethically acceptable, and cost-effective.

### 4.2.5 **Results: assessment of implementation**

#### 4.2.5.1 **Characteristics of included studies**

Table 13 presents a summary of study characteristics of the twelve studies{#395}[3, 12, 13])[5-11, 25]included in the review of implementation of high-throughput NIPT testing. Most of these were also included in the diagnostic accuracy and/or clinical effectiveness reviews. These studies were conducted in six countries including Denmark, UK, Spain, Netherlands and Sweden. Fetal RhD screening programmes were implemented nationally in the Netherlands and Denmark, and regionally in England, Sweden and Spain. Most of the included studies were large cohort studies reporting implementation data alongside with diagnostic accuracy data, while one study was a survey based at UK (London). The sample size ranged from 282 to 18,383 participants.

**Table 13 Study characteristics of implementation studies**

| Study | Location | Study dates | Sample size[a] | Gestational age at time of NIPT testing (weeks, median/range) |
|---|---|---|---|---|
| Finning (2008) [2] | England Birmingham and Sheffield centre of the National Blood Service | NR | 1869 | 28 (8-38) |
| Soothill (2015)[3] | England, South West, 3 NHS Trusts | 04/2013-09/2013 | 526 | 15-17 (mostly) |
| Oxenford (2013)[13] | England, 4 hospitals (Birmingham, London, Newcastle, Sunderland) | NR | 289 (270 survey respondents, 19 interviews/focus groups) | >12 |
| Banch-Clausen (2014)[5]; Banch-Clausen (2012)[9]; Banch-Clausen (2013)[25]; Damkjaer (2012)[12] | Denmark, nationwide 5 regions | 2010-2011 | 14,547 | 25 (73% between 23 & 28) |
| de Haas (2012)[10]; Thurik (2015) [6] | Netherlands, nationwide | 07/2011- 01/2012 | 18383* | 26 |
| Grande (2013)[7] | Spain, Barcelona, 6 maternity care unit | 02/2010-10/2011 | 282 | 24-26 |
| Wikman (2012)[8]<br><br>Tiblad 2013[11] | Sweden, Stockholm, 83 maternity care centres, 6 delivery units | 09/2009 - 03/2012 (reference cohort: 2004-2008) | 8374[#] | 8-40 |

[a] Number of blood samples undergoing NIPT unless otherwise specified; * number of participants undergoing NIPT; [#] number of pregnancies undergoing NIPT

#### 4.2.5.2 **Results of implementation studies**

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

One UK study (Soothill 2014) conducted in the South West of England stated that it is feasible to implement routine cffDNA 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 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 (2014) [5] | National programme delivered in five regions in Denmark | Very good screening accuracy (see diagnostic review) FNRs mainly due to poor DNA yields or handling error. FPRs due to contamination and genetic variants. Inconclusive due to weak D genotypes. High compliance with anti-D/ Moderate compliance with NIPT test (see effectiveness review) | The challenges to implement the prenatal RHD screening programme are related to programme anti-D prophylaxis compliance. | Implementing external quality assurance programmes as well as regular in-house testing to optimise effectiveness of the screening programme.<br>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 immunizations.<br><br>Issuing focused statements to GPs to avoid sending samples from early pregnancy to help reduce false negative results.<br><br>Increasing information given directly to pregnant women, GPs, midwives, and obstetricians, and systems such as a reminder system integrated into the GPs' software, may help to increase women's compliance with the programme. | None |
| | Banch Clausen 2012 [9] | 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 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. |
| | Banch | Paper focused | Total DNA declines | Not applicable. The paper did | The aim should be for a | None |

| Screening programme (country) | Study | Details of screening programme | General results | Issues to implementation | Authors' practical advice | Authors' research recommendation |
|---|---|---|---|---|---|---|
| | Clausen 2013 [25] | on issues around transportation of blood samples in the Danish screening programme | over time from sampling. Fetal DNA was not generally unaffected over time from sampling. | not consider implementation of the screening programme as a whole. | transportation time of up to 4 days, and no more than 7 | |
| | Damkjaer 2012 [12] | Earlier report on Danish screening programme, focused on compliance issues. | Compliance with NIPT testing was around 90%, improving over time | No additional implementation issues reported. | For GPs: a) Higher level of physician information regarding antenatal RHD screening and tRAADP<br>b) Use of new maternity reports with separate text boxes for information on antenatal RHD screening and the injection of anti-D, which standardizes the communication between departments.<br><br>For midwives: a) increased attention to documentation<br>in the maternity report; b) obligatory disclosure<br>to the patient of the information letter from the Danish<br>National Board of Health at the first meeting with the<br>midwife;<br><br>For patients: encouragement to make an appointment with their GP at 25 weeks for blood sample collection for antenatal RhD screening.<br><br>For obstetricians: to give extra antenatal prophylaxis in case of | None |

| Screening programme (country) | Study | Details of screening programme | General results | Issues to implementation | Authors' practical advice | Authors' research recommendation |
|---|---|---|---|---|---|---|
| | | | | | potentially sensitizing events and to register whether extra doses are given. | |
| UK (Bristol) | Finning (2008) (#395) | Two regions in England - the Birmingham and Sheffield centre of the National Blood Service for routine ABO and RhD blood grouping and antibody screening | Very good diagnostic accuracy (see review). Inconclusive results often due to 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 an 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.<br><br>Commencement of anti-D treatment at 30 weeks' gestation rather than 28 weeks has been considered as 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.<br><br>Testing only samples that are less than seven 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 (2015) (#4) | 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.<br><br>The requirements of patient information, patient consent, | 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 Ig. Continued use of anti-D in women who can be | 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 |
|---|---|---|---|---|---|---|
| | | | | sample handling, sample transfer and implementation of the changed management were all successfully met. | shown to have RhD negative fetuses may be unethical. | |
| UK (London) | Oxenford (2013) (#132) | Survey conducted in one hospital in London, UK. | This study investigated women's preferences and information needs for routine implementation of NIPT testing. Around 290 women included. 92.1% agreed that NIPT testing 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 cell-free fetal DNA, but women's current knowledge of Rhesus blood groups and anti-D administration was found to be limited.<br><br>Although women may agree to extra appointments for the test, health professionals (n=13) thought this may be impractical. | Developing information leaflets and health professional training will be critical for successful implementation. | None |
| Spain | Grande (2013) (#144) | Six health centres of Barcelona-West health district in Spain. | High diagnostic accuracy (see diagnostic review) False negative results were mainly been related to specific DNA extraction methods, prolonged stored time before sample processing, and early gestational age | No issues to implementation were reported. | High-throughput NIPT testing of exons 5, 6, 7 and 10, before 28 weeks of gestation in their mixed population should be considered for further clinical application. | None |
| Netherlands | Thurik (2015) [6] | One region in 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 to document a vanishing twin at any early pregnancy scan and | Prospective studies in pregnancies with a vanishing twin will be required to test whether discrepant NIPT results may be compatible with a vanishing co-twin as source |

| Screening programme (country) | Study | Details of screening programme | General results | Issues to implementation | Authors' practical advice | Authors' research recommendation |
|---|---|---|---|---|---|---|
| | | | | | to counsel against non-invasive prenatal testing. False positive findings will have little impact in NIPT testing as it causes only unnecessary anti-D use. | of a third genomic cell line. |
| | De Hass 2012 [10] | Earlier report on Netherlands screening programme | Compliance with NIPT screening was around 95%. The false-positive rate 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 week of 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. |
| Sweden | Wikman (2012) (#172) | 83 maternity care centres in the Stockholm area, Sweden. | NIPT testing had high diagnostic accuracy with over 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 testing 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 2013 [11] | See Wikman 2012 | 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. |

A number of studies reported issues related to implementing prenatal RhD screening programmes. For example, Banch Clausen (2014)[5] stated that the challenges to implement the prenatal RhD screening programme were related to programme anti-D prophylaxis compliance. Another study by Banch Clausen 2012[9] noted that 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 general practitioner.

The UK-based survey (Oxenford 2013) investigated 290 women's preferences and information needs for routine implementation of NIPT testing. 92.1% women agreed that NIPT testing should be offered but only 75.9% stated that they would accept the test. Women preferred having the test when 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 the NIPT test, 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 the limited knowledge of women in 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.

Several studies offered practical advice for implementing high-throughput NIPT. For example, Finning (2008) 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 an RhD positive fetus. Another recent UK (Bristol) study (Soothill 2015) stated that this service should be extended to the whole 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 (2012)[9] recommended continuing cord blood typing in practice in order to ensure that postnatal anti-D is given if the NIPT testing compliance is poor. Damkjaer 2012 [12] suggested improvement in relevant knowledge on prenatal RhD screening among general practitioners and midwives in Denmark.

Banch Clausen (2013)[25] 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 four days, and no more than seven days. Wikman (2012) 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 testing and the importance of anti-D immunisation is important. Any nationwide NIPT screening programme will require careful logistical management to ensure that blood sample are transported to laboratories and tested quickly, and that results are reliably returned to general practitioners and midwives. NIPT testing 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.

## 4.3 Clinical Effectiveness Summary and Conclusions

### 4.3.1 **Diagnostic accuracy**

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

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

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

The false positive rate found is mostly a consequence of treating women who have an inconclusive test result (approximately 7% of NIPT tests in the UK) as if they had a positive test. Excluding these women from analysis gave a lower false positive rate of 1.26% (95% CI 0.87 to 1.83). It may therefore be possible to reduce the false positive rate 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 testing is insufficiently accurate before around 11 week's gestation (i.e. in first trimester), but is accurate at any time after the end of the first trimester. One study (Chitty 2014) also suggested that 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.

### 4.3.2 **Clinical effectiveness**

Seven studies were included in the clinical effectiveness review. Only two studies had a control group. All studies were judged to be at high risk of bias. As all except one were conducted in non-UK countries, the generalisability of their findings to the UK settings is limited due to variations in national guidelines and health policies between countries (e.g. prescription of routine antenatal anti-D prophylaxis). One large prospective cohort study [11] 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 more than 95% in an established national programme in Denmark. Uptake rates of routine antenatal anti-D prophylaxis 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, ranging from 92% to 99.7%.

Three non-comparative studies evaluated changes in anti-D use following the implementation of NIPT testing. All found that the use of NIPT reduced the total use of anti-D Ig doses, particularly falling by 29% in one UK study (Soothill 2015), 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 likely clinical consequences of implementing NIPT testing. Its results were broadly consistent with the review evidence. It suggested that NIPT testing, when compared to 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 to universal anti-D use) also depends on compliance. Sensitisation rates may increase by 3 to 15 sensitisations per 100,000 women if postpartum cord blood testing is continued, or 13 to 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 an NIPT test are still offered, and receive, antenatal anti-D will minimise the number of additional sensitisations.

### 4.3.3 **Implementation**

Twelves studies were included in the review of implementation. Most of the included studies were large cohort studies reporting implementation data alongside with diagnostic accuracy data, while 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 due to 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 testing among physicians and midwives.

A UK-based survey (Oxenford 2013) revealed that, while most of the women surveyed supported the idea of NIPT testing, their knowledge of Rhesus blood groups and anti-D administration was limited, which could be a barrier to implementation.

### 4.3.4 **Conclusions**

High throughput NIPT testing for fetal RhD status is an accurate diagnostic test, if performed after 11 week's gestation. It has a false negative rate (women remain at risk of sensitisation) of around 0.2%, and a false positive rate (women receive unnecessary anti-D) of around 5.7%. The test gives an inconclusive result in around 7% of women, in the UK. Treating these inconclusive tests as if they were positive is the cause of most false positive results. Giving antenatal anti-D immunoglobulin on the basis of the NIPT test, 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 an RhD negative fetus. Some women will, however, continue to receive anti-D unnecessarily due to an inconclusive test result.

Although the evidence was limited, it appears that using the NIPT test will lead, at worst, to only a small increase in the number of sensitisations when compared to 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 an NIPT test) 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 the NIPT testing.

# 5 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 this data to inform UK practice and the current assessment, as set out in the NICE scoping documentation. 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 post-natal administration of anti-D immunoglobulin. The findings from the review informed the development of a new decision analytic model reported in the following chapter.

## 5.1 Methodology of the cost-effectiveness review

### 5.1.1 **Searches**

In addition to the searches conducted for the review of clinical evidence (see Section 4), the following databases were searched up to December 2015 for cost-effectiveness evidence: NHS Economic Evaluation Database (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.

### 5.1.2 **Selection criteria**

A broad range of studies were 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.

### 5.1.3 **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.

### 5.1.4 **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.

#### 5.1.5 **Critical appraisal**

A quality appraisal was carried out using the checklist of Drummond and Jefferson [60]. This checklist evaluates the extent to which each review result provides detail on different aspects such as study design, data collected and its 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.

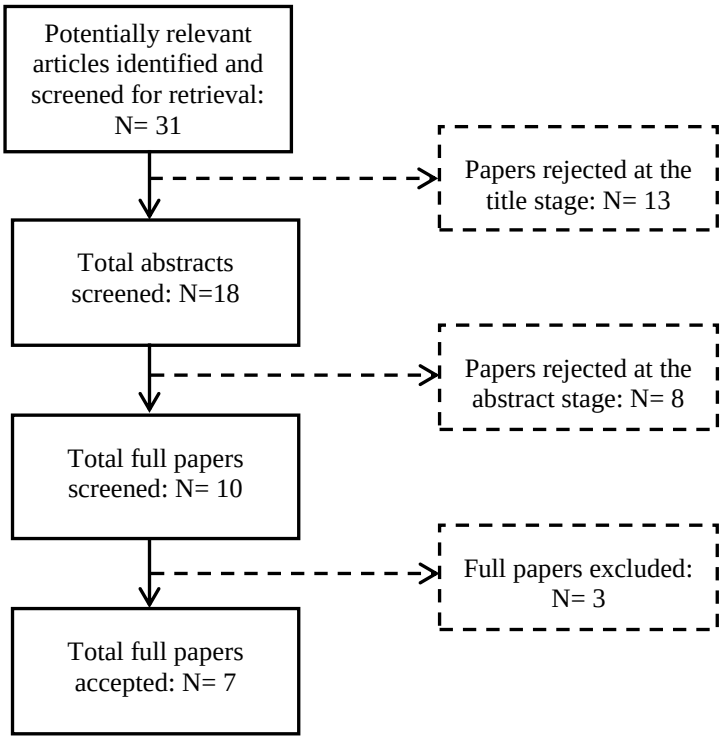
## 5.2 Results of review of existing cost-effectiveness evidence

### 5.2.1 **Quantity of research available**

#### 5.2.1.1 **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 paper screening. Of those, seven met the selection criteria and were included in the review.[58, 61-66] A flow diagram of the selection process is reported in Figure 10.

**Figure 10: Assessment of cost effectiveness: Summary of study selection and exclusion**



#### 5.2.1.2 **Number and type of studies excluded**

A list of full-text papers that were excluded is given in Appendix 10.10. These papers were excluded because they failed to meet one or more of the inclusion criteria, including lack of full text publication and ineligible study design.

### 5.2.2 **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 [63] and Hawk et al [65] stated that their analysis considered RhD-negative pregnant women, but were not clear about women's sensitisation status at study entry. Only two studies [61, 66] 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-througput studies. One study [64] explicitly focused on providing NIPT to all RhD-negative women as the test for sensitisation is only conducted if the NIPT test result is positive.

Most studies [58, 61, 64-66] evaluated the cost-effectiveness of introducing NIPT in the management pathway of RhD-negative pregnant women compared to alternative strategies. These studies explored a range of alternative strategies to prevent sensitisation. Except for Szczepura et al [61] and Macher et al [63], 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 [64] also explored the immunological 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 8-12 weeks' gestation. Benachi and colleagues [62] compared alternative timings of the NIPT by considering the cost consequences of performing NIPT during the first and the third gestation trimesters. With the exception of Duplantie et al [64], where insufficient information is provided, all cost-effectiveness studies evaluated the consequences of introducing NIPT in terms of avoiding RAADP but also how it impacted on post-partum treatment.

Three studies [58, 61, 66] aimed at evaluating the short-term costs and consequences of sensitisation in RhD-negative women. Duplantie et al [64] and Hawk et al [65], however, estimated long-term outcomes relating to morbidity and mortality attributable to haemolytic disease of the fetus and/or newborn. Furthermore, two studies [58, 64] explicitly considered in their analysis women's first and subsequent pregnancies, presenting cost-effectiveness results for each scenario.

Benachi et al [62] and Macher et al [63] 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, anti-body testing, etc.

The cost-effectiveness studies evaluated different strategies in different health systems, including England and Wales, Canada, Sweden and the United States. Except for Sweden, where only post-partum administration of anti-D (conditional on 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 [62, 63] evaluated the cost implications of introducing NIPT in the French and Spanish (namely, Andalusia region) health care settings. Current guidance on the prevention of sensitisation in these countries was not clearly stated. Macher et al [63] focused mainly on addressing questions relating to the accuracy and implementation of different NIPT methodologies into current clinical practice in Spain.

**Table 15 Cost-effectiveness study characteristics**

| Study | Objectives | Setting / perspective | Population | Analytical approach | Diagnostic comparators | Outcomes | Main results |
|---|---|---|---|---|---|---|---|
| Szczepura, 2011 [61] | Cost-effectiveness analysis of NIPT implementation in England and Wales | NHS England and Wales | 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 to RAADP. | Two scenarios compared:<br>1. Assumed that all RhD-negative women will routinely receive an NIPT at approximately 28 weeks and that RAADP will be withheld if an RhD-negative fetus is identified (prophilatyc anti-D for potentially sensitising events assumed withheld); post-partum testing and anti-D prophylaxis assumed to be unaffected; and<br>2. Assumed that, in addition to scenario 1, post-delivery blood cord serology and fetal-maternal haemorragae test will be withheld if NIPT result has identified an 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 - number of sensitisations was estimated not to fall appreciably; sensitisations expected to rise if NIPT sensitivity is below 99.9%. |
| Benachi, 2012 [62] | Cost-minimisation analysis of NIPT on the costs of managing RhD-negative pregnant women, whether or not they are sensitised | French National Health Service | Unsensitised RhD-negative pregnant women | A prospective follow-up of RhD-negative women during their pregnancy | Four scenarios compared:<br>1. RAADP at 28-32 weeks' gestation;<br>2. RAADP and additional 300 µg anti-D administration at 28 weeks' gestation;<br>3. NIPT performed during the 1st trimester in order to detect women not at-risk (i.e. carrying an RhD-negative fetus);<br>4. NIPT performed during the 3rd trimester in order to offer RAADP only to women carrying an RhD-positive fetus.<br>For strategies 3 and 4 systematic (i.e. to all) and targeted (i.e. conditional on test results) newborn 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 and beginning of second trimester) was found to be cost saving compared to RAADP during the third trimester. |

| Study | Objectives | Setting / perspective | Population | Analytical approach | Diagnostic comparators | Outcomes | Main results |
|---|---|---|---|---|---|---|---|
| Macher, 2012 [63] | Cost-minimisation analysis of NIPT (multiplex real-time PCR assay for fetal cell-free DNA) in pregnant women plasma | Andalusian government, Spain | RhD-negative pregnant women | 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.<br>Three ways of detecting fetal RhD using NIPT were compared:<br>1. Exon 5;<br>2. Exon 7; and<br>2. SRY<br>Testing was performed on RhD-negative women in weeks 10–28 of pregnancy.<br>Consequences of test results 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 improving the response of the laboratory, saving time and reagent costs, opening the door to a complete automatisation of the process. |

| Study | Objectives | Setting / perspective | Population | Analytical approach | Diagnostic comparators | Outcomes | Main results |
|---|---|---|---|---|---|---|---|
| Duplantie, 2013 [64] | Cost-effectiveness analysis of strategies to prevent RhD alloimunisation | Public health care system of Quebec | Unsensitised RhD-negative pregnant women | Computer-based simulation model with virtual population of 10,000 RhD-negative pregnant women.<br>Two decision trees: a) applied to the first pregnancy of an RhD-negative woman; and b) applied to an eventual second pregnancy in 55% of those women. | Four scenarios compared:<br>1. Systematic prophylaxis: RAADP at around 28 weeks' gestation (recommended by the Canadian guidelines);<br>2. NIPT at around 12 weeks' and/or at 28 weeks' gestation. RAADP and post-partum anti-D withheld for RhD-negative fetus result;<br>3. Immunological determination of the father's Rh type; and<br>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 post-partum anti-D withheld for RhD-negative fetus result.<br>Prophilatyc anti-D for potentially sensitising events not discussed but assumed withheld for a RhD-negative fetus result in scenarios 2 and 4. | Clinical:<br>a) number of babies without haemolytic disease; and b) number of surviving infants.<br>Economic:<br>a) cost per 10,000 pregnancies; b) cost per number of babies without haemolytic disease; and c) cost per number of surviving babies.<br>Outcomes obtained for first and second pregnancies. | 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.<br>RAADP remained the preferred option for the prevention of maternal sensitisation. |

| Study | Objectives | Setting / perspective | Population | Analytical approach | Diagnostic comparators | Outcomes | Main results |
|---|---|---|---|---|---|---|---|
| Hawk, 2013 [65] | Cost-effectiveness of NIPT for targeted prophylaxis | United States Health system (Medicaid and Medicare) | RhD-negative women | Decision tree model using a decision tree structure comparing three relevant scenarios | Three scenarios compared:<br>1. RAADP at 28 weeks' gestation and post-partum prophylaxis guided by cord blood typing (current approach in most of the US);<br>2. Non-invasive fetal RhD typing performed early in pregnancy (1st trimester assumed) with prophylaxis (i.e. for potentially sensitising events, RAADP and post-partum anti-D administration) guided by test results;<br>3. No screening or prophylaxis. | Costs per RhD woman, morbidity and mortality attributable to haemolytic disease | Non-invasive fetal RhD testing was found not to provide any economic benefit for the management of RhD-negative women. RAADP and post-partum prophylaxis guided by cord blood typing remained the most cost-beneficial option for the management of RhD-negative women. |
| Neovius, 2015 [58] | Cost-effectiveness of first trimester NIPT for targeted antenatal versus no RAADP or versus 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:<br>1. First trimester NIPT followed by targeted RAADP at 29 weeks' gestation as well targeted post-partum anti-D;<br>2. Historical comparators of no RAADP, only post-partum anti-D in case of an RhD-positive baby; and<br>3. Non-targeted RAADP and post-partum 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, 2015 [66] | 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 during 1 year | Two scenarios compared:<br>1. RAADP for all unsensitised pregnant women – inc. 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); and<br>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 an 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 / pregnancy in 1 year, cost per pregnancy. | Implementation of a program 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 program) the cost per patient was expected to decrease due to economies of scale. |

### 5.2.3 **Quality of included studies**

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

#### 5.2.3.1 **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 analysis 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, while Duplantie et al [64] and Hawk et al [65] explicitly dealt with not just sensitisations but also 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 provision of the NIPT or administration of anti-D immunoglobulin. None of the studies considered the effectiveness and/or cost-effectiveness of NIPT in ethnic minority groups. Except for one study[61], most studies were not explicit in considering that most NIPT performance assessments have been done in white Caucasian populations and, thus, its realiability 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.

#### 5.2.3.2 **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 mainly based on diagnostic studies aimed at verifying test performance; including three studies [58, 62, 63] which considered evidence collected from subjects in the underlying cohort studies. These type of observational studies are inherently prone to bias and tools exist to appraise them (e.g. STARD [67], QADAS [68] or the more recent update QADAS-2 [26]). To our knowledge, these tools were not used to appraise the study findings. Sources for the effectiveness of anti-D immunoglublin varied across the different studies and were not based on systematic reviews but mainly on jurisdiction-specific sensitisation estimates. Studies which considered broader outcomes associated with sensitisation (i.e. haemolytic disease and impact of future pregnancies), populated these parameters with relevant published evidence [69-71].

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 any price and currency conversion adjustments were made. One study [65]

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 of the anti-D immunoglubin treatment. The cost of NIPT varied significantly across studies from approximately €20 [63] (2012 prices) to US$450 [65] (2013 prices) per sample; some including blood type, RhD determination and anti-body screen. The NIPT cost range in the studies that explicitly stated that a high-throughput method was being assessed varied from £16.25 [61] (2011 prices) to CAD$34.45 [66] (2015 prices). This may indicate that studies reporting a high unit cost for NIPT [64, 65] were not based on a high-throughput process. The majority of studies that provided a reference for the NIPT cost figures obtained these from government [58, 64, 66] or from laboratory genetic test companies [65]. A relevant consideration in relation to the cost of NIPT is whether the test is also subject to additional royalty fees which 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 and colleagues [61] explicitly considered this aspect by exploring the robustness of the results by varying the the fee from zero to £46.5; the latter cost being the unit cost of a commercial testing kit which includes the royalty. Significant variation was also found over the unit costs per dose of anti-D immunoglobulin; varying from £33.5 [61] (2011 prices) to US$462 [65] (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 as to test implications.

#### 5.2.3.3 **Analysis and interpretation of results**

The two cost-minimisation studies [62, 63] took a simple approach and evaluated direct medical costs associated with the management of the RhD-negative pregnant women. From 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 by the majority of studies [58, 64-66] 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 [61], 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 [62] found that greater cost savings were possible when the NIPT was given in the 1st trimester compared to the 3rd trimester due 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 to performing the test later in pregnancy (third trimester), saving, on average, €38 per patient (2012 prices).

Teitelbaum et al [66] and Szczepura et al [61] were the only two research studies which factored in their analysis the issue of NIPT fetal RhD genotyping producing inconclusive results and therefore performing sensitivity analysis over the inconclusive rate. Their analyses assumed that inconclusive test results would be treated as positive test results and thus, 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 and 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.

**Table 16: Quality assessment of studies included in the economic review using the checklist of Drummond and Jefferson**

| Criteria | Szczepura, 2011 [61] | Benachi, 2012 [62] | Macher, 2012 [63] | Duplantie, 2013 [64] | Hawk, 2013 | Neovius, 2015 [58] | Teitelbaum, 2015 [66] |
|---|---|---|---|---|---|---|---|
| ***Study design*** | | | | | | | |
| The research question is stated | Y | Y | Y | Y | Y | Y | Y |
| The economic importance of the research question is stated | Y | Y | Y | Y | Y | Y | Y |
| The viewpoint(s) of the analysis are clearly stated and justified | Y | Y | N | Y | Partial | Y | N |
| The rationale for choosing alternative programmes or interventions compared is stated | Y | Y | Y | Y | Y | Y | Y |
| The alternatives being compared are clearly described | Y | Y | Y | Y | Y | Y | Y |
| The form of economic evaluation used is stated | Partial | Partial | N | Y | Partial | Y | Partial |
| The choice of form of economic evaluation is justified in relation to the question addressed | N | N | N | Partial | N | Partial | N |
| ***Data collection*** | | | | | | | |
| The source(s) of effectiveness estimates used are stated | Y | NA | NA | Y | Y | Y | Y |
| Details of the design and results of the effectiveness study are given (if based on a single study) | N | NA | NA | N | N | Y | N |
| Details of the methods of synthesis or meta-analysis of estimates are given (if based on a synthesis of a number of effectiveness studies) | N | NA | NA | NA | NA | NA | N |

| Criteria | Szczepura, 2011 [61] | Benachi, 2012 [62] | Macher, 2012 [63] | Duplantie, 2013 [64] | Hawk, 2013 | Neovius, 2015 [58] | Teitelbaum, 2015 [66] |
|---|---|---|---|---|---|---|---|
| The primary outcome measure(s) for the economic evaluation are clearly stated | Y | N | N | Partial | Partial | Y | Y |
| Methods to value benefits are stated | NA | NA | NA | N | N | N | NA |
| Details of the subjects from whom valuations were obtained are given | NA | NA | NA | NA | NA | NA | NA |
| Productivity changes (if included) are reported separately | N | N | N | N | N | N | N |
| The relevance of productivity changes to the study question is discussed | NA | NA | NA | NA | NA | NA | NA |
| Quantities of resource use are reported separately from their unit costs | Y | Y | Y | Y | N | Y | Y |
| Methods for the estimation of quantities and unit costs are described | Y | N | Y | Y | Partial | N | Partial |
| Currency and price date are recorded | Y | N | Y | Y | Partial | Y | Partial |
| Details of currency of price adjustments for inflation or currency conversion are given | N | N | N | N | N | Partial | N |
| Details of any model used are given | N | N | N | Y | Y | Y | Y |
| The choice of model used and the key parameters on which it is based are justified | NA | NA | NA | Partial | Partial | N | Partial |
| ***Analysis and interpretation of results*** | | | | | | | |
| Time horizon of costs and benefits is stated | N | N | N | Partial | N | Y | N |
| The discount rate(s) are stated | N | N | N | NA | N | Y | N |
| The choice of discount rate(s) is justified | NA | NA | N | NA | NA | Y | NA |

Y, yes; N, no; NA, not applicable.

### 5.2.4 **Results of included studies**

In terms of conclusions, conflicting results were reported across the existing economic studies. Three studies [61, 64, 65] reported NIPT fetal RhD genotyping not to be cost-effective or of no economic benefit. Hawk et al [65] and Szczepura et al [61] 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 [61] 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 the NIPT sensitivity fell below 99.9%.

Two studies [58, 62] reported that NIPT is cost-saving compared to no RAADP (i.e. compared to post partum anti-D only). Only one study [66] found NIPT for targeted RAADP to be cost-saving compared to non-targeted RAADP, which also estimated no increase in the risk of sensitisation if NIPT were to be used. Duplantie et al [64] found that targeting of RAADP based on immunological RhD typing of the father is cost-effective compared to the use of NIPT.

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

### 5.2.5 **Relevance to NHS and current decision problem**

One of the key aspects of this review is to address how relevant to the UK study assumptions and findings are. None of the study approaches and findings reviewed were 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 immunoglubin treatment routinely and post-partum; and the impact of sensitisation on infant health and/or on subsequent pregnancies. Only one [61] 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. Also, it assumed that post-partum testing and treatment would be unaffected by NIPT results. Furthermore, no assessment of the timing of NIPT was done, nor any consideration of the impact on subsequent pregnancies. 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 US; contries in which similar guidance to the UK exists on the prevention of sensitisation, their relevance to the UK and generalisability of findings can be questioned due to crucial health care system differences and how anti-D immunoglubulin policies have been implemented over recent decades.

# 6 Independent economic assessment

## 6.1 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 Section 5.1 and the independent economic model used to inform NICE TA156 on the clinical and cost-effectiveness of RAADP [72]. The model provides a framework for the synthesis of diagnostic accuracy reported in Section 4 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 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 [72]. 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 10.11.

The decision model is populated using the results from the systematic clinical review on the diagnostic accuracy of high throughput NIPT as described in Section 4 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 the 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. fetal-maternal haemorrhage) as well as the administration of prophylactic anti-D immunoglobulin after a potentially sensitising event where the test result indicates an RhD-negative fetus. In addition, high-throughput NIPT for fetal RhD status determination may impact post-partum testing (i.e. cord blood typing and fetal-maternal haemorrhage) and post-partum 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.

### 6.1.1 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 post-delivery testing and post-partum anti-D immunoglobulin administration.

### 6.1.2 Intervention and comparator pathways

Current NICE clinical guidance on antenatal care [20] 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 [16].

Routine Antenatal Anti-D Prophylaxis (RAADP) 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 [16, 21]. Potentially sensitising events include those which may lead to fetal-maternal haemorrhage such as medical interventions (e.g. chorionic villus sampling, amniocentesis or external cephalic version), terminations, late miscarriages, antepartum haemorrhage and abdominal trauma  Following a potentially sensitisation event, the recommended minimum dosage of anti-D immunoglobulin increases with gestational age (i.e. higher dose for more than 20 weeks gestation) and fetal-maternal haemorrhage 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 fetal-maternal haemorrhage test 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 the 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:
    i. Avoidance of RAADP;
    ii. Avoidance of prophylactic anti-D immunoglobulin and fetal-maternal haemorrhage tests following potentially sensitising events;
    iii. 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:
    i. Avoidance of cord serology testing in favour of routine fetal-maternal haemorrhage testing and post-partum anti-D immunoglobulin following delivery.

## 6.2 Model Structure

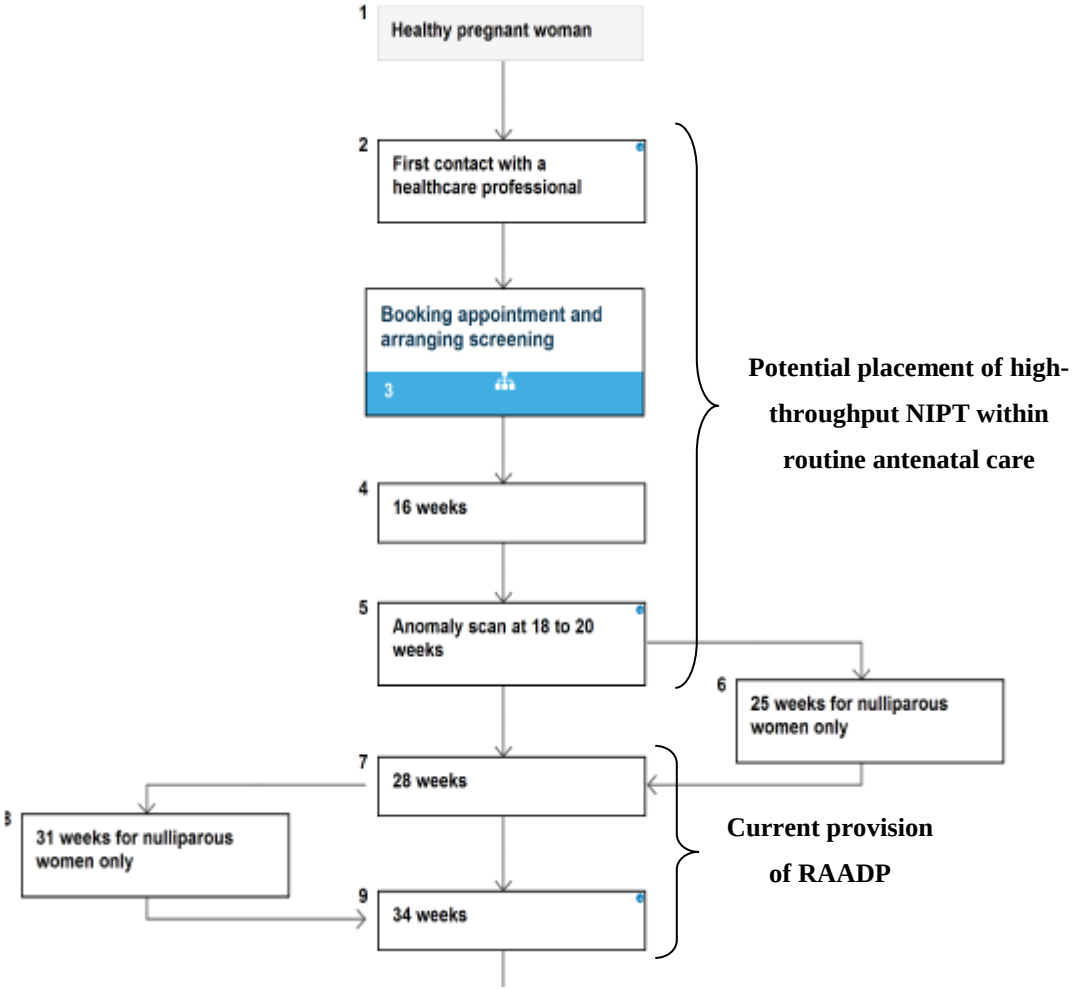
### 6.2.1 Modelling methodology and scope

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 immunoglobluin and, if after 20 weeks'

gestation, fetal-maternal haemorrhage 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 fetal-maternal haemorrhage tests and the administration of post-partum 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.

**Figure 11: Excerpt from NICE schedule of appointments in routine antenatal care**




Additionally to the first contact/ 8-12 weeks' gestation booking appointment, the points of routine contact at which blood could be drawn for the NIPT are the 16 weeks' visit and 18-20 weeks' scan (at which outstanding routine screening tests are offered). Other opportunities may include attendance to receive the 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 only be subsequently offered 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 the NIPT indicate a RhD-positive baby. We assume that provision of the 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 that evaluated 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, fetal maternal haemorrhage tests and anti-D immunoglobulin for potentially sensitising events, cord serology, fetal maternal haemorrhage tests and post-natal anti-D immunoglobulin). This determines where receipt of anti-D immunoglubulin is appropriate (true positive in terms of NIPT test result, and/or post-natal cord serology and inconclusive result but pregnant with RhD positive fetus), where avoidance of anti-D immunoglobulin is appropriate (true negative in terms of NIPT test result), where anti-D immunoglobluin is uneccessary (false positive or inconclusive in terms of NIPT test result and carrying a RhD negative fetus) and where avoidance of anti-D immunoglobulin is potentially harmful (false negative in terms of NIPT test 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 post-partum care pathway were considered:

(a) Post-partum scenario 1 (NIPT PP1): post-partum cord blood typing and fetal-maternal haemorrhage testing would continue to be performed, as per current guidelines, in all women regardless of the fetal RhD status identified through high-throughput NIPT;

(b) Post-partum scenario 2 (NIPT PP2): post-partum cord blood typing, fetal-maternal haemorrhage 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;

(c) Post-partum scenario 3 (NIPT PP3): post-partum cord blood typing would be performed if high-throughput NIPT of fetal RhD status identifies a RhD-negative fetus. Fetal-maternal haemorrhage testing and post-delivery anti-D immunoglobulin would be administered if high-throughput NIPT was inconclusive or identifies a RhD-positive fetus; and

(d) Post-partum scenario 4 (NIPT PP4): post- partum cord blood typing not performed in any women. Fetal-maternal haemorrhage testing and post-delivery anti-D immunoglobulin administered if high-throughput NIPT was inconclusive or had identified a RhD-positive fetus.

The impact that post-delivery testing has on the cost-effectiveness results are 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: (i) RAADP and supplementary anti-D immunoglobulin (as required based on potentially sensitising events) offered to all RhD-negative pregnant women; (ii) further post-partum anti-D immunoglobulin offered to all RhD-negative women whose baby RhD status is confirmed to be positive after cord blood typing.

A schematic representation of the model is provided in **Error! Reference source not found.**. Note hat this figure does not provide a comprehensive representation of all components being considered in each alternative strategy, including the post-partum scenarios. The four post-partum scenarios for how the introduction of the NIPT could impact on the use of cord serology, fetal maternal haemorrage tests and anti-D immunoglobulin use following delivery are detailed in Table 17.

The model considers the total number of children that 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 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 affect 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 Section 6.3.14), and any further RhD positive fetuses are at risk of adverse health consequences (see Sections 6.3.10 and 6.3.14). 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:

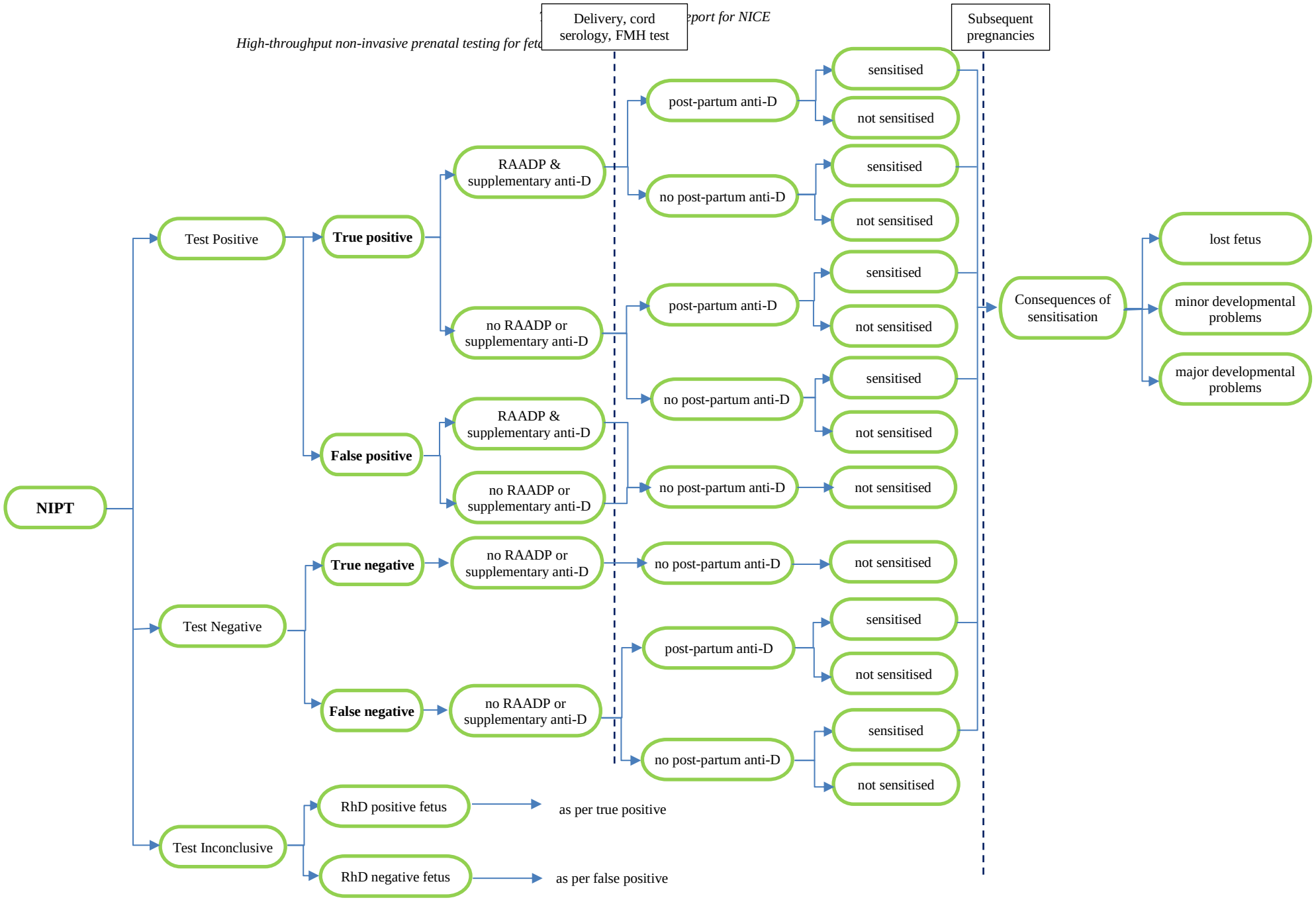
- number of sensitisations and the associated costs;
- number of affected fetuses following sensitisation;
- number of fetuses lost and associated QALY loss;
- cost per life-year gained.

**Table 17 Characteristics of the post-partum scenarios.**

| Scenarios | High-throughput NIPT result | Cord serology | FMH | Post-partum Anti-D |
|---|---|---|---|---|
| **Post-partum scenario 1** | Any | Yes | Yes if CS+ | As guided by CS and FMH |
| **Post-partum scenario 2** | T- | No | No | No |
| | T+, inc | Yes | Yes if CS+ | As guided by CS and FMH |
| **Post-partum scenario 3** | T- | Yes | Yes if CS+ | As guided by CS and FMH |
| | T+, inc | No | Yes | Yes with additional dose per FMH |
| **Post-partum scenario 4** | T- | No | No | No |
| | T+, inc | No | Yes | Yes with additional dose per FMH |

'-' indicates negative high-throughput NIPT result; '+' indicates positive high-throughput NIPT result; 'inc' indicates inconclusive high-throughput NIPT result; CS- cord serology; FMH- fetal-maternal haemorrhage test.

**Figure 12: Decision analytic model schematic representation of RhD-negative pregnant women pathways: (i) no high-throughput NIPT and RAADP (current practice, no test and RAADP); and (ii) high-throughput NIPT and targeted RAADP.**


Delivery, cord serology, FMH test
Subsequent pregnancies
NIPT
Test Positive
Test Negative
Test Inconclusive
True positive
False positive
True negative
False negative
RhD positive fetus
RhD negative fetus
as per true positive
as per false positive
RAADP & supplementary anti-D
no RAADP or supplementary anti-D
post-partum anti-D
no post-partum anti-D
sensitised
not sensitised
Consequences of sensitisation
lost fetus
minor developmental problems
major developmental problems

### 6.2.2 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 shows 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. While the majority of these are thought to occur in the third trimester (weeks 29 to 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 indicates 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.[21] Also, post-partum anti-D immunoglobulin current uptake is believed to be also close to 100%.[21] However, data relating to the uptake of routine and post-partum anti-D immunoglobulin in the presence of fetal RhD status identification is scarce – see Section 4. Finally, we consider alternative scenarios for the proportion of women in whom the NIPT result is inconclusive. The rate of inconclusive results may reach more than 14% and these are typically managed as RhD-positive results – see Section 4.2.2. 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.

## 6.3 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 showed on**

Table 23.

### 6.3.1 Target population

The number of pregnancies in RhD-negative women in England was estimated to be of 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 1,000 women per year [73] and assumes that 15% of the population is RhD-negative [74].

### 6.3.2 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 [75], 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 TA 156 [72], the model assumes that the proportion of RhD-positive babies born to RhD-negative women is a function of: (i) 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); (ii) the proportion of heterozygous fathers; and (iii) the proportion of heterozygous fathers having RhD-positive babies. While 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 anticipated to be determined on the basis of 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 Table 1 below.

**Table 18 Probability of RhD-positive baby following delivery of a RhD-positive baby**

| Parameter | Mean value | S.E. | Distribution | Source / calculation |
|---|---|---|---|---|
| **Total number of births** | 659,213 | --- | --- | Office for National Statistics, 2013 [76] |
| **Proportion of pregnancies accounted for by Rh-negative women (a)** | 15.0% | --- | --- | Hospital Episode Statistics Analysis and Health and Social Care Information Centre, 2013-2014 [74] |
| **Proportion of heterozygous fathers (b)** | 55.0% | 10.0% | Normal | Roman and Pernell, 2002 [77] |
| **Proportion of heterozygous fathers having RhD-positive babies (c)** | 50.0% | --- | --- | Assumption |
| **Proportion of RhD-positive babies in Rh-negative women (1st 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 (1st baby) (d) |

### 6.3.3 Diagnostic accuracy of NIPT

Data on the diagnostic accuracy of high-throughput NIPT are based on the meta-analyses summarised in Section 4.2.3. The base case utilises the pooled results for the subgroup of UK (Bristol-based) studies where inconclusive results are considered as test positive. These were considered to be the most relevant to the English setting. Sensitivity, specificity (with 95% confidence intervals) and the correlation between these two test accuracy dimensions (on the log-odds scale) were used to inform 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). Sensitivity analyses 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 UK studies only, shows a lower false-negative rate and a slightly higher false-positive rate compared to other scenarios.

Only one study [1] 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 to 13 weeks, 14 to 17 weeks and 18 to 23 weeks – see section 6.5.2.

**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 (0.991-0.999) | 0.987 (0.981-0.991) | 0.461 | Log-Normal |
| **All studies (treating inconclusive results as if testing positive)** | 0.997 (0.992-0.999) | 0.962 (0.943-0.975) | -0.316 | Log-Normal |
| **Only studies reporting inconclusive results* (treating inconclusive results as if testing positive)** | 0.996 (0.989-0.998) | 0.957 (0.932-0.972) | -0.074 | Log-Normal |
| **UK Bristol studies only (treating inconclusive results as if testing positive)** | 0.998 (0.992-0.999) | 0.942 (0.92-0.959) | -1.000 | Log-Normal |

* Excluding Thurik et al [6] and Grande et al [7].

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

| NIPT accuracy per gestation age, Chitty et al [1] | Sensitivity (mean, SE) | Specificity (mean, SE) | Distribution |
|---|---|---|---|
| ***Treating inconclusive results as if testing positive*** | | | |
| **Less than 11 weeks** | 0.9685 (0.0079) | 0.9440 (0.0123) | Log-Normal |
| **Between 11 and 13 weeks** | 0.9983 (0.0023) | 0.9525 (0.0114) | Log-Normal |
| **Between 14 and 17 weeks** | 0.9967 (0.0045) | 0.9534 (0.0141) | Log-Normal |
| **Between 18 and 23 weeks** | 0.9982 (0.0003) | 0.9304 (0.0138) | Log-Normal |
| **More than 24 weeks** | 1.0000 (0.0010) | 0.9574 (0.0076) | Log-Normal |
| ***Excluding inconclusive results*** | | | |
| **Less than 11 weeks** | 0.9615 (0.0079*) | 0.9970 (0.0123*) | Log-Normal |
| **Between 11 and 13 weeks** | 0.9981 (0.0023*) | 0.9884 (0.0114*) | Log-Normal |
| **Between 14 and 17 weeks** | 0.9963 (0.0045*) | 0.9956 (0.0141*) | Log-Normal |
| **Between 18 and 23 weeks** | 0.9980 (0.0003*) | 0.9847 (0.0138*) | Log-Normal |
| **More than 24 weeks** | 1.000 (0.0010*) | 0.9900 (0.0076*) | Log-Normal |

* In the absence of information the SEs were assumed the same as in the approach where inconclusive results were treated as positive results.

### 6.3.4 NIPT inconclusive results

In the UK studies that inform the base case for the decision model the pooled proportion of inconclusive NIPT results was 6.2%. Across all diagnostic studies which report the number of inconclusive results this proportion is lower at 3.9%. The results of the diagnostic accuracy studies suggest that the probability of an RhD-positive baby is higher among women in whom the high-throughput NIPT is inconclusive compared to the probability across all RhD-negative women – see Section 4.2.3.4. In section 6.3.4 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 69.7%, irrespective of the pregnancy. This probability is slightly reduced (69.6%) if only UK studies are considered.

### 6.3.5 Effectiveness of Anti-D immunoglobulin

The introduction of the 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 incorrectly withheld due to a false negative high-throughput NIPT result; and
- the costs and adverse effects associated with administration of anti-D immunoglobulin.

The clinical and cost-effectiveness of RAADP in RhD-negative women has been previously established in NICE TA41 [78] and most recently in NICE TA156 [72]. No new systematic reviews of RAADP with studies not considered in TA156 were identified. We maintain consistency between the NICE Technology Appraisal 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 [79] published after NICE TA156 had been completed is explored within a separate sensitivity analysis. Evidence for the clinical effectiveness of the post-partum use of anti-D immunoglobulin was sourced from a previous Cochrane review [80]. The clinical effectiveness estimates of RAADP and post-partum use of anti-D immunoglobulin reported across these separate sources are reported in Table 21.

**Table 21 Effectiveness of anti-D immunoglobulin when routinely administered and post-partum.**

| | **Odds ratio: sensitisation with RAADP* (95% CI)** | **Odds Ratio: sensitisation at birth, follow-up up to 6 months, with post-partum Anti-D † (95% CI)** | **(Baseline) Sensitisation rate of no RAADP* (95% CI)** | **Sensitisation rate of RAADP (pooled using meta-analysis) (95% CI)** | **Sensitisation rate of no RAADP and no post-partum Anti-D (95% CI)** |
|---|---|---|---|---|---|
| **NICE TA 156 [72]** | 0.37 (0.21-0.65) | --- | 0.95% (0.18%-1.71%) | 0.35% (0.29%-0.40%) | --- |
| **Turner et al [79]** | 0.31 (0.17-0.56) | --- | 0.95%[#] (0.18%-1.71%) | 0.40% (0.16%-0.70%) | --- |
| **Turner et al [79] (Single-dose‡)** | 0.42 (0.17-0.73) | --- | 0.95%[#] (0.18%-1.71%) | 0.30% (0.16%-0.53%) | --- |
| **Turner et al [79] (Two-dose§)** | 0.31 (0.09-0.65) | --- | 0.95%[#] (0.18%-1.71%) | 0.31% (0.09%-0.62%) | --- |
| **Crowther et al [80] [$]** | --- | 0.08 (0.06-0.11) | 0.95%[#] (0.18%-1.71%) | --- | 10.7% (8.0%-13.8%) |

* *versus* no RAADP, conditional on receiving post-partum anti-D immunoglobulin; † *versus* no post-partum anti-D immunoglobulin, conditional on no RAADP; [#] Baseline sensitisation rate of no RAADP assumed the same; ‡ single-dose (1500 IU) at 28-30 weeks, conditional on receiving post-partum anti-D immunoglobulin; § Two-doses (500 IU) at 28 and 34 weeks, conditional on receiving post-partum anti-D immunoglobulin; [$] Sensitisation after 6 months of delivery, irrespective of ABO status.

#### 6.3.5.1 **NICE Technology Appraisal on RAADP**

Ten studies evaluating the clinical effectiveness of RAADP were found to be relevant in NICE TA156; these varied in terms of their patient selection criteria and dosage regimens. Despite the heterogeneity across studies, there was consistency across the synthesis results based on different subsets of the evidence. The result of a fixed-effect meta-analysis of two non-randomised community-based UK studies that used a dosage regimen of 500 IU 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%-1.71%) to 0.35% (95% CI, 0.29%-0.40%). These sensitisation rates are conditional on anti-D immunoglobulin treatment being provided also at potentially sensitising events. This gives an odds ratio for the risk of sensitisation of 0.37 (95% CI, 0.21-0.65) for RAADP compared to no RAADP, and an absolute reduction in risk of sensitisation in RhD-negative mothers at risk (i.e. of carrying an 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.

#### 6.3.5.2 **Turner et al 2012**

Following the publication of the NICE TA156, Turner and colleagues [79] revisited the effectiveness of RAADP for preventing sensitisation in pregnant RhD-negative women. This publication used alternative meta-analytic 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, while 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 the uncertainty about the extent of bias.

After adjusting for differences in study quality and design, the pooled odds ratio for sensitisation was estimated as to be 0.31 (95% CI, 0.17-0.56), with no evidence of heterogeneity ($I^2$ = 0%). Pooled results were similar to the ones obtained from the NICE TA 156 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 sensitivity analysis for the *de-novo* model presented here.

#### 6.3.5.3 **Post-partum use of Anti-D**

Current anti-D immunoglobulin post-partum prophylaxis states that following 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 fetal-maternal haemorrhage and additional dose(s) given as guided by fetal-maternal haemorhage tests [16, 20].

A Cochrane systematic review was identified which assessed the effectiveness of anti-D immunoglobulin in RhD-negative women who had given birth to RhD-positive babies [80]. Data on six

eligible studies, comparing post-partum anti-D immunoglobulin prophylaxis with no treatment or placebo, were synthesised. The estimated odds ratio for sensitisation six months after birth with post-partum anti-D immunoglobulin was 0.08 (95% CI, 0.06-0.11). The estimated odds ratio for sensitisation in subsequent pregnancies with post-partum anti-D immunoglobulin was 0.12 (95% CI, 0.07-0.19). While the former was estimated on 5 studies with approximately 7,500 participants, the latter was based on 4 studies with approximately 1,000 patients. Thus, on the basis of a larger sample size we assumed the former estimate to be the most representative of the effectiveness of post-partum anti-D immunoglobulin in the target population (reported in the last row of results in Table 20). Estimated benefits of post-partum anti-D immunoglobulin administration were observed irrespective of the ABO status of mother and child.

### 6.3.6 **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 [20] 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 less than 12 weeks gestation. A minimum dose of 250 IU 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 72hrs with additional dose as guided by a test for fetal-maternal-haemorrhage.

Evidence on reported number of potentially sensitising event was found in the recent audit on anti-D immunoglobulin prophylaxis [21]. The probability of women having at least one (reported) potentially sensitising event was estimated to be 15.5%. From these, 69.3% were estimated to have had a fetal-maternal-heamorrhage test and 95.8% 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 anti-D immunoglobulin. For the remaining 20% events (pre 20 weeks' gestation events), we assumed that women received the minimum required dose of 250 IU anti-D immunoglobulin.

The audit on anti-D immunoglobulin prophylaxis [21] 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 post-partum 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 prohylatic anti-D immunoglobulin treatment and will not subject to fetal-maternal haemorrhage testing. This is an issue particularly for the the false negatives (RhD-negative women with a RhD-positive fetus, but for which the test result was negative), as these women will at most only receive post-partum treatment. For women with false negative NIPT test results who receive only post partum anti-D immunoglobulin the model assumes a rate of sensitisation of 0.95%. This is likely to be an underestimate as it is 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 post partum treatment (10.7%) is likely to be a large overestimate as the majority of events occur at birth (Table 21). 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%.

### 6.3.7 Compliance with RAADP and post-partum anti-D immunoglobulin

The National Comparative Audit of Blood Transfusion 2013 on Anti-D Immunoglobulin Prophylaxis [21] reported that, out of all eligible women, 99% received at least one RAADP injection. Full compliance (i.e. correct dose / correct time) was found to better with the single-dose regime (90%) compared to the two-dose regime (59%). Also, the audit shows that a very high proportion of eligible women (98.4%) received post-partum 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 is points to higher compliance with the single-dose regimen than with the two-dose regimen and 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% [21]. Thus we did not adjust the compliance rate across RAADP regimen. In the model it has been also assumed that post-partum anti-D immunoglobulin compliance rate is 98.4%, again following evidence from the recent audit [21]. 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).

### 6.3.8 Compliance with NIPT given RAADP and post-partum anti-D immunoglobulin

The evidence around the compliance with high-throughput NIPT is scarce, particularly in health systems where the test is introduced after RAADP guidance is in place – see Section 4.2.3. 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% for post-partum received at least one dose of anti-D immunoglobulin, respetively), we subsequently assume that the use of high-throughput NIPT has no additional impact on compliance. Therefore, it has been assumed that RAADP and post-partum anti-D immunoglobulin compliance is 99.0% and 98.4%, respectively, the same as in the no high-throughput NIPT scenarios.

### 6.3.9 Sensitisation outcomes

As for the independent economic developed for NICE TA156 on RAADP, the current economic model considered a set of input parameters directly related to the consequences of sensitisation towards the fetus and the newborn, namely the implications of haemolytic disease. Three of these model input parameters were key to an appropriate representation of the possible health states, namely: (i) the fetal loss rate per RhD-negative women at risk; (ii) the proportion of babies affected by haemolytic disease which resulted in minor developmental problems (these include, for instance, myopia, squint or delay in language and fine motor skills); and (iii) the proportion of babies affected by haemolytic disease which result in major developmental problems (these include, for instance, severe permanent neurodevelopmental delay such as cerebral palsy). Given the long-term consequences of these two later 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, further to the ones found in the NICE TA 156. The literature review focused particularly on the anti-D immunoglobulin systematic reviews [80-82] and the high-throughput NIPT diagnostic accuracy studies (see Section 4.2.3) as potential sources of data associated with the consequences of sensitisation. Apart from the study published by Finning and colleagues [2], 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, the duration of these problems and relating to the life expectancy of people with major developmental problems, we used the same evidence as NICE TA 156 with updated costs. It should be noted that due to the small number of haemolitic 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 TA 156 [72].

### 6.3.10 Cost of high-throughput NIPT

For the base case analysis the cost of high-throughput NIPT per sample was estimated to be [redacted]. This unit cost takes into account consumables, staffing, equipment, indirect and overhead costs. This is the company's estimated cost of testing at full capacity, i.e. dealing with at least 100,000 samples. An estimated royalty payment of [redacted] 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 [redacted]. The cost of high-throughput NIPT is discounted according to the pregnancy number it is being performed in, 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 delivery of blood samples for testing. Szczepura et al [61] included a postage cost of £1.10 per sample in their analysis, while recognising that cost would be much reduced if existing NHS transport service system was to be used.

### 6.3.11 Cost of RAADP, of anti-D immunoglobulin for potentially sensitising events and post-partum

The cost of anti-D immunoglobulin was taken from the BNF [83]. Currently two brands (D-Gam® and Rhophylac®) 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 which 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 [21]. The cost of RAADP was estimated to be £41.58, representing a weighted average of single (1500 IU) and two (2x 500 IU) dose regimens and their associated market share – 92.6% *versus* 7.4%, respectively [21]. 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%) [21]. 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 the previous NICE TA 156 [72], an administration cost of anti-D immunoglobulin was set to £5.

### 6.3.12 Cost of post-partum health resources used

Following birth, in current practice a cord serology test should be performed to confirm the baby's RhD type. Additionally, maternal blood samples should be tested for fetal-maternal haemorrhage. The costs, updated to 2015 prices, for post-partum serology (£4.18) and associated phlebotomy (£3.32), were obtained from Szczepura et al [61]. The cost of fetal-maternal haemorrhage testing was provided by personal communication with and estimated to be £128.10 (for test by flow cytometry, NHS Blood and Transport Red Cell Immunohaemotology). This cost was subject to sensitivity analysis as Szczepura et al [61] 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.

### 6.3.13 Cost of management of sensitisation

The list of relevant interventions in the management of maternal and neonatal sensitisation was taken from the previous NICE TA 156 [72]. 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 the previous NICE TA 156 [72] (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 the same as in the previous NICE TA 156, but updated to 2015 prices. Unit costs were sourced from the NHS reference costs 2014-15 [84]. The total average cost per sensitisation is estimated to be £3,167. Note that due to the multiplicity of factors affecting sensitisation and its management, the uncertainty associated with this parameter was taken from the NICE TA 156 [72] and assumed to be substantial (standard error £700).

**Table 22 Cost of management of sensitisation.**

| Intervention | | 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 used for the unit costs |
|---|---|---|---|---|---|---|---|
| **Management of maternal sensitisation** | **Blood tests, bilirubin, monitoring etc.** | 100% | 6 | 1 | £195 | £1,172 | 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 | £1,121 | 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 | £1,176 | £294 | Code XA01Z - Neonatal Critical Care, Intensive Care - Critical Care |
| **Total** | | | | | | **£3,167** | |

### 6.3.14 Model parameters and main assumptions

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

Within the model the following assumptions are consistent with NICE TA 156 [72]:

- sensitisations do not affect the pregnancy in which they occur;
- anti-D immoglobulin used within one pregnancy has no effect in reducing sensitisations during the next pregnancy;
- the proportion of RhD-negative women is based on the Caucasian population given that this group makes up over 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 assumed the same irrespective of pregnancy number;
- all NIPT are assumed to be performed early enough to determine the use of RAADP at 28 weeks' gestation;
- routine and prophylactic anti-D immunoglobulin is only offered to women in whom the NIPT result indicates that their fetus is RhD-positive or in whom the results are inconclusive;
- in women with an inconclusive NIPT result we assume that the existing care pathway is unchanged and that 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 assumed independent of previous sensitisation within the same pregnancy;
- women with false negative test results but who are provided with cord serology and post-partum anti-D immunoglobulin are assumed to have a sensitisation rate of 0.95% despite forgoing anti-D immunoglobulin treatment for potentially sensitising events;
- compliance with RAADP is assumed the same with and without NIPT; similarly, compliance for post-partum anti-D immoglobulin is assumed the same with or without NIPT;

**Table 23 Model parameters**

| Parameter | Mean value | S.E. | Distribution | Source / calculation |
|---|---|---|---|---|
| ***Discounting*** | | | | |
| Discount rate for utilities | 3.5% | --- | --- | NICE methods guidance [85] |
| Discount rate for costs | 3.5% | --- | --- | NICE methods guidance [85] |
| ***Target population characteristics*** | | | | |
| Population of England(a) | 54,316,600 | --- | --- | Office for National Statistics - Annual Mid-year Population Estimates, 2014 [86] |
| Crude birth rate in England: all births per 1,000 population of all ages (b) | 12.18 | --- | --- | Office for National Statistics - Births in England and Wales, 2014[73] |
| Proportion of pregnancies accounted for by Rh-negative women (c) – reiterated from Table 18 | 15.0% | --- | --- | Hospital Episode Statistics Analysis and Health and Social Care Information Centre, 2013-14 [74] |
| Number of women requiring treatment | 99,225 | --- | --- | Estimate based on information above [ =(a*(b/1000)*c) ] |
| Proportion of 1st pregnancies proceeding to next pregnancy | 91.4% | --- | --- | Office for National Statistics - Birth Summary Tables, England and Wales - Characteristics of Mother 2, England and Wales – average over 5 years (2009 to 2013) [87] |
| Proportion of 2nd pregnancies proceeding to next pregnancy | 40.5% | --- | --- | Office for National Statistics - Birth Summary Tables, England and Wales - Characteristics of Mother 2, England and Wales – average over 5 years (2009 to 2013) [87] |
| Proportion of 3rd pregnancies proceeding to next pregnancy | 58.3% | --- | --- | Office for National Statistics - Birth Summary Tables, England and Wales - Characteristics of Mother 2, England and Wales – average over 5 years (2009 to 2013) [87] |
| Median time between pregancies (in years) | 3.17 | --- | --- | Office for national statistics - Birth Summary Tables, England and Wales 2014 - Characteristics of Mother 2, England and Wales, 2013 [87] |
| ***Compliance*** | | | | |
| Compliance with RAADP | 99.0% | 0.1% | Beta | National Comparative Audit of Blood Transfusion - 2013 Audit of Anti-D Immunoglobulin Prophylaxis [21] |
| Compliance with RAADP if high-throughput NIPT performed | 99.0% | 0.1% | Beta | Assumed the same as compliance with RAADP |
| Compliance with post-partum Anti-D immunoglobulin (dose of at least 500 IU given within 3 days of delivery) | 98.0% | 0.2% | Beta | National Comparative Audit of Blood Transfusion - 2013 Audit of Anti-D Immunoglobulin Prophylaxis[21] |
| ***High-throughput NIPT inconclusive results*** | | | | |
| Proportion of high-throughput NIPT inconclusive results: All studies reporting inconclusives | 6.2% | 0.4% | Beta | Diagnostic accuracy review (see section 4 above) |
| Proportion of high-throughput NIPT inconclusive results: UK | 3.9% | 0.1% | Beta | Diagnostic accuracy review (see section 4 above) |

| Parameter | Mean value | S.E. | Distribution | Source / calculation |
|---|---|---|---|---|
| Bristol studies | | | | |
| Proportion of RhD-positive babies in high-throughput NIPT inconclusive results: All studies reporting inconclusives | 69.7% | 0.7% | Beta | Diagnostic accuracy review (see section 4 above) |
| Proportion of RhD-positive babies in high-throughput NIPT inconclusive results: UK Bristol studies | 69.6% | 0.3% | Beta | Diagnostic accuracy review (see section 4 above) |
| ***Sensitisation events*** | | | | |
| Probability of having at least 1 potentially sensitising event | 15.5% | 0.5% | Beta | National Comparative Audit of Blood Transfusion - 2013 Audit of Anti-D Immunoglobulin Prophylaxis [21] |
| Probability of performing a FMH test given at least 1 potentially sensitising event | 69.3% | 1.4% | Beta | National Comparative Audit of Blood Transfusion - 2013 Audit of Anti-D Immunoglobulin Prophylaxis [21] |
| Probability of receiving Anti-D after having at least 1 potentially sensitising event | 95.8% | 0.6% | Beta | National Comparative Audit of Blood Transfusion - 2013 Audit of Anti-D Immunoglobulin Prophylaxis [21] |
| Probability of women having a miscarriage (including stillbirth and intrauterine death) | 4.7% | 0.3% | Beta | National Comparative Audit of Blood Transfusion - 2013 Audit of Anti-D Immunoglobulin Prophylaxis [21] |
| ***Consequences of sensitisation*** | | | | |
| Fetal loss rate per woman at risk | 5.0% | 1.0% | Beta | Finning et al 2008 [2] and previous NICE assessment (TA 156) [72] |
| Proportion of babies affected by HDN with minor developmental problems | 6.0% | 2.0% | Beta | Previous NICE assessment (TA 156) [72] |
| Duration of minor developmental problems (years) | 16 | 5 | Beta | Previous NICE assessment (TA 156) [72] |
| Proportion of babies affected by HDN with major developmental problems | 5.0% | 1.0% | Beta | Finning et al 2008 [2] and previous NICE assessment (TA 156) [72] |
| Life expectancy for person with major developmental problems | 59.5 | Range 40-79 | Uniform | Previous NICE assessment (TA 156) [72] |
| ***Utilities*** | | | | |
| Utility for 'normal' person | 0.88 | 0.02 | Beta | Previous NICE assessment (TA 156) [72] |
| Utility for minor development problems | 0.85 | 0.02 | Beta | Previous NICE assessment (TA 156) [72] |
| Utility for major development problems | 0.42 | 0.03 | Beta | Previous NICE assessment (TA 156) [72] |
| ***Costs*** | | | | |
| Cost of high-throughput NIPT | [redacted] | --- | --- | Provided by the company – commercial in confidence |
| Royalty fee of high-throughput NIPT | [redacted] | --- | --- | Provided by the company – commercial in confidence |
| Cost of RAADP | £41.58 | --- | --- | BNF [83] and National Comparative Audit of Blood Transfusion - 2013 Audit of Anti-D Immunoglobulin Prophylaxis [21] - weighted average of single- and two-dose anti-D |

| Parameter | Mean value | S.E. | Distribution | Source / calculation |
|---|---|---|---|---|
| | | | | regimen costs and their market share |
| Cost of potentially sensitising events anti-D immunoglobulin | £31.69 | --- | --- | BNF [83] and National Comparative Audit of Blood Transfusion - 2013 Audit of Anti-D Immunoglobulin Prophylaxis [21] - weighted average of dose anti-D regimen cost and the likelihood of pre and post-20 weeks events |
| Cost of post-partum anti-D immunoglobulin | £35.69 | --- | --- | BNF [83] and National Comparative Audit of Blood Transfusion - 2013 Audit of Anti-D Immunoglobulin Prophylaxis [21] - 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 (TA 156) [72] |
| Cost of post-partum blood cord serology | £4.18 | --- | --- | Szczepura et al [61], updated to 2015 |
| Cost of feto-maternal haemorrhage testing | £128.10 | --- | --- | Provided by clinical experts |
| Cost of phlebotomy | £3.32 | --- | --- | Szczepura et al [61], updated to 2015 prices |
| Cost of management of a sensitised woman and sensitised neonate | £3,166.72 | £700.00 | Gamma | Previous NICE assessment (TA 156) [72] |
| Yearly cost of minor developmental problems | £110.58 | £35.00 | Gamma | Previous NICE assessment (TA 156) [72]}, updated to 2015 prices |
| Yearly cost of major developmental problems | £573.72 | £405.73 | Gamma | Previous NICE assessment (TA 156) [72], updated to 2015 prices |



## 6.4 Analytic methods

In exploring the alternative means by which the introduction of high-throughput NIPT could impact on the post-partum care pathway, we first present results for each post partum 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 23. 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 attributable to the model being structured around the specificity and sensitivity of the 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 are used to summarise the cost-effectiveness results in addition to the cost-effectiveness ratio. Net health benefits (NHB) are calculated for cost-effectiveness thresholds of £20,000 and £30,000 as shown in the equation below:

$$Net\ Health\ Benefit = QALYs - \frac{Costs}{Cost{-}effectiveness\ threshold}$$

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 ICERs against the threshold. They are useful to summarise results when there are small differences in health between strategies and where the new intervention may be less effective and less costly compared to 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 compared to 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 Section 6.3.1. 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' compared to 'no test and no RAADP' in order to illustrate the impact of the adjustments made to the model used in NICE TA 156 [72] and to establish the baseline comparability in terms of the cost-effectiveness of the current practice, 'no test and RAADP'. This was required as the benefits of a diagnostic test are reliant on there being a cost-effective treatment available. Results of this analysis are shown in Appendix 10.11. 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 (EVPI), 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 sensitivity analysis as described further in Section 6.4.2.

### 6.4.1 Base case analysis

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

**Table 24 Main base case assumptions**

| Parameter | Assumption / Evidence source |
|---|---|
| **High-throughput NIPT accuracy** | Bivariate meta-analysis of UK (Bristol) studies – see section 4; Diagnostic test assumed to be performed at first contact with health services. |
| **Effectiveness of RAADP (vs no RAADP)** | Sensitisation rate=0.35% (NICE TA 156 [72]) |
| **Uptake of RAADP (with and without high-throughput NIPT performed)** | 99.0% (National Comparative Audit of Blood Transfusion - 2013 Audit of Anti-D Immunoglobulin Prophylaxis [21]) |
| **Uptake of post-partum anti-D immunoglubin (with and without high-throughput NIPT performed)** | 98.4% (National Comparative Audit of Blood Transfusion - 2013 Audit of Anti-D Immunoglobulin Prophylaxis [21]) |
| **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 [redacted] with a [redacted] royalty fee added: [redacted] |
| **Cost of anti-D immunoglobulin** | Potentially sensitising event: £31.69; RAADP: £41.58; Post-partum: 35.69 |
| **Cost of fetal-maternal haemorrhage test** | £128.10 (personal communication with clinical experts) |
| **Further post-partum scenario on the management of high-throughput NIPT inconclusive results** | Inconclusive results are treated post-delivery as positive test results |

### 6.4.2 Sensitivity analyses

A series of scenario and sensitivity analysis were also conducted. We focussed on parameters and assumptions to which we expected that the ICER would be the most sensitive and where the available evidence was limited. The sensitivity analyses (SAs) are described in detail below and summarised in Table 25. We focus on the comparison of current practice with the best performing post partum scenario in all cases unless the results of the sensitivity analysis affect the rank order of post partum

scenarios or suggest that multiple post partum scenarios could potentially provide the highest net health benefit.

**SA1**. 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;

**SA2**. We explored the use of high-throughput NIPT at different gestation periods. Performance results from a recent UK study [1] were used to assess the cost and consequences of introducing high-throughput NIPT at 11 to 13 weeks, 14 to 17 weeks and 18 to 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 these costs that would have to occur prior to the NIPT test in order for the ICER to cross a threshold of £20,000 per QALY;

**SA3**. The base case analysis incorporates the rate of inconclusive high-throughput NIPT results found in the UK (Bristol) studies. The rate of inconclusive results will vary according to the local population demography because they are more likely in certain ethnic groups such as those of African ethnic origin. The rate of inconclusive results may also vary if the operation of the NIPT is different in a trial setting compared to in routine use, for example if less time is spent on reprocessing initially inconclusive test results. Increasing the rate of inconclusive test results where 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 use of high-throughput NIPT;

**SA4**. The base case analysis utilised the same rate of sensitisation with 'no test and RAADP' as was used in the NICE TA 156 [72]. Subsequent to NICE TA 156 a further meta-analysis was performed by Turner et al [79], 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;

**SA5**. 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 the high-throughput NIPT will not alter the proportion of women who comply with 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. While 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 if offered RAADP. Some women who are told 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 sensitivity analysis in which the uptake of RAADP is decreased or increased alongside the reduction of the uptake of post-partum anti-D immunoglobulin;

**SA6**. We conduct a two-way sensitivity analysis 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: (a) the unit cost varies by throughput and so will depend on the total uptake of the NIPT; (b) the unit cost of the test must be considered alongside other potential additional costs relating to transport of blood samples for testing, whether additional antenatal visits are required to draw blood and deliver test counselling and results; and (c) 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 [redacted] and a royalty fee of [redacted] ([redacted]). 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 the 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;

**SA7**. Since the introduction of RAADP there has been a move from the two-dose to the single-dose regimens for a variety of reasons as indicated in the recent anti-D immunoglobulin prhophylaxis audit. We conducted a sensitivity analaysis that assumes a 100% use of the cheaper of the two regimens, i.e. the single-dose.

**SA8**. A further alternative way in which the use of high-throughput NIPT may impact on the existing post-partum care pathway is considered. This strategy, rather than grouping high-throughput NIPT inconclusive results with positive results it regards them as distinct from those on whom the NIPT indicated a RhD positive fetus. In this scenario post-partum 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. Fetal-maternal haemorrhage testing and post-delivery 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 sensitivity analysis performed is listed in Table 25 below.

**Table 25 Summary of sensitivity analysis performed**

<table>
<tr><th>Parameter</th><th>Assumption / Evidence source</th></tr>
<tr><td>High-throughput NIPT accuracy</td><td>SA1. Bivariate meta-analysis of all studies – see section 4;<br>SA2. High-throughput NIPT performace assessed at different gestation periods, using evidence from Chitty et al [1]</td></tr>
<tr><td>Effectiveness of RAADP (vs no RAADP)</td><td>SA3. Sensitisation rate=0.30% (Turner et al [79])</td></tr>
<tr><td>Compliance with RAADP (with and without high-throughput NIPT performed)</td><td>SA4a. 87.5% (National Comparative Audit of Blood Transfusion - 2013 Audit of Anti-D Immunoglobulin Prophylaxis [21])</td></tr>
<tr><td>Compliance with post-partum anti-D immunoglubin (with and without high-throughput NIPT performed)</td><td>SA4b. 91.6% (National Comparative Audit of Blood Transfusion - 2013 Audit of Anti-D Immunoglobulin Prophylaxis [21])</td></tr>
<tr><td>High-throughput NIPT inconclusive results</td><td>SA5. Pooled estimates for the sensitivity and specificity replaced with the individual study results</td></tr>
<tr><td>Cost of high-throughput NIPT</td><td>SA6a. Varied between £13.20 and £24.20 (including a [redacted] royaly fee)</td></tr>
<tr><td>Cost of anti-D immunoglobulin</td><td>SA6b. All varied ±20%</td></tr>
<tr><td>Cost of fetal-maternal haemorrhage test</td><td>SA7. £3.17 (Szczepura et al [61] updated to 2015 prices)</td></tr>
<tr><td>Further post-partum scenario on the management of high-throughput NIPT inconclusive results</td><td>SA8.
<table>
<tr><th>NIPT result</th><th>Cord serology</th><th>FMH</th><th>Post-partum Anti-D</th></tr>
<tr><td>T-</td><td>Yes</td><td>Yes if CS+</td><td>As guided by CS and FMH</td></tr>
<tr><td>T+</td><td>No</td><td>Yes</td><td>Yes with additional dose per FMH</td></tr>
<tr><td>Inconclusive</td><td>Yes</td><td>Yes if CS+</td><td>As guided by CS and FMH</td></tr>
</table></td></tr>
</table>

Legend: SA= sensitivity analysis

### 6.4.3 Model validation

PS developed the model and SG checked the model for errors. Comparisons across strategies were done to identify inconsistencies. Comparisons with the previous NICE TA 156 were also done to identify the sources of any potential discrepancy.

## 6.5 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 post-partum strategies are shown first, followed by the results of performing sensitivity analysis on key model input parameters. All results are based on the probabilistic analysis. Detailed characteristics of each post-partum scenario are provided in **Error! Reference source not found.**.

### 6.5.1 Base case results

Table 26 presents the results for each post-partum 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 net health benefits 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 0.84 QALYs. Any difference in QALYs between strategies is attributable wholly to the difference in the number of sensitisations.

Post-partum scenario 1 (NIPT PP1) describes the use of NIPT to guide RAADP only, with all women continuing to receive cord serology with FMH and post-partum anti-D immunoglobulin as required, irrespective of NIPT test result. This is estimated to reduce costs by £344,000 per 100,000 pregnancies and to result in lower health benefits (0.6 QALYs) than current practice.

Post-partum scenario 2 (NIPT PP2) describes the use of NIPT to guide both RAADP and post-partum care to women who test postive or in whom the results are inconclusive, where cord serology is provided only in these women to guide FMH and post-partum anti-D immunoglobulin as required. This is estimated to reduce costs compared to current practice by approximately £409,000 but to result in a loss of 23.3 QALYs per 100,000 pregnancies.

Post-partum scenario 3 (NIPT PP3) describes the use of NIPT to guide RAADP and post-partum anti-D immunoglobulin to women who test positive or inconclusive, and where cord serology is used to guide FMH and post-partum anti-D immunoglobulin as required only to women in whom the NIPT

indicates a RhD negative fetus. This is estimated to reduce costs compared to current practice by £296,000 but to result in a loss of 0.6 QALYs per 100,000 pregnancies.

Post-partum scenario 4 (NIPT PP4) describes the use of NIPT to guide both RAADP and post-partum FMH and anti-D immunoglobuline to women who test postive or inconclusive, and where cord serology is not provided. This is estimated to reduce costs compared to current practice by approximately £362,000 but results in a loss of 23.3 QALYs per 100,000 pregnancies.

All post-partum 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 – see **Figure 13**. The least effective strategies are those that omit cord serology for women who test negative on the NIPT. Without cord serology false negatives are not picked up at delivery and are not provided with post-partum anti-D immunoglobulin. In the model, the additional health gains are determined by the management of high-throughput NIPT false negative test results.



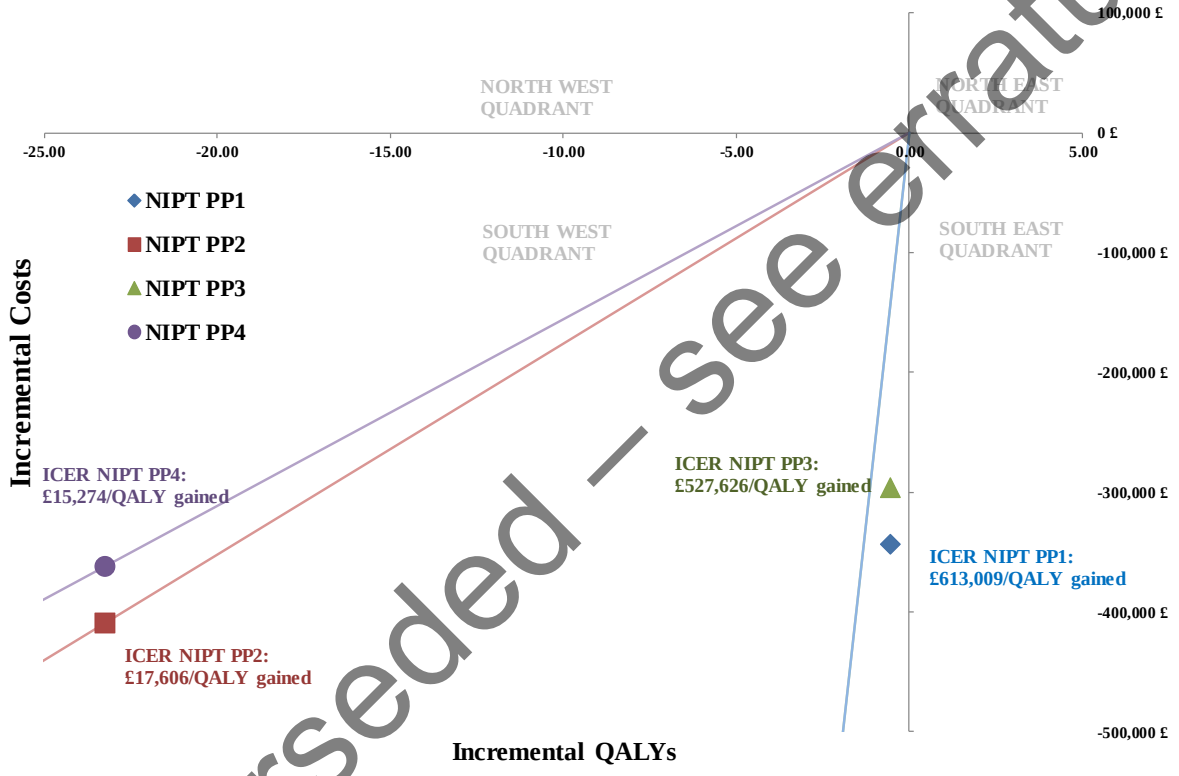
**Table 26 Incremental cost-effectiveness outcomes associated with high-throughput NIPT *vs* other strategies (base case post-partum scenarios) – probabilistic results**

| Strategies | Total costs | Total QALYs | Increm. Costs | Increm. QALYs | ICER (£/ QALY gained) | Population NHB (λ=£20,000) | Population NHB (λ=£30,000) |
|---|---|---|---|---|---|---|---|
| **Current clinical practice** | | | | | | | |
| **No Test and RAADP** | £16,679,607 | 2,433,116 | --- | --- | --- | 2,432,282 | 2,432,560 |
| **Post-partum scenario 1 (NIPT PP1)** | | | | | | | |
| **Test and RAADP (T+ only) vs No Test and RAADP** | £16,335,599 | 2,433,115 | -£344,008 | -0.56 | £613,009 | 2,432,299 | 2,432,571 |
| **Post-partum scenario 2 (NIPT PP2)** | | | | | | | |
| **Test and RAADP (T+ only) vs No Test and RAADP** | £16,270,284 | 2,433,093 | -£409,323 | -23.25 | £17,606 | 2,432,279 | 2,432,550 |
| **Post-partum scenario 3 (NIPT PP3)** | | | | | | | |
| **Test and RAADP (T+ only) vs No Test and RAADP** | £16,383,514 | 2,433,115 | -£296,093 | -0.56 | £527,626 | 2,432,296 | 2,432,569 |
| **Post-partum scenario 4 (NIPT PP4)** | | | | | | | |
| **Test and RAADP (T+ only) vs No Test and RAADP** | £16,317,529 | 2,433,093 | -£362,078 | -23.25 | £15,574 | 2,432,277 | 2,432,549 |

Due 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 to high-throughput NIPT. Hence where the ICER is above the cost-effectiveness threshold this would support the use of NIPT (No test and RAADP vs NIPT PP1, ICER approximately

£613,000 per QALY gained). The cost-effectiveness threshold can be used to present results in terms of net health benefits (NHB), in which case the comparison is more straightforward as the strategy with the highest NHB is preferred. Except for NIPT PP1 and NIPT PP3, all other NIPT strategies have an expected NHB lower than No test and RAADP, both at threshold values of £20,000 and £30,000. Compared to No test and RAADP, NIPT PP1 has greater NHB (incremental NHB at £20,000 of approximately 17; incremental NHB at £30,000 of approximately 11, vs No test and RAADP).

**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 for 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 sensitivity analysis around 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 7.9/20000 = 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 the NIPT test, the cost of managing potentially sensitising events, the cost of RAADP, the cost of post-partum tests and anti-D immunoglobulin and the cost consequences of sensitisations, and this is shown in Table 27. While the added NIPT cost is similar across strategies at approximately £1,585,000 per 100,000 pregnancies, it is accummulated 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 the NIPT is recommended to 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 £1,370,000 per 100,000 pregnancies) and management of potentially sensitising events (approximately £560,000 per 100,000 pregnancies). The NIPT strategies vary more markedly in their impact on post-partum testing and anti-D immunoglobulin costs. Here NIPT PP1 is essentially the same as current practice, except for the small reduction in costs due to increased sensitisations, which makes women ineligible for FMH and anti-D immunoglobulin. NIPT PP2 decreases post-partum 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 nor provided with post-partum fetal maternal haemorrhage tests and anti-D immunoglobulin. NIPT PP3 increases post partum care costs because while cord serology is avoided for those who test positive, this results in unnecessary use of fetal maternal haemorrhage tests and anti-D immunoglobulin amongst women who test false positive (which includes those who test inconclusive but carry a RhD negative baby). NIPT PP4 decreases post-partum 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 £2,000 per 100,000 pregnancies).

**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 testing cost** | £1,584,874 | £1,584,581 | £1,584,874 | £1,584,581 |
| **PSE management costs** | -£556,667 | -£558,244 | -£556,667 | -£558,244 |
| **RAADP costs** | -£1,374,104 | -£1,374,946 | -£1,374,104 | -£1,374,946 |
| **Post-partum test and anti-D costs** | -£52 | -£140,252 | £47,863 | -£93,007 |
| **Sensitisation costs** | £1,941 | £79,539 | £1,941 | £79,539 |
| **Total incremental cost** | -£344,008 | -£409,323 | -£296,093 | -£362,078 |

The assumption that the results of the NIPT can be used to avoid all costs associated with the management of potentially sensitising events is favourable to NIPT, and £560,000 represents the maximum cost saving in this regard. If this cost saving is reduced to £228,000, i.e. if 60% of potentially sensitising events occur prior to the results of the NIPT being known, the ICER for No test and RAADP compared to 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 where it would be provided in week 20 or earlier (see Figure 11 for schedule of appointments) could avoid upward of 80% of the cost of managing potentially sensitising events.

We calculated the probability that each strategy would be cost-effective compared to No test and RAADP for each pair-wise comparison. NIPT PP1 and NIPT PP3 both have 90% probability of being cost-effective ata threshold of £20,000 per QALY. NIPT PP2 and NIPT PP4 have a lower probability of being cost-effective at £20,000 per QALY, no higher than 60% when compared to No test and RAADP.

Table 28 presents the fully incremental cost-effectiveness probabilistic results for high-throughput NIPT vs other strategies. Fully incremental results do not compare each NIPT strategy to 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 at the bottom rows of the table. Incremental costs, incremental QALYs and consequently the incremental cost effectiveness ratio (ICER) are incremental to the strategy in the row above. The same applies to the incremental net health benefits (INHB) at £20,000 and £30,000 threshold values.

In NIPT PP2 cord serology is used to identify false positive results, thereby avoiding unnecessary FMH and anti-D immunoglobulin in these women, but is withheld in women for whom the NIPT indicates a RhD negative fetus. Using the negative results of high-throughput NIPT to rule out post-partum cord serology, FMH and anti-D immunoglobulin (NIPT PP2 and NIPT PP4) has lower QALYs compared to No test and RAADP, NIPT PP1 and NIPT PP3. While there are further cost savings from avoiding post-partum cord serology and anti-D immonoglobulin, the majority of sensitisations occur and can be prevented by the administration of anti-D immonoglubulin 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 CS to all women, as with NIPT PP1, will identify both the false positive (the small number of false positives and the proportion of women with inconclusive results who are carrying RhD

negative babies) and false negative results. While NIPT PP1 has higher costs compared to NIPT PP2 due to the additional cord serology tests, these are offset somewhat by cost savings from avoiding sensitisations in false negatives. Compared to NIPT PP2, NIPT PP1 is estimated to provide approximately 23 additional QALYs per 100,000 pregnancies, at approximately £65,000 additional cost, corresponding to an ICER of around £3,000 per QALY gained.

In NIPT PP3 cord serology is used to identify false negative results, but withheld in women with inconclusive results or for whom the NIPT indicates a RhD positive fetus (in favour of FMH and anti-D immunoglobulin). Compared to NIPT PP1, the QALY gain is not affected as the model assumes no adverse health benefits from unnecessary use of anti-D immonoglubulin. 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 0.6 QALYs per 100,000 pregnancies compared to NIPT PP1, at an additional cost of £344,000. This means that the ICER for No Test and RAADP compared to NIPT PP1 is £613,000. Using high-throughput NIPT and performing cord serology irrespective of the result (NIPT PP1) has higher NHB compared to any other strategy

**Table 28 Fully incremental cost-effectiveness outcomes associated with high-throughput NIPT *vs* other strategies (base case post-partum scenarios) – probabilistic results**

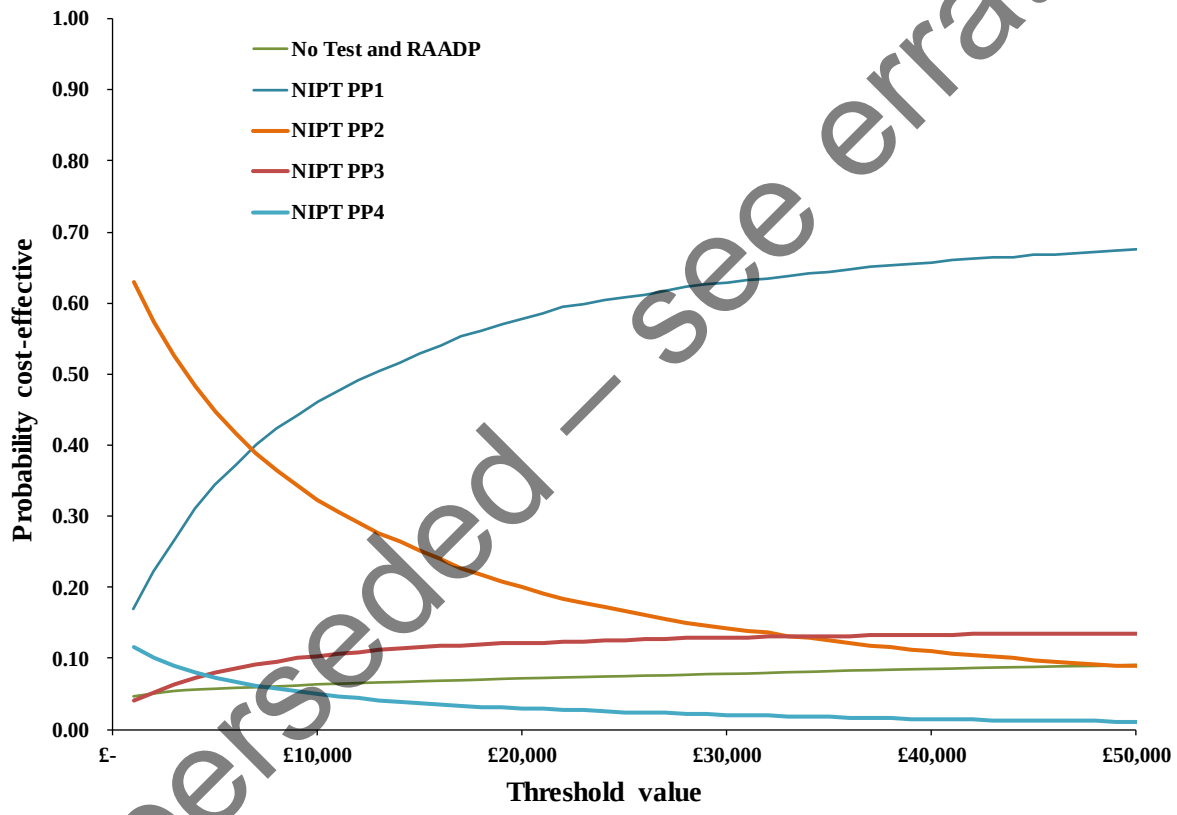
| Strategies | Total costs | Total QALYs | Incr. Costs | Incr. QALYs | ICER (£/QALY gained) | Population INHB (λ=£20,000) | Population INHB (λ=£30,000) |
|---|---|---|---|---|---|---|---|
| **NIPT PP2** | £16,270,284 | 2,433,093 | --- | --- | --- | --- | --- |
| **NIPT PP1** | £16,335,599 | 2,433,115 | £65,314 | 22.69 | £2,879 | 19 | 21 |
| **No Test and RAADP** | £16,679,607 | 2,433,116 | £344,008 | 0.56 | £613,009 | -17 | -11 |
| **NIPT PP4** | £16,317,529 | 2,433,093 | --- | --- | Dominated | --- | --- |
| **NIPT PP3** | £16,383,514 | 2,433,115 | --- | --- | Dominated | --- | --- |



The decision uncertainty can be shown graphically with a cost-effectiveness acceptability curve (CEAC). Figure 14 shows the CEACs 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 simultaneously compared, for threshold values of £20,000 and £30,000, the highest probability of

being cost-effective is obtained by NIPT PP1 with 0.58 and 0.63, respectively. For the same threshold values, the probability of NIPT PP2 being cost-effective is 0.20 and 0.14, respectively. NIPT PP1 is the alternative with the highest probability of being cost-effective and also expected cost-effective alternative for thresholds above £7,000. An estimate of the maximum value of further research, the EVPI, is estimated to be approximately £230,000 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 more than £230,000 this suggests that it would not represent a good investment.

**Figure 14: Cost-effectiveness acceptability curves of current practice (No Test and RAADP) and alternative NIPT scenarios (PP1 to PP4).**






### 6.5.2 Sensitivity analyses results

Several sensitivity analyses 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 [1], assessed the performance of high-throughput NIPT at different gestation periods. An analysis over the NIPT inconclusive results was also performed by replacing the pooled estimates for the sensitivity and specificity with the individual study results. Sensitivity analysis was performed on the effectiveness of RAADP by using a different sensitisation rate pooled from a larger number of studies. An assessment was also done over the uptake rates for RAADP and post-partum anti-D

immunoglobulin, with and without NIPT, decreasing these to the circunstances when the correct dose at the correct time was administered according to recent evidence [21]. Additionaly, 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 fetal-maternal haemorrhage test and, under an alternative post-partum 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 relatively to the base case findings.

6.5.2.1 **SA1: Sensitivity analysis over the NIPT 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%, while the pooled sensitivity levels are reduced by only 0.2% – see section 4.2.2. Compared to 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 £10,000 and £100,000 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 to the base case, this results in a further loss of approximately 15 QALYs per 100,000 pregnancies. Compared to No test and RAADP, NIPT PP2 and NIPT PP3 are still found to be cost saving (approximately £400,000 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 38 with NIPT PP2. NIPT PP1 and NIPT PP3 are the only strategies to offer increased net health benefits compared to No Test and RAADP, with ICERs for No Test and RAADP of approximately £450,000.

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

| Strategies | Total costs | Total QALYs | Increm. Costs | Increm. QALYs | ICER (£/ QALY gained) | Population NHB (λ=£20,000) | Population NHB (λ=£30,000) |
|---|---|---|---|---|---|---|---|
| **Current clinical practice – all NIPT accuracy evidence pooled** | | | | | | | |
| **No Test and RAADP** | £16,679,607 | 2,433,116 | --- | --- | --- | 2,432,282 | 2,432,560 |
| **Post-partum scenario 1 (NIPT PP1) – all NIPT accuracy evidence pooled** | | | | | | | |
| **Test and RAADP (T+ only) vs No Test and RAADP** | £16,293,588 | 2,433,115 | -£386,019 | -0.92 | £420,095 | 2,432,300 | 2,432,572 |
| **Post-partum scenario 2 (NIPT PP2) – all NIPT accuracy evidence pooled** | | | | | | | |
| **Test and RAADP (T+ only) vs No Test and RAADP** | £16,258,279 | 2,433,077 | -£421,328 | -37.77 | £11,154 | 2,432,265 | 2,432,536 |
| **Post-partum scenario 3 (NIPT PP3) - all NIPT accuracy evidence pooled** | | | | | | | |
| **Test and RAADP (T+ only) vs No Test and RAADP** | £16,251,580 | 2,433,115 | -£428,026 | -0.92 | £465,810 | 2,432,303 | 2,432,573 |
| **Post-partum scenario 4 (NIPT PP4) - all NIPT accuracy evidence pooled** | | | | | | | |
| **Test and RAADP (T+ only) vs No Test and RAADP** | £16,214,684 | 2,433,077 | -£464,923 | -37.77 | £12,309 | 2,432,268 | 2,432,538 |



6.5.2.2 **SA2: Sensitivity analysis over the NIPT accuracy at different timings using Chitty et al**

Table 30 presents the results of providing the high-throughput NIPT test at different gestation periods. These are based on the analysis by Chitty et al (see Section 4.2.2), with the sensitivity and specificity repeated in 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 due to random chance rather than systematic variation between these time points. While this analysis does not directly take into consideration the impact of the test timing on the potential to avoid costs associated with the management of a potentially sensitising events, we estimate the threshold amount of these costs that would have to occur prior to the NIPT in order for the ICER to cross the threshold of £20,000 per QALY gained. Thus, results are only shown for the best NIPT strategy within each period.

As for the base case, the introduction of high-throughput NIPT results in lower health benefits when compared to No test and RAADP. This happens irrespectively of the timing at which the test is carried out. The QALY loss is slightly greater when performing NIPT at 14-17 weeks' gestation due to 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 to current practice, rather than a loss of approximately 0.5 QALYs if NIPT is provided between 11-13 weeks or 18-23 weeks. The cost saving is greatest at 14-17 weeks' due to the increase in specificity as fewer false positive results result in less unnecessary treatment. A reduction in false positive results favours NIPT PP3 more than NIPT PP1, and so when the test is performed prior to 18 weeks NIPT PP3 becomes less costly than NIPT PP1 for the same QALY gain. NIPT PP3 is estimated to result in higher population net health benefits than current practice or any other NIPT post-partum strategy for the earliear gestation periods. The increase in the false positive rate of about 2% for the 18-23 weeks' gestation implies that NIPT PP3 is no longer the strategy with highest population net benefits and the results are more in line with the base case. For this gestation period, NIPT PP1 is cheaper than NIPT PP3 and No test and RAADP. NIPT PP1 is considered the best post-partum strategy, estimated to bring 5 and 15 additional population net health benefit units than NIPT PP3 and No test and RAADP, respectively.

**Table 30 Incremental cost-effectiveness outcomes associated with high-throughput NIPT at different timings vs other strategies (post-partum scenarios) – based on Chitty et al – probabilistic results**

| Strategies | Sensitivity | Specificity | Total costs | Total QALYs | Increm. Costs | Increm. QALYs | ICER (£/ QALY gained) | Pop. NHB (λ=£20,000) | Pop. NHB (λ=£30,000) |
|---|---|---|---|---|---|---|---|---|---|
| **Current clinical practice – irrespective of NIPT test timing (Chitty et al [1])** | | | | | | | | | |
| **No Test and RAADP** | --- | --- | £16,679,607 | 2,433,116 | --- | --- | --- | 2,432,282 | 2,432,560 |
| **Best post-partum scenario when NIPT testing performed at 11-13 weeks' gestation (Chitty et al [1])** | | | | | | | | | |
| **NIPT PP3** (vs No Test and RAADP) | 0.9983 | 0.9525 | £16,313,534 | 2,433,116 | -£366,073 | -0.48 | £762,510 | 2,432,300 | 2,432,572 |
| **Best post-partum scenario when NIPT testing performed at 14-17 weeks' gestation (Chitty et al [1])** | | | | | | | | | |
| **NIPT PP3** (vs No Test and RAADP) | 0.9967 | 0.9534 | £16,303,029 | 2,433,115 | -£376,577 | -0.94 | £400,439 | 2,432,300 | 2,432,572 |
| **Best post-partum scenario when NIPT testing performed at 18-23 weeks' gestation (Chitty et al [1])** | | | | | | | | | |
| **NIPT PP1** (vs No Test and RAADP) | 0.9982 | 0.9304 | £16,361,476 | 2,433,116 | -£318,130 | -0.44 | £717,752 | 2,432,298 | 2,432,570 |



The base case results suggest that NIPT PP1 provides savings of £560,000 from avoiding the costs of managing potentially sensitising events. The audit [21] indicates that 80% of potentially sensitising events occur after week 20. If NIPT PP1 is provided between 18-23 weeks' gestation and £310,000 or 55% 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 11-13 weeks' or 14-17 weeks' gestation, then approximately £360,000 or 64% 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.

#### 6.5.2.3 SA3: Sensitivity analysis on the effectiveness of RAADP using Turner et al

Findings from Turner et al [79] estimated a pooled odds ratio estimate for sensitisation under RAADP (*vs* No RAADP, only post-partum anti-D immonogubulin) of 0.31 rather than 0.37 as in the NICE TA 156 [72] (Table 31) Table 31. Compared to base case results (Table 26) the marginal reduction on the sensitisation rate (less 0.05%) 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.

**Table 31 Incremental cost-effectiveness outcomes associated with high throughput NIPT vs other strategies (post-partum scenarios) – based on Turner et al [79] pooled RAADP effectiveness – probabilistic results**

| Strategies | Total costs | Total QALYs | Increm. Costs | Increm. QALYs | ICER (£/ QALY gained) | Population NHB (λ=£20,000) | Population NHB (λ=£30,000) |
|---|---|---|---|---|---|---|---|
| **Current clinical practice – Turner et al [79] pooled RAADP effectiveness** | | | | | | | |
| **No Test and RAADP** | £16,610,418 | 2,433,137 | --- | --- | --- | 2,432,307 | 2,432,584 |
| **Post-partum scenario 1 (NIPT PP1) – Turner et al [79] pooled RAADP effectiveness** | | | | | | | |
| **Test and RAADP (T+ only) vs No Test and RAADP** | £16,266,181 | 2,433,136 | £344,237 | -0.61 | £563,641 | 2,432,323 | 2,432,594 |
| **Post-partum scenario 2 (NIPT PP2) – Turner et al [79] pooled RAADP effectiveness** | | | | | | | |
| **Test and RAADP (T+ only) vs No Test and RAADP** | £16,200,872 | 2,433,107 | -£409,546 | -23.30 | £17,575 | 2,432,304 | 2,432,574 |
| **Post-partum scenario 3 (NIPT PP3) – Turner et al [79] pooled RAADP effectiveness** | | | | | | | |
| **Test and RAADP (T+ only) vs No Test and RAADP** | £16,314,104 | 2,433,136 | -£296,314 | -0.61 | £485,173 | 2,432,321 | 2,432,593 |
| **Post-partum scenario 4 (NIPT PP4) – Turner et al [79] pooled RAADP effectiveness** | | | | | | | |
| **Test and RAADP (T+ only) vs No Test and RAADP** | £16,248,125 | 2,433,107 | -£362,293 | -23.30 | £15,547 | 2,432,302 | 2,432,572 |



#### 6.5.2.4 SA4: Sensitivity analysis on the uptake of RAADP and post-partum anti-D immonogublin

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, ignorance of the father's status and remain pregnant to receive RAADP. The National Comparative Audit of Blood Transfusion 2013 on Anti-D Immunoglobulin Prophylaxis [21] 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 post-partum 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 if NIPT was previously accepted/administered.

Table 32 presents the incremental cost-effectiveness outcomes for each alternative scenario when different RAADP and post-partum anti-D immunoglobulin uptake rates are used. As the sensitivity analysis does not impact on the rank order of the alternative post partum scenarios, the results are shown for NIPT PP1 only. i.e. out of the five alternatives being compared, the results for the best strategy is shown together with current practice. Base case results correspond to 99.0% and 98.4% uptake with RAADP and post-partum anti-D immunoglubin, 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, while the QALY loss associated with additional sensitisations is slightly reduced.

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

| Strategies | Total costs | Total QALYs | Increm. Costs | Increm. QALYs | ICER (£/ QALY gained) | Population NHB (λ=£20,000) | Population NHB (λ=£30,000) |
|---|---|---|---|---|---|---|---|
| **Base case anti-D immunoglobulin uptake rates – RAADP at 99.0% and post-partum at 98.4%** | | | | | | | |
| **No Test and RAADP** | £16,679,607 | 2,433,116 | --- | --- | --- | 2,432,282 | 2,432,560 |
| **NIPT PP1 (vs No Test and RAADP)** | £16,335,599 | 2,433,115 | -£344,008 | -0.56 | £613,009 | 2,432,299 | 2,432,571 |
| **Anti-D immunoglobulin uptake rates – RAADP at 87.5% and post-partum at 98.4%** | | | | | | | |
| **No Test and RAADP** | £16,768,351 | 2,433,088 | --- | --- | --- | 2,432,250 | 2,432,529 |
| **NIPT PP1 (vs No Test and RAADP)** | £16,424,649 | 2,433,088 | -£343,702 | -0.50 | £690,895 | 2,432,266 | 2,432,540 |
| **Anti-D immunoglobulin uptake rates – RAADP at 99.0% and post-partum at 91.6%** | | | | | | | |
| **No Test and RAADP** | £16,732,394 | 2,433,099 | --- | --- | --- | 2,432,263 | 2,432,542 |
| **NIPT PP1 (vs No Test and RAADP)** | £16,388,567 | 2,433,099 | -£343,826 | -0.52 | £657,058 | 2,432,279 | 2,432,553 |
| **Anti-D immunoglobulin uptake rates – RAADP at 87.5% and post-partum at 91.6%** | | | | | | | |
| **No Test and RAADP** | £16,814,981 | 2,433,073 | --- | --- | --- | 2,432,233 | 2,432,513 |
| **NIPT PP1 (vs No Test and RAADP)** | £16,471,440 | 2,433,073 | -£343,541 | -0.46 | £740,399 | 2,432,249 | 2,432,524 |

#### 6.5.2.5 **SA5: Sensitivity analysis on NIPT 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 an RhD-negative fetus. In order to undertake a sensitivity analysis 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 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 antenatal anti-D immunoglobulin continues to be provided in these women carrying a RhD-negative fetus.

One study produced no inconclusive results and no false negative results, and so we omit this from the sensitivity analysis[7]. In general, the net health benefits associated with the NIPT strategies fall as the rate of inconclusive results increases, but at no point do the net health benefits fall below those offered with No Test and RAADP. Figure 16 shows the net health benefits for all of the NIPT strategies. When the rate of inconclusive results is low, NIPT PP3 offers the highest net health benefit. This is because the amount of unnecessary post-partum 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 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 [88] and Wikman [8] diverge from the remaining studies in terms of the number of false negative results and the sensitivity, and this impacts on the net health benefits of the strategies that do not identify false negatives through cord serology (NIPT PP2 and NIPT PP4). Figure 17 hows how the net health benefits for NIPT PP1 only varies with the rate of inconclusives.

**Figure 15: Specificity by rate of high-throughput NIPT inconclusive results per study.**

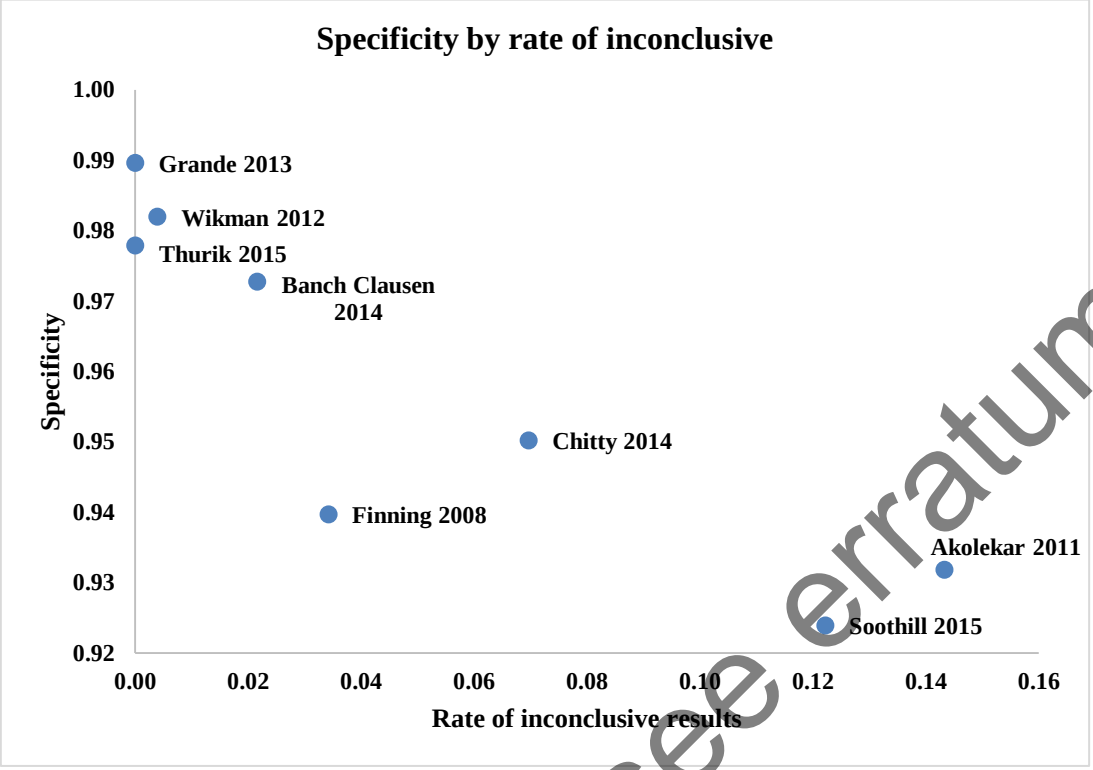



**Figure 16: Population net health benefits for all NIPT strategies by rate of NIPT inconclusive results per study.**

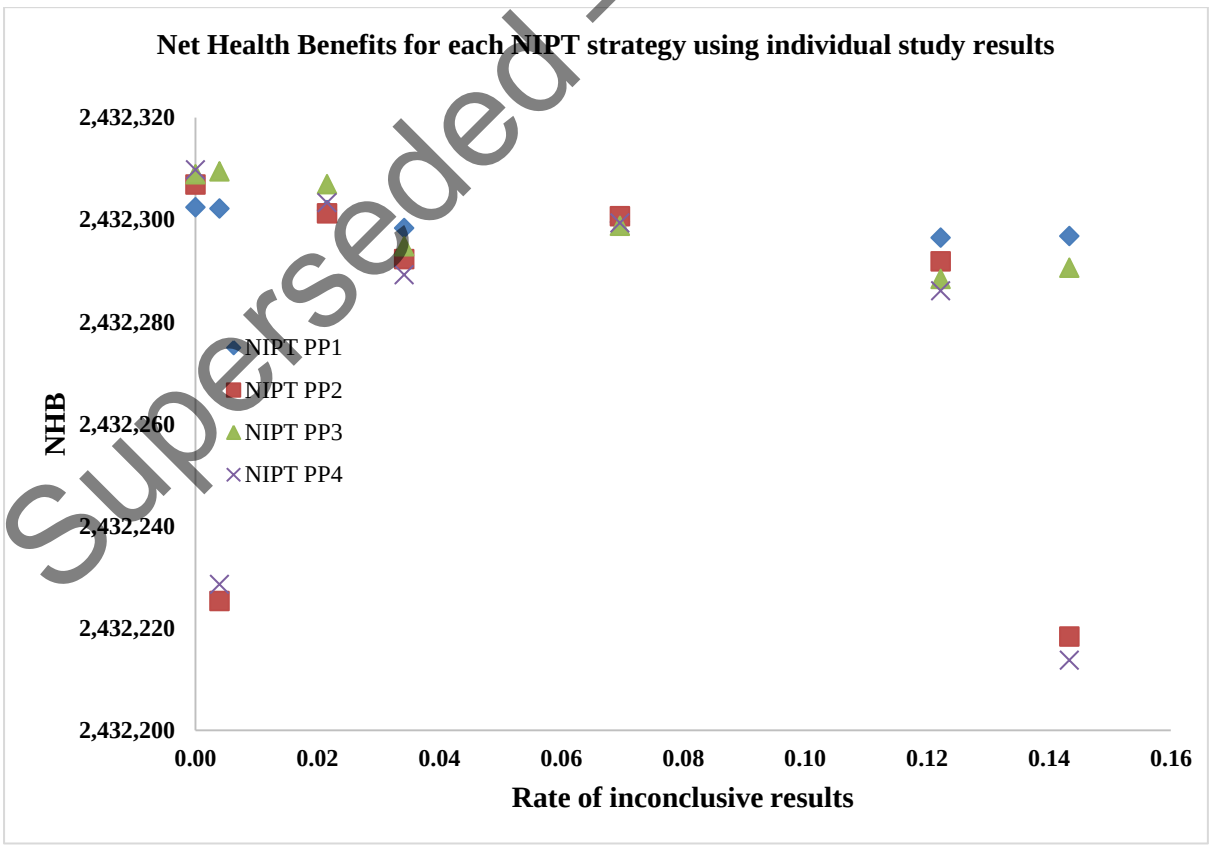
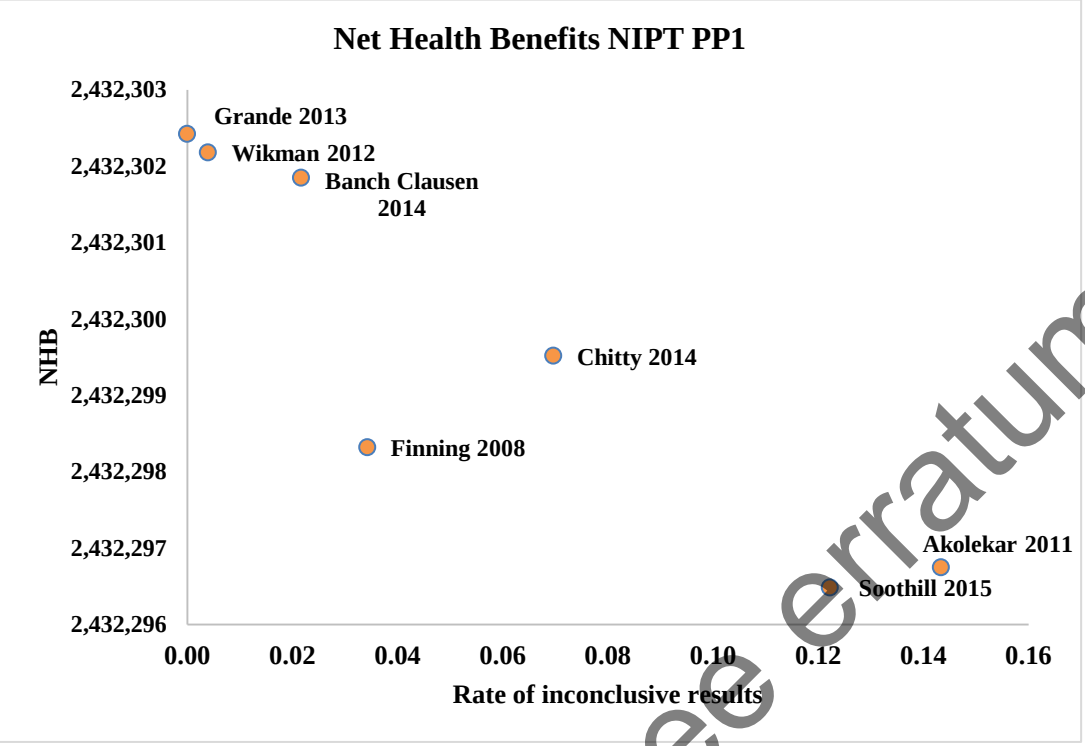


**Figure 17: Population net health benefits for NIPT PP1 by rate of NIPT inconclusive results per study.**






### 6.5.2.6 SA6: Sensitivity analysis on NIPT and Anti-D costs

The unit cost of an 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 the 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 only indicative as the estimated cost of anti-D for potentially sensitising events and for post-partum, as described in Section 6.3.12, are ommited. The y-axis represents the range of costs per high-throughput NIPT test from £13.2 to £24.2 (which may for example be interpreted as a range between [redacted] and [redacted] with an additional [redacted] royalty fee). A small increase in price of high-

throughput NIPT or a small fall in the price of anti-D immunoglobulin would result in NIPT PP1 no longer offering the highest population net health benefit. In fact, raising the cost per high-throughput NIPT test to £21.89 implies a switch to No test and RAADP being the strategy offering highest net health benefits. Similar results were found when the cost-effectiveness threshold is £20,000 or £30,000. NIPT PP1 strategy is always preferred over other post-partum strategies (PP2, PP3 or PP4). At no point would the price of anti-D immunoglobulin be high enough to make the omission of post-partum anti-D immunoglobulin (NIPT PP2 and NIPT PP 4) look cost-effective.

**Figure 18: Cost-effectiveness outcomes associated with NIPT high throughput vs other strategies (post-partum scenarios) across a range of NIPT* and Anti-D costs** – probabilistic results for thresholds of £20,000/QALY gained and £30,000/QALY.**



* NIPT cost includes a royalty fee of [redacted] over the NIPT price;
** The decrease/increase of RAADP cost was applied to the different RAADP dosages used routinely at 28-32 wks, at potentially sensitising events or post-partum. For illustrative purposes, however, the decrease/increase shown is for an anti-D of 1500 UI (Rhophylac – BNF price);
*** Location of the base case with a cost of high-throughput NIPT of [redacted].

#### 6.5.2.7 SA7: Sensitivity analysis over the Fetal-maternal haemorrhage test cost

Reducing the cost of fetal-maternal haemorrhage test to £3.17 (Szczepura et al [61], updated to 2015 prices) halves the estimated total costs of all strategies when compared to the total costs of the base case scenarios - see Table 33. Estimated total QALYs are similar to base case findings. NIPT PP1 is now dominated by current practice, offering fewer benefits at higher costs. This is explained by the use of fetal-maternal haemorrhage test in the management of potentially sensitising events. When the cost of fetal-maternal haemorrhage test is reduced, the savings from avoiding the management of potentially sensitising events are reduced from £560,000 to £170,000 across all NIPT strategies. NIPT PP2 still reduces costs compared to No test and RAADP, but only by £2,000 and at a loss 23 QALYs per 100,000 pregnancies. Strategy NIPT PP3 still offers savings compared to current practice, but these are reduced from £296,000 in the base case to £120,000 per 100,000 pregnancies. The reduction in savings attributable to avoiding potentially sensitising event management costs are partially offset by the impact on post-partum costs. With the cost of fetal-maternal haemorrhage test reduced, NIPT PP3 offers cost savings post-partum. This is because the benefits of avoiding cord serology among the false positives now outweigh the cost of unnecessary fetal-maternal haemorrhage

testing among false positives, and this effect is seen also for NIPT PP4. NIPT PP3 is the only strategy to offer greater net health benefits compared to No test and RAADP. For NIPT PP1, PP2 and PP4, the ICER when comparing to No test and RAADP is below £20,000 per QALY.

**Table 33 Incremental cost-effectiveness outcomes associated with high-throughput NIPT *vs* other strategies (post-partum scenarios) – Fetal-maternal haemorrhage test cost reduced – probabilistic results**

| Strategies | Total costs | Total QALYs | Increm. Costs | Increm. QALYs | ICER (£/ QALY gained) | Population NHB (λ=£20,000) | Population NHB (λ=£30,000) |
|---|---|---|---|---|---|---|---|
| **Current clinical practice** | | | | | | | |
| **No Test and RAADP** | £8,371,549 | 2,433,116 | --- | --- | --- | 2,432,697 | 2,432,835 |
| **Post-partum scenario 1 (NIPT PP1)** | | | | | | | |
| **Test and RAADP (T+ only) vs No Test and RAADP** | £8,416,529 | 2,433,115 | £44,980 | -0.56 | Dominated | 2,432,695 | 2,432,835 |
| **Post-partum scenario 2 (NIPT PP2)** | | | | | | | |
| **Test and RAADP (T+ only) vs No Test and RAADP** | £8,369,431 | 2,433,093 | -£2,118 | -23.25 | £91 | 2,432,674 | 2,432,814 |
| **Post-partum scenario 3 (NIPT PP3)** | | | | | | | |
| **Test and RAADP (T+ only) vs No Test and RAADP** | £8,252,361 | 2,433,115 | -£119,188 | -0.56 | £212,389 | 2,432,703 | 2,432,840 |
| **Post-partum scenario 4 (NIPT PP4)** | | | | | | | |
| **Test and RAADP (T+ only) vs No Test and RAADP** | £8,205,101 | 2,433,093 | -£166,448 | -26.25 | £7,160 | 2,432,683 | 2,432,819 |



#### 6.5.2.8 **SA8: Sensitivity analysis on post-partum management of inconclusive results**

The post-partum scenarios specified in the decision problem applied cord serology, fetal-maternal haemorrhage testing and post-partum anti-D immunoglobulin according to whether the results of the NIPT were positive or negative. In this regard, we grouped inconclusive results with NIPT positive results. However, in terms of post-partum management it may be worthwhile to regard those with inconclusive results as distinct from those on whom the NIPT indicates an 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 cord serology to be withheld in women with in whom the NIPT indicates a RhD positive fetus. This would result in total costs of £16,125,098 and 2,433,115 QALYs per 100,000 pregnancies. This post-partum approach would dominate all other NIPT strategies, and the ICER for No test and RAADP versus this strategy would be £988,113 per QALY gained.

Table 34 summarises the results of the base case analysis and the key sensitivitiy analyses.

**Table 34 Summary of base case and key sensitivity analysis results**

| | Total | | *vs* No test and RAADP (current practice) | *vs* next best strategy | |
|---|---|---|---|---|---|
| **Analysis** | **Cost** | **QALYs** | **ICER** | **ICER** | **Comparator** |
| *Base Case* | | | | | |
| No test and RAADP | £16,679,607 | 2,433,116 | --- | £613,009 | NIPT PP1 |
| NIPT PP1 | £16,335,599 | 2,433,115 | £613,009 | £2,879 | NIPT PP2 |
| NIPT PP2 | £16,270,284 | 2,433,093 | £17,606 | --- | --- |
| NIPT PP3 | £16,383,514 | 2,433,115 | £527,626 | --- | --- |
| NIPT PP4 | £16,317,529 | 2,433,093 | £15,574 | --- | --- |
| *SA1 - Bivariate meta-analysis of all studies* | | | | | |
| No test and RAADP | £16,679,607 | 2,433,116 | --- | £465,810 | NIPT PP3 |
| NIPT PP1 | £16,293,588 | 2,433,115 | £420,095 | --- | --- |
| NIPT PP2 | £16,258,279 | 2,433,078 | £11,154 | --- | --- |
| NIPT PP3 | £16,251,580 | 2,433,115 | £465,810 | £1,001 | NIPT PP4 |
| NIPT PP4 | £16,214,684 | 2,433,078 | £12,309 | --- | --- |
| *SA2 - High-throughput NIPT performance assessed at different gestation periods (Chitty et al 2014)* | | | | | |
| *11 – 13 weeks' gestation* | | | | | |
| No test and RAADP | £16,679,607 | 2,433,116 | --- | £762,510 | NIPT PP3 |
| NIPT PP1 | £16,316,016 | 2,433,116 | £757,339 | --- | --- |
| NIPT PP2 | £16,242,836 | 2,433,096 | £21,308 | --- | --- |
| NIPT PP3 | £16,313,534 | 2,433,116 | £762,510 | £3,656 | NIPT PP4 |
| NIPT PP4 | £16,240,355 | 2,433,096 | £21,429 | --- | --- |
| *14 – 17 weeks' gestation* | | | | | |
| No test and RAADP | £16,679,607 | 2,433,116 | --- | £400,439 | NIPT PP3 |
| NIPT PP1 | £16,308,605 | 2,433,115 | £394,510 | --- | --- |
| NIPT PP2 | £16,276,465 | 2,433,076 | £10,164 | --- | --- |
| NIPT PP3 | £16,303,029 | 2,433,115 | £400,439 | £830 | NIPT PP4 |
| NIPT PP4 | £16,270,893 | 2,433,076 | £10,304 | --- | --- |
| *18 – 23 weeks' gestation* | | | | | |
| No test and RAADP | £16,679,607 | 2,433,116 | --- | £717,752 | NIPT PP1 |
| NIPT PP1 | £16,361,476 | 2,433,116 | £717,752 | £3,987 | NIPT PP2 |
| NIPT PP2 | £16,287,775 | 2,433,097 | £20,699 | --- | --- |
| NIPT PP3 | £16,468,004 | 2,433,116 | £477,410 | --- | --- |
| NIPT PP4 | £16,394,285 | 2,433,097 | £15,072 | --- | --- |
| *SA3 - Sensitisation rate from Turner et al 2012* | | | | | |
| No test and RAADP | £16,610,418 | 2,433,137 | --- | £563,641 | NIPT PP1 |
| NIPT PP1 | £16,266,181 | 2,433,137 | £563,641 | £2,878 | NIPT PP2 |
| NIPT PP2 | £16,200,872 | 2,433,114 | £17,575 | --- | --- |
| NIPT PP3 | £16,314,104 | 2,433,137 | £485,173 | --- | --- |
| NIPT PP4 | £16,248,125 | 2,433,114 | £15,547 | --- | --- |
| *SA4 - Uptake with RAADP (with and without high-throughput NIPT performed)* | | | | | |
| *Uptake of RAADP at 87.5%* | | | | | |
| No test and RAADP | £16,768,351 | 2,433,088 | --- | £690,895 | NIPT PP1 |
| NIPT PP1 | £16,424,649 | 2,433,088 | £690,895 | £2,880 | NIPT PP2 |
| NIPT PP2 | £16,359,331 | 2,433,065 | £17,646 | --- | --- |

| | Total | | *vs* No test and RAADP (current practice) | *vs* next best strategy | |
|---|---|---|---|---|---|
| **Analysis** | **Cost** | **QALYs** | **ICER** | **ICER** | **Comparator** |
| NIPT PP3 | £16,472,554 | 2,433,088 | £594,599 | --- | --- |
| NIPT PP4 | £16,406,565 | 2,433,065 | £15,609 | --- | --- |
| *Uptake of post-partum anti-D immunoglobulin at 91.6%* | | | | | |
| No test and RAADP | £16,732,394 | 2,433,099 | --- | £657,058 | NIPT PP1 |
| NIPT PP1 | £16,388,567 | 2,433,099 | £657,058 | £2,879 | NIPT PP2 |
| NIPT PP2 | £16,323,251 | 2,433,076 | £17,630 | --- | --- |
| NIPT PP3 | £16,436,477 | 2,433,099 | £565,503 | --- | --- |
| NIPT PP4 | £16,370,490 | 2,433,076 | £15,595 | --- | --- |
| *Uptake of RAADP at 87.5% and post-partum anti-D immunoglobulin at 91.6%* | | | | | |
| No test and RAADP | £16,814,981 | 2,433,073 | --- | ££740,399 | NIPT PP1 |
| NIPT PP1 | £16,471,440 | 2,433,073 | £740,399 | £2,880 | NIPT PP2 |
| NIPT PP2 | £16,406,120 | 2,433,050 | £17,668 | --- | --- |
| NIPT PP3 | £16,519,339 | 2,433,073 | £637,166 | --- | --- |
| NIPT PP4 | £16,453,349 | 2,433,050 | £15,627 | --- | --- |
| *SA5 – High-throughput NIPT inconclusive results rate* | | | | | |
| Please see section above on SA5 | | | | | |
| *SA6 – Cost of high-throughput NIPT and anti-D immunoglobulin* | | | | | |
| Please see section above on SA6 | | | | | |
| *SA7 – Cost of fetal-maternal haemorrhage test* | | | | | |
| No test and RAADP | £8,371,549 | 2,433,116 | --- | £212,389 | NIPT PP3 |
| NIPT PP1 | £8,416,529 | 2,433,115 | Dominated | --- | --- |
| NIPT PP2 | £8,369,431 | 2,433,093 | Dominated | --- | --- |
| NIPT PP3 | £8,252,361 | 2,433,115 | £212,389 | £2,083 | NIPT PP4 |
| NIPT PP4 | £8,205,101 | 2,433,093 | £7,160 | --- | --- |
| *SA8 – Post-partum management of high-throughput NIPT inconclusive results* | | | | | |
| Please see section above on SA8 | | | | | |



## 6.6 Discussion of the independent economic assessment

The evidence to support the diagnostic accuracy of the NIPT is of good quality. We can combine this with established evidence for the efficacy of RAADP and post-partum anti-D immunoglobulin in order to estimate the impact of introducing NIPT testing 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 £3,167 and is associated with a loss of approximately 0.8 QALYs, but these estimates are subject to uncertainty and incorporate expert opinion.

There exists uncertainty regarding the cost of introducing the high-throughput NIPT. The unit cost will vary with throughput, and may be subject to an additional royalty fee. Unless the NIPT can be

incorporated seemlessly into routine antenatal care, it may result in additional costs for blood draw, transport of samples, and antenatal care visits to administer the test and deliver counselling and results. We conducted extensive sensitivity analysis to address this uncertainty and to identify the threshold cost per NIPT. The cost of high-throughput NIPT has to increase by only [redacted] 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 the cost-effectiveness. While there is uncertainty as to 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 net health benefits of using the NIPT to target care are reduced as the rate of inconclusive results is increased. However, our sensitivity analysis 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. While 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 such as 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 post-partum strategy that would offer the highest net health benefit.

There a numerous ways in which the results of the high-throughput NIPT could be used to guide post-partum testing and administration of anti-D immunoglobulin. We have compared four alternative post-partum scenarios, and the results indicate that cord serology testing should be retained in women for whom the 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 post-partum strategy that distinguishes between inconlusive results and positive results offers the greatest cost-savings.

If the cost of fetal-maternal haemmorhage 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 fetal-maternal haemorrhage test and unnecessary post-partum 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 would be revealed to be carrying a RhD-negative fetus. In contrast where the results of the 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 post-partum care. This would allow for a post-partum scenario where cord serology was applied to women who test negative and to those who test inconclusive, but where fetal-maternal haemorrhage tests and anti-D immunoglobulin is provided without cord serology in women who test positive.

## 6.7 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 to 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 the 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 [redacted] or more per test would cause the ICER for No test and RAADP to reduce below £20,000 per QALY.

# 7 Discussion

## 7.1 Statement of principal findings

### 7.1.1 Diagnostic accuracy

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

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

A subgroup analysis of three high-quality studies based at Bristol (UK) showed a slightly lower false negative rate of 0.21% (95% CI 0.09 to 0.48), and a higher false positive rate of 5.73% (95% CI 4.58 to 7.16). This suggests that the Bristol NIPT testing 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 false positive rate 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 false positive rate of 1.26% (95% CI 0.87 to 1.83). We were unable to conduct the subgroup analysis based on ethnicity due to 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 testing 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 might be due to low concentration of cell-free fetal DNA in early pregnancy[89] but an increased concentration of cell-free fetal DNA after the end of the first trimester.[90]

### 7.1.2 Clinical effectiveness

Seven studies were included in the clinical effectiveness review. Only two studies had a control group, but both studies were judged to be at high risk of bias. One large prospective cohort study [11] 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 (Soothill 2014, Banch Clausen 2014, Grande 2013) reported outcome measures relating to anti-D doses administrated. All studies found that the use of NIPT reduced the total use of anti-D Ig doses, particularly decreasing by 29% in one UK study by Soothill et al., because around 35% of RhD-negative women avoided unnecessary anti-D administration.

Four studies reported moderate to high compliance with antenatal anti-D Ig administration. The compliance with antenatal anti-D administration after a positive NIPT result ranged from 86% to 96.1% (four studies). High-throughput NIPT testing uptake rates ranged from 70% to over 95% (seven studies).

The results from the simulation study suggested that the use of NIPT testing 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 28 per 100,000 women if cord blood testing is withdrawn and postpartum anti-D given on the basis of the NIPT result. Sensitisation rates are minimised by ensuring women who do not receive an NIPT test are still offered, and receive, antenatal anti-D. The results suggest that NIPT test 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.

### 7.1.3 **Implementation**

Twelves studies were included in the review of implementation. Most of the included studies were large cohort studies reporting implementation data alongside with diagnostic accuracy data, while one study was a survey based at the UK (London). All the large cohort studies reported high diagnostic accuracy of high-throughput NIPT and suggested that high throughput RhD genotyping of foetuses 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. Some studies highlighted the importance of short transport times of samples and the need for effective management of transporting samples. Some studies also identified the need for greater knowledge of NIPT testing among physicians and midwives.

A UK-based survey (Oxenford 2013) revealed that, while most of the women surveyed supported the implementation of NIPT testing, their current knowledge of Rhesus blood groups and anti-D administration was limited, which could be a barrier to implementation.

### 7.1.4 **Cost effectiveness**

Seven cost-effectiveness studies were included in the review. Conflicting results were identified across the existing economic studies with 3 of the studies reporting that NIPT fetal RhD genotyping

did not appear 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 1 of the studies 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. Of the studies undertaken outside the UK, differences in health care systems and implementation of anti-D immunoglubulin policies limit their relevance to UK practice. In conclusion, none of the existing studies were considered to be sufficiently generalisable to inform the specific the 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 post-partum care pathway were evaluated (cord serology, fetal-maternal haemorrhage testing and post-partum anti-D immunoglobulin). These included scenarios in which the result of the NIPT was only used to guide RAADP only (with all women continuing to receive receive cord serology with fetal-maternal haemorrhage testing and post-partum anti-D immunoglobulin as required, irrespective of NIPT test result) and scenarios where the NIPT result guided both RAADP and separate aspects of post-partum care. A series of additional sensitivity and scenario analyses were also performed.

Our *de-novo* economic model indicated that the use of high-throughput NIPT to guide the prenatal and post-partum provision of anti-D immunoglobulin prophylaxis is estimated to be cost saving compared to current practice of providing RAADP to all women who are RhD-negative. The magnitude of the cost saving appears highly sensitive to the cost of the 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 to current practice. However, even a small increase in the cost imposed on the NHS of [redacted] or more per test would alter these conclusions.

In the base-case analysis, all four separate post-partum 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 £296,000 and £409,000. The magnitude of the QALY loss varied between 0.6 QALYs and 23.3 QALYs (per 100,000 pregancies). Although the

magnitude of the cost-savings was sufficient to outweigh the associated QALY loss when each post-partum scenario was separately compared to 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 fetal-maternal haemorrhage testing and post-partum 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 0.58 and 0.63, 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 post-partum strategy was also shown to vary across several of the main sensitivity analysis.

A post-partum strategy that distinguishes between inconlusive 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 post-partum care. This could allow for a post-partum scenario where cord serology was applied to women who test negative and to those who test inconclusive, but where fetal-maternal haemorrhage tests and anti-D immunoglobulin is provided without cord serology in women who test positive.

## 7.2 Strengths and limitations of the assessment

### 7.2.1 Clinical effectiveness

Extensive literature searches were conducted with 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.

For limitations, only studies in English were included, therefore some potentially relevant non-English language studies may have been missed. There was very limited evidence relating to the clinical effectiveness of high-throughput NIPT testing. No studies were identified reporting adverse effects of high-throughput NIPT testing. 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 (Akolekar and Thurik) did not report the number of inconclusive results of the test and some studies did not report detailed reasons for inconclusive results.

### 7.2.2 **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 TA 156 on RAADP ensuring consistency between the separate diagnostic and technology appraisals. A broad range of scenario and sensitivity analyses were undertaken to address key assumptions and uncertainties.

## 7.3 Uncertainties

### 7.3.1 **Clinical effectiveness**

In this assessment we identified very limited data on the evaluation of clinical effectiveness for using high-throughput NIPT testing to detect fetal RhD status in RhD negative women. Therefore, the potential role of high-throughput NIPT testing 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 measure.

Due to a lack of sufficient data from included studies, we were unable to conduct subgroup analyses based on ethnicity. Therefore, whether the diagnostic performance of high-throughput NIPT testing differs between different ethnic groups remains unclear.

In terms of implementing high-throughput NIPT testing in healthcare settings, no studies were identified reporting compliance rates to prenatal anti-D treatment in the 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 due to variations in national guidelines and health policies between different countries.

### 7.3.2 **Cost effectiveness**

There exists uncertainty regarding the cost of introducing the high-throughput NIPT. The unit cost will vary with throughput, and may be subject to an additional royalty fee. Unless the NIPT can be incorporated seemlessly into routine antenatal care, it may result in additional costs for blood draw, transport of samples, and antenatal care visits to administer the test and deliver counselling and results. We conducted extensive sensitivity analysis to address this uncertainty and to identify the

threshold cost per NIPT. The cost of high-throughput NIPT has to increase by only [redacted] above that modelled in the base case in order for current practice to be the preferred strategy.

While there remains uncertainty as to the timing of the test, our analysis suggests that this 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.

Althouh the evidence to support the diagnostic accuracy of the 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.

## 7.4 Other relevant factors

Due to a lack of relevant evidence, we have not considered any adverse health impacts from provision of a blood based product. While widespread global use of anti-D immunoglobulin would suggest that is it safe, there remains uncertainty as to 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 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 the NIPT could inadvertently reveal mistaken paternity of the child in cases where 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 pre-natal test could potentially have adverse impacts on uptake of other antenatal care if the overall quality of care is comprimised by the additional test burden.

# 8 Conclusions

## 8.1 Implications for service provision

The findings from this assessment demonstrated high diagnostic performance of high-throughput NIPT testing for the detection of fetal RhD status in RhD negative women, with very low false positive rate and false negative rate. About 0.7% of women will have an incorrect test result and approximately 7% will have an inconclusive result. Sensitivity analyses did not materially alter the results. These findings have important implications for service provision.

The use of high-throughput NIPT testing 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 put 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 anticipated cost savings is estimated to range between £296,000 and £409,000 depending on the impact of high-throughput NIPT on post-partum management.

## 8.2 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 RHD-pseudogene,[14] prenatal detection of fetal RhD type from maternal blood would lead to higher rates of false positives results in this particular population.

Further research to improve the test itself would be useful, particularly for reducing the number of inconclusive test results.

Given the limited evidence on the clinical impact of NIPT testing, further cohort studies comparing the use of high-throughput NIPT testing with universal antenatal anti-D administration are required. These should focus on recording clinical outcomes, such as sensitisation rates, test and anti-D compliance, and costs and quality of life. There is also limited existing evidence on the impact of sensitisations in terms of their long term health and cost consequences. Further reseach to comprehensively assess the full impact of sensitisations over mothers and children appears warranted.

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# 10 Appendices

## 10.1 Search strategy

**MEDLINE**
via Ovid http://ovidsp.ovid.com/
1946 to October Week 5 2015
Searched on: 5th November 2015
Records retrieved: 1815

The search was updated on 26th February 2016 retrieving 77 records from MEDLINE and 40 records from MEDLINE In-Process.

1 Rh-Hr Blood-Group System/ (10006)
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 (13812)
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$)).ti,ab. (1074)
12 ((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) adj2 immuni#ation).ti,ab. (80)
13 ((rh or rhesus) adj2 (immuni#ation or autoimmuni#ation)).ti,ab. (695)
14 or/7-13 (4428)
15 exp Erythroblastosis, Fetal/ (11006)
16 ((hemolytic or haemolytic) adj2 (disease$ 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$).ti,ab. (760)
21 or/15-20 (13551)
22 6 or 14 or 21 (25723)
23 Prenatal Diagnosis/ (33273)
24 Maternal Serum Screening Tests/ (153)
25 Hematologic Tests/ (5564)
26 ((prenatal or pre-natal or antenatal or ante-natal) adj3 (test$ or screen$ or diagnos$ or determin$ or detect$)).ti,ab. (32925)
27 ((fetal or foetal or fetus$ or foetus$) adj3 (test$ or screen$ or diagnos$ or determin$ or detect$)).ti,ab. (20036)
28 (NIPD or NIPT).ti,ab. (328)
29 or/23-28 (69981)
30 Genotyping Techniques/ (2761)
31 ((genotype$ 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$)).ti,ab. (789)
33 ((genotype$ 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. (946538)
41 39 not 40 (1824)
42 exp animals/ not humans/ (4137930)
43 41 not 42 (1815)

**Key:**
/ = indexing term (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 (CINAHL Plus)**
via EBSCO https://www.ebscohost.com/
Inception to 5th November 2015
Searched on: 6th November 2015
Records retrieved: 290

The search was updated on 26th 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 | 2,737 |
| S35 | TI cffDNA OR AB cffDNA | 20 |
| S34 | TI "cell-free f#etal DNA" OR AB "cell-free f#etal DNA" | 124 |
| 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" | 2,529 |

| | | |
|---|---|---|
| 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*) ) | 2,644 |
| 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*) ) | 5,033 |
| S25 | (MH "Noninvasive Procedures") | 1,538 |
| S24 | (MH "Hematologic Tests") | 11,530 |
| S23 | (MH "Prenatal Diagnosis") | 5,562 |
| S22 | S6 OR S14 OR S21 | 1,924 |
| 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 |
| 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 (CENTRAL)**
via Wiley http://onlinelibrary.wiley.com/
Issue 10 of 12, October 2015
Searched on: 6th November 2015
Records retrieved: 16

The search was updated on 26th 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
#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

NB: The strategy above was used to search CENTRAL and CDSR. 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 (CDSR)**
via Wiley http://onlinelibrary.wiley.com/
Issue 11 of 12, November 2015
Searched on: 6th November 2015
Records retrieved: 8

See above under CENTRAL for search strategy used.

The search was updated on 26th February 2016 retrieving 9 records from CDSR.

**Database of Abstracts of Reviews of Effects (DARE)**
via http://www.crd.york.ac.uk/CRDWeb/
Inception – 31st March 2015
Searched on: 6th November 2015
Records retrieved: 9

The strategy below was used to search DARE, NHS EED and 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 |
| 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/
1974 to 2015 November 04
Searched on: 05/11/15
Records retrieved: 3092

The search was updated on 26th 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 (15398)
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$)).ti,ab. (1306)
13 ((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) adj2 immuni#ation).ti,ab. (90)
14 ((rh or rhesus) adj2 (immuni#ation or autoimmuni#ation)).ti,ab. (772)
15 or/8-14 (5218)
16 exp newborn hemolytic disease/ (11867)
17 ((hemolytic or haemolytic) adj2 (disease$ 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$).ti,ab. (739)
22 rhesus incompatibility/ (1131)
23 or/16-22 (16217)
24 7 or 15 or 23 (30562)
25 prenatal diagnosis/ (50220)
26 prenatal screening/ (6356)
27 maternal serum screening test/ (145)
28 blood examination/ (10293)
29 non invasive procedure/ (17457)
30 diagnostic accuracy/ (195290)
31 ((prenatal or pre-natal or antenatal or ante-natal) adj3 (test$ or screen$ or diagnos$ or determin$ or detect$)).ti,ab. (40821)
32 ((fetal or foetal or fetus$ or foetus$) adj3 (test$ or screen$ or diagnos$ or determin$ or detect$)).ti,ab. (25280)
33 (NIPD or NIPT).ti,ab. (561)
34 or/25-33 (301546)
35 genotyping technique/ (4081)
36 ((genotype$ 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)
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. (1117567)
46 44 not 45 (3107)
47 animal/ (1701987)
48 exp animal experiment/ (1895782)
49 nonhuman/ (4645212)
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. (4564702)
51 47 or 48 or 49 or 50 (7266921)
52 exp human/ (16514549)
53 human experiment/ (344858)
54 52 or 53 (16515997)
55 51 not (51 and 54) (5693442)
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 (HTA)**
via http://www.crd.york.ac.uk/CRDWeb/
Inception – 31st March 2015
Searched on: 6th November 2015
Records retrieved: 3

See above under DARE for search strategy used.

**Maternity and Infant Care**
via Ovid http://ovidsp.ovid.com/
1971 to September 2015
Searched on: 5th November 2015
Records retrieved: 238

The search was updated on 26th 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$)).ti,ab. (4763)
28 (NIPD or NIPT).ti,ab. (89)
29 25 or 26 or 27 or 28 (12193)
30 ((genotype$ 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)
38 24 and 36 (67)
39 37 or 38 (245)
40 (editorial or commentary).pt. (14906)
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 (NHS EED)**
via http://www.crd.york.ac.uk/CRDWeb/
Inception – 31st March 2015
Searched on: 6th November 2015
Records retrieved: 6

See above under DARE for search strategy used.

**PubMed**
http://www.ncbi.nlm.nih.gov/pubmed/
Searched on: 26th 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/
1900 – 4th November 2015
Searched on: 6th November 2015
Records retrieved: 801

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 26th February 2016 retrieving 811 records.

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

| | | |
|---|---|---|
| # 32 | 821 | #31 OR #30<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 31 | 287 | #29 AND #19<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 30 | 744 | #23 AND #19<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 29 | 2,378 | #28 OR #27 OR #26 OR #25 OR #24<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 28 | 79 | TS=cffDNA<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 27 | 543 | TS=("cell-free foetal DNA" or "cell-free fetal DNA")<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 26 | 204 | TS=((genotype* or genotyping) NEAR/2 (noninvasive or non-invasive))<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 25 | 1,222 | TS=((genotype* or genotyping) NEAR/2 (maternal or pregnan*))<br>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))<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 23 | 51,060 | #22 OR #21 OR #20<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 22 | 632 | TS=(NIPD or NIPT)<br>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*))<br>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*))<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 19 | 15,143 | #18 OR #12 OR #5<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 18 | 5,220 | #17 OR #16 OR #15 OR #14 OR #13<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 17 | 581 | TS=((erythroblastoses or erythroblastosis) NEAR/2 f$etal*)<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 16 | 413 | TS=((rhesus or rh or RhD) NEAR/2 (incompatib* or antagonism))<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 15 | 1,248 | TS=((rhesus or rh) NEAR/2 (disease* or disorder*))<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 14 | 102 | TS=HDFN<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 13 | 3,593 | TS=((hemolytic or haemolytic) NEAR/2 (disease* or disorder*))<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 12 | 2,937 | #11 OR #10 OR #9 OR #8 OR #7 OR #6<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |

| | | |
|---|---|---|
| # 11 | 565 | TS=((rh or rhesus) NEAR/2 (immuni?ation or autoimmuni?ation))<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 10 | 32 | TS=((fetomaternal or feto-maternal or foetomaternal or foeto-maternal) NEAR/2 immuni?ation)<br>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*))<br>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*))<br>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*))<br>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*))<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 5 | 8,522 | #4 OR #3 OR #2 OR #1<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 4 | 5,198 | TS=((rh or rhesus) NEAR/2 (factor or factors or antigen* or system or group))<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 3 | 121 | TS=("Rhesus negative" or "Rhesus positive")<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 2 | 479 | TS=(Rh-negative or Rh-positive)<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |
| # 1 | 3,491 | TS=(RhD or "rhesus D" or "Rh(D)" or "Rh-(D)" or "Rh D" or "Rh-D")<br>Indexes=SCI-EXPANDED, CPCI-S Timespan=All years |

***On-going, unpublished or grey literature search strategies***

**ClinicalTrials.gov**
https://clinicaltrials.gov/
Searched on: 10th 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 26th February 2016 retrieving 2 new records.

**Conference Proceedings Citation Index: Science**
via Web of Science, Thomson Reuters
http://thomsonreuters.com/thomson-reuters-web-of-science/
1990 – 4th November 2015
Searched on: 6th November 2015
Records retrieved: 801

See above under 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 26th February 2016 retrieving 811 records.

**EU Clinical Trials Register**
https://www.clinicaltrialsregister.eu/ctr-search/search
Searched on: 10th 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 26th February 2016 but no new records were retrieved.

**PROSPERO**
http://www.crd.york.ac.uk/PROSPERO/
Searched on: 10th November 2015
Records retrieved: 4

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

The search was updated on 26th February 2016 retrieving 1 new record.

**WHO International Clinical Trials Registry Platform**
http://www.who.int/ictrp/search/en/
Searched on: 10th November 2015
Records retrieved: 29

RhD OR rhesus OR Rh-negative OR Rh-positive

The search was updated on 26th 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 4th March 2016, however no new guidelines were retrieved.

**National Guidelines Clearinghouse**
http://www.guideline.gov/
Searched on: 17th 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)

23 results were retrieved and browsed for relevance. 18 relevant guidelines were found.

**National Institute for Health and Care Excellence**
https://www.nice.org.uk/
Searched on 13th November 2015

1. Browsed for relevant guidance in the fertility, pregnancy and childbirth section:
https://www.nice.org.uk/guidance/conditions-and-diseases/fertility--pregnancy-and-childbirth

2. Searched NICE website using gereral search box with keyword RhD

3.Searched NICE website using gereral search box with keyword Rhesus

4 relevant guidelines found.

**NHS Evidence**
https://www.evidence.nhs.uk/
Searched on: 17th 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.

81 results were retrieved and browsed for relevance. 7 relevant guidelines were found.

**Royal College of Obstetricians and Gynaecologists**
https://www.rcog.org.uk/en/
Searched on 13th November 2015

1. Browsed all guidelines.

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

4 relevant guidelines found.

**TRIP database**
https://www.tripdatabase.com/
Searched on: 17th November 2015

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

37 results were retrieved and browsed for relevance. 17 relevant guidelines were found.

**UK National Screening Committee**
https://www.gov.uk/government/groups/uk-national-screening-committee-uk-nsc
Searched on: 13th November 2015

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

1 relevant report found.

Search strategies - systematic reviews of antenatal anti-D prophylaxis

MEDLINE(R) In-Process & Other Non-Indexed Citations and MEDLINE(R)
via Ovid http://ovidsp.ovid.com/
1946 to October Week 5 2015
Searched on: 18th January 2016
Records retrieved: 45

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

1 systematic$ review$.ti,ab. (75835)
2 meta-analysis as topic/ (14365)
3 meta-analytic$.ti,ab. (4298)
4 meta-analysis.ti,ab,pt. (89180)
5 metanalysis.ti,ab. (140)
6 metaanalysis.ti,ab. (1210)
7 meta analysis.ti,ab. (70616)
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$).ti,ab. (2200)
21 evidence based review.ti,ab. (1467)
22 comprehensive review.ti,ab. (8251)
23 critical review.ti,ab. (11964)
24 quantitative review.ti,ab. (517)
25 structured review.ti,ab. (542)
26 realist review.ti,ab. (102)
27 realist synthesis.ti,ab. (73)
28 or/1-27 (187703)
29 review.pt. (2049547)
30 medline.ab. (68680)
31 pubmed.ab. (46181)
32 cochrane.ab. (39786)
33 embase.ab. (40092)
34 cinahl.ab. (12936)
35 psyc?lit.ab. (879)
36 psyc?info.ab. (10559)
37 (literature adj3 search$).ab. (32390)
38 (database$ adj3 search$).ab. (30393)
39 (bibliographic adj3 search$).ab. (1461)
40 (electronic adj3 search$).ab. (11252)
41 (electronic adj3 database$).ab. (13910)
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. (44510)
47 selection criteria.ab. (22215)
48 predefined criteria.ab. (1258)
49 predetermined criteria.ab. (787)
50 (assess$ adj3 (quality or validity)).ab. (48127)
51 (select$ adj3 (study or studies)).ab. (43640)
52 (data adj3 extract$).ab. (34903)

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. (121595)
58 (intervention$ adj2 evaluat$).ab. (7046)
59 confidence interval$.ab. (258288)
60 heterogeneity.ab. (106141)
61 pooled.ab. (53158)
62 pooling.ab. (8496)
63 odds ratio$.ab. (171463)
64 (Jadad or coding).ab. (133119)
65 or/30-64 (923716)
66 29 and 65 (141974)
67 review.ti. (299976)
68 67 and 65 (62549)
69 (review$ adj4 (papers or trials or studies or evidence or intervention$ or evaluation$)).ti,ab. (119221)
70 28 or 66 or 68 or 69 (340645)
71 letter.pt. (897674)
72 editorial.pt. (391059)
73 comment.pt. (647299)
74 71 or 72 or 73 (1445828)
75 70 not 74 (331856)
76 exp animals/ not humans/ (4171020)
77 75 not 76 (321762)
78 "Rho(D) Immune Globulin"/ (1190)
79 (immune adj2 globulin adj2 rh$).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
adj = terms next to each other (order specified)
adj2 = terms within two words of each other (any order)

Cochrane Database of Systematic Reviews (CDSR)
via Wiley http://onlinelibrary.wiley.com/
Issue 1 of 12, January 2016
Searched on: 18th January 2016
Records retrieved: 6

The search was updated on 4th March 2016 retrieving 6 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 (DARE)
via http://www.crd.york.ac.uk/CRDWeb/
Inception – 31st March 2015
Searched on: 20th 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 (HTA)
via http://www.crd.york.ac.uk/CRDWeb/
Inception – 31st March 2015
Searched on: 20th January 2016
Records retrieved: 7

See above under DARE for search strategy used.

PubMed
http://www.ncbi.nlm.nih.gov/pubmed/
Searched on: 20th January 2016
Records retrieved: 57

The search was updated on 4th 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/
1886 to November 2015
Search on: 4th 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)

**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 EED**
via http://www.crd.york.ac.uk/CRDWeb/
Inception – 31st March 2015
Searched on: 4th 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 | 17 |

| | | |
|---|---|---|
| | isoimmune or iso-immune) ) | |
| 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

**RePEc : Research Papers in Economics**
http://repec.org/
Searched on: 4th 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

## 10.2 List of included studies

| **Included studies – Diagnostic accuracy** | | | |
|---|---|---|---|
| **Study (Author name, date)** | **Full title** | **Country** | **Linked publications** |
| Akolekar (2011) [4] | Fetal RHD genotyping in maternal plasma at 11-13 weeks of gestation. Fetal Diagnosis & Therapy. 29: 301-6. | UK (London) | None |
| Banch Clausen | Routine noninvasive prenatal screening for fetal RHD in plasma of RhD-negative pregnant women-2years | Denmark | Full paper: #173 ; #185 |

| | | | |
|---|---|---|---|
| (2014)[5] | of screening experience from Denmark. Prenatal Diagnosis. 34: 1000-5. | | Abstract: #1512 ; #1482 |
| Chitty (2014)[1] | Diagnostic accuracy of routine antenatal determination of fetal RHD status across gestation: population based cohort study. BMJ. 349: g5243. | UK (Bristol) | Full paper: None<br><br>Abstract: #1604; #1532; #1480 |
| Finning (2008) [2] | 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: 816-8. | UK (Bristol) | None |
| Grande (2013) [7] | Clinical application of midtrimester non-invasive fetal RHD genotyping and identification of RHD variants in a mixed-ethnic population | Spain | None |
| Soothill (2015) [3] | Use of cffDNA to avoid administration of anti-D to pregnant women when the fetus is RhD-negative: implementation in the NHS."BJOG: an international journal of obstetrics & gynaecology 122(12): 1682-1686. | UK (Bristol) | None |
| Thurik (2015) [6] | Analysis of false-positive results of fetal RHD typing in a national screening program reveals vanishing twins as potential cause for discrepancy | Netherlands | Full paper: #3368<br><br>Abstract: #1834; #1835; #1839; #1840; #1842; #3150; #1481; #1466 |
| Wikman (2012) [8] | Noninvasive single-exon fetal RHD determination in a routine screening program in early pregnancy. Obstetrics & Gynecology. 120: 227-34. | Sweden | Full paper: None<br><br>Abstract: #1830; #1831; #1832; #1833; #1837 |
| **Included studies – Clinical effectiveness** | | | |
| **Study (Author name, date)** | **Full title** | **Country** | **Linked publications** |

| | | | |
|---|---|---|---|
| Banch-Clausen (2014)[5] | Routine noninvasive prenatal screening for fetal RHD in plasma of RhD-negative pregnant women-2years of screening experience from Denmark. Prenatal Diagnosis, 34, pp.1000-5. | Denmark | Full paper: [9, 12]<br>Abstract: [35-39] |
| Banch-Clausen (2012) [9] | 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, pp.752-8. | Denmark | Full paper: [5, 12]<br>Abstract: [35-39] |
| Damkjaer 2012 [12] | Study of compliance with a new, targeted antenatal D immunization prevention programme in Denmark. Vox Sanguinis, 103, pp.145-9. | Denmark | Full paper: [5, 9]<br>Abstract: [35-39] |
| De Haas (2012)[10] | A nation-wide fetal RHD screening programme for targeted antenatal and postnatal anti-D." ISBT Science Series 7: 164-167. | Netherlands | Full paper: #5<br>Abstract: [46, 47]<br>[40-45] |
| Grande (2013) [7] | Clinical application of midtrimester non-invasive fetal RHD genotyping and identification of RHD variants in a mixed-ethnic population | Spain | None |
| Soothill (2015)[3] | Use of cffDNA to avoid administration of anti-D to pregnant women when the fetus is RhD-negative: implementation in the NHS."BJOG: an international journal of obstetrics & gynaecology 122(12): 1682-1686. | UK (Bristol) | Full paper: None.<br>Abstract: #1360; #1305 |
| Tiblad (2013) [11] | 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 paper: None<br>Abstract: #1849; #1836; #1838 |
| **Included studies - Implementation** | | | |
| **Study (Author name, date)** | **Full title** | **Country** | **Linked publications** |
| Banch Clausen (2014) [5] | Routine noninvasive prenatal screening for fetal RHD in plasma of RhD-negative pregnant women-2years of screening experience from Denmark. Prenatal | Denmark | Report: #185<br>Full paper: #115; #173 |

| | | | |
|---|---|---|---|
| | Diagnosis. 34: 1000-5. | | Abstract: #1615 |
| Banch-Clausen 2012 [9] | "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**(4): 752-758. | Denmark | Linked to above |
| Banch-Clausen 2013 [25] | "Pre-analytical conditions in non-invasive prenatal testing of cell-free fetal RHD." PLoS ONE [Electronic Resource] **8**(10): e76990. | Denmark | Linked to above |
| Damkjaer 2012 [12] | "Study of compliance with a new, targeted antenatal D immunization prevention programme in Denmark." Vox Sanguinis **103**(2): 145-149. | Denmark | Linked to above |
| Brojer 2005[91] | Noninvasive determination of fetal RHD status by examination of cell-free DNA in maternal plasma." TRANSFUSION 45(9): 1473-1480. | Poland | None |
| Finning (2008) [2] | 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: 816-8. | UK (Bristol) | Full paper: None<br>Abstract: #1360; #1305 |
| Grande (2013) [7] | Clinical application of midtrimester non-invasive fetal RHD genotyping and identification of RHD variants in a mixed-ethnic population | Spain | None |
| Thurik (2015) [6] | Analysis of false-positive results of fetal RHD typing in a national screening program reveals vanishing twins as potential cause for discrepancy | Netherlands | Full paper: None<br>Abstract: #1834 |
| De Hass 2012 [10] | "A nation-wide fetal RHD screening programme for targeted antenatal and postnatal anti-D." ISBT Science Series 7: 164-167. | Netherlands | Linked to Thurik |
| Oxenford (2013) [13] | 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." Prenatal Diagnosis 33(7): 688-694. | UK(London) | None |
| Soothill (2015) [3] | Use of cffDNA to avoid administration of anti-D to pregnant women when the fetus is RhD-negative: implementation in the NHS."BJOG: an international journal of obstetrics & gynaecology 122(12): 1682-1686. | UK (Bristol) | None |

| | | | |
|---|---|---|---|
| Wikman (2012) [8] | Noninvasive single-exon fetal RHD determination in a routine screening program in early pregnancy. Obstetrics & Gynecology. 120: 227-34. | Sweden | Full paper: #126<br><br>Abstract: #1831; #1830 |
| Tiblad 2013 [11] | "Targeted routine antenatal anti-D prophylaxis in the prevention of RhD immunisation--outcome of a new antenatal screening and prevention program." PLoS ONE [Electronic Resource] **8**(8): e70984. | Sweden | Linked to Wikman |

## 10.3 List of excluded studies

### 10.3.1 **Not high-throughput NIPT (123 references)**

Abildinova G, Baynova M, Kamalieva B, and Kostina A. (2011). 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 Berlin , pp.S691.

Achargui S, and Benchemsi N. (2009). Fetal rhesus D genotyping by PCR using plasma from rhd negative pregnant women. Vox Sanguinis, Conference: 19th Regional Congress of the ISBT - Eastern, pp.144.

Achargui S, Tijane M, and Benchemsi N. (2011). [Fetal RHD genotyping by PCR using plasma from D negative pregnant women]. Transfusion Clinique et Biologique, 18(1), pp.13-19.

Ahangari G, Zeinali S, Ebrahimi M, Mohsani F, and Saremi A T. (2003). Analysis of fetal sex and RhD gene in fetal cells DNA from maternal blood by polymerase chain reaction. Middle East Fertility Society Journal, 8, pp.263-268.

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## 10.4 Characteristics of diagnostic accuracy studies

| Short Title | Country | Study dates | Number tested [a] | Number analysed [a] | Gestational age (weeks, median/range) | Ethnicity (%) | Multiple pregnancies included? | DNA extraction tool | PCR technology | Multiple testing performed? |
|---|---|---|---|---|---|---|---|---|---|---|
| Akolekar (2011)[4] | England | NR | 591 | 586 | 12.4 (11-14) | European white 77.3; Asian 1.2; African 19.3; Mixed: 2.2 | No | MDx BioRobot (Qiagen) | ABI 7900 detection system (Applied Biosystems) | Yes (for RHD variants) |
| Banch Clausen (2014)[5] | Denmark | 2010-2011 | 14,547 | 12,668 | 25 (73% between 23–and 28) | NR | NR | QIAsymphony SP; MagNA Pure LC; MagNA Pure Compact Instrument (Roche) | ABI 7900 detection system (Applied Biosystems) LightCycler 480 (Roche) PCR ABI 7500 (Applied BioSystems) | NR |
| Chitty (2014)[1] | England | 2009-2012 | 4913 | 4913 | 19 (5 to 35) | European white 78; Asian 6; Black or mixed race 4; Unknown 12 | No | MDx BioRobot (Qiagen) | ABI Prism 7900HT (Applied Biosystems) | Up to 4 samples per woman |
| Finning (2008)[2] | England | NR | 1997* | 1869 | 28 (8-38) (92% at 26-32) | European white 55; Asian 8; African 2; Other: 2 | Yes | MDx BioRobot (Qiagen) | ABI Prism 7900HT (Applied Biosystems) | NR |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Unknown:33 | | | | |
| Grande (2013)[7] | Spain | 02/2010-10/2011 | 284 | 282 | 24-26 | European white 84; Asian 1.5; African 1.8; Latin American 12; other 0.7 | Yes | COBAS AmpliPrep (Roche) | 7300 Real-Time PCR System (Applied Biosystems) | Yes 2 independent assays performed in triplicate for all. |
| Soothill (2015)[3] | England | 04/2013-09/2013 | 526* | 499* | 15-26 | NR | No | MDx BioRobot (Qiagen) | NR | NR |
| Thurik (2015)[6] | Netherlands | 07/2011-10/2012 | 24986* | 18383* | 26 | NR | No | MagNa Pure 96 (Roche) | StepOnePlus Real-Time PCR System (Applied Biosystems) | Yes (state reasons) PCR in triplicate |
| Wikman (2012)[8] | Sweden | 09/2009-05/2011 | 4118 | 3291 | 10 (3-40) (75.5% 1st trimester) | NR | Yes | MagNA Pure LC (Roche) | PCR ABI 7500 (Applied BioSystems) | Yes PCR on all samples in triplicate<br><br>211 samples re-analysed due to uninterpretable results |

## 10.5 Risk of bias assessment of diagnostic accuracy studies

### 10.5.1 **Patient selection**

| **Study** | **Was a consecutive sample of patients enrolled?** | **Did the study avoid inappropriate exclusions?** | **Were key study population characteristics reported? (incl. ethnicity, GA, multiple pregnancies)** | **Risk of bias** | **Applicability: Are there concerns that the included patients do not match the target population?** |
|---|---|---|---|---|---|
| Akolekar (2011) | Unclear | No<br>Excluded multiple pregnancies* | Yes | High. Reporting of selection process limited, much higher proportion of African than general population (19.3%) | Yes<br>Much higher proportion of African than general population (19.3%) |
| Banch-Clausen (2014) | Unclear<br>Not stated but seems likely | Unclear<br>Appears fine | No<br>Population characteristics (including ethnicity) NR | Low | Unclear<br>Population characteristics (including ethnicity) NR |
| Chitty (2014) | Unclear<br>Not stated but seems likely | No<br>Excluded multiple pregnancies* | Yes | Low | No |
| Finning (2008) | Unclear<br>Not stated but seems likely | Yes | Yes | Low | No |
| Grande (2013) | Unclear | Yes | Yes | Low | Yes<br>Ethnic distribution differs from general UK population (12% Latin American) |
| Soothill (2015) | Unclear<br>Not stated but seems likely | Yes | No<br>Ethnicity and multiple pregnancy NR. Gestational range could be inferred but was not clearly reported. | Low | No |
| Thurik (2015) | Unclear<br>Not stated but seems likely | No<br>multiple pregnancies excluded and treated as positive.* | No<br>Ethnicity and number of multiple pregnancies NR | Low | Yes<br>exclusion of multiple pregnancies |
| Wikman (2012) | Unclear<br>Not stated but seems likely | Unclear<br>exclusion criteria not reported | No<br>Ethnicity NR | Low | Unclear<br>ethnicity unknown |

### 10.5.2 **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 pre-specified?** | **Were results from replicate samples dealt with appropriately?** | **Were results from multiple pregnancies dealt with appropriately** | **RoB: 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 (2011) | Unclear<br>likely not | Unclear<br>Thresholds were reported, but unclear if pre-specified | Yes | N/A<br>only singleton pregnancies | High<br>inconclusive results were not included in the main analysis. This may have inflated the accuracy estimates | Low | No |
| Banch-Clausen (2014) | Yes | Yes | Unclear (NR) | Unclear (NR) | Low | Low | No |
| Chitty (2014) | Yes | Yes | Unclear (NR) | N/A<br>only singleton pregnancies | Low | Low | No |
| Finning (2008) | Yes | Unclear.<br>Unclear if pre-specified | Unclear (NR) | Yes | Low | Low | No |
| Grande (2013) | Unclear | Unclear.<br>Unclear if pre-specified | Yes | Yes | Low | Low | No |
| Soothill (2015) | Unclear<br>Presumably as in Chitty 2014 | Unclear<br>Presumably as in Chitty 2014 | Unclear (NR) | Unclear (NR) | Unclear<br>Presumably as in Chitty 2014 | Low | No |
| Thurik (2015) | Unclear<br>unclear for back-up plasma analysis, yes for samples not re-analysed | No<br>Prediction algorithm is judged daily and ajusted 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<br>all treated as positive and prescribed anti-D | High<br>Change of diagnostic algorithm after start of study may have introduced bias | Low | No |
| Wikman | Unclear | Unclear | Yes | Yes | Low | High | No |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| (2012) | likely not | | | | | only exon 4 was targeted | |

### 10.5.3 **Reference standard**

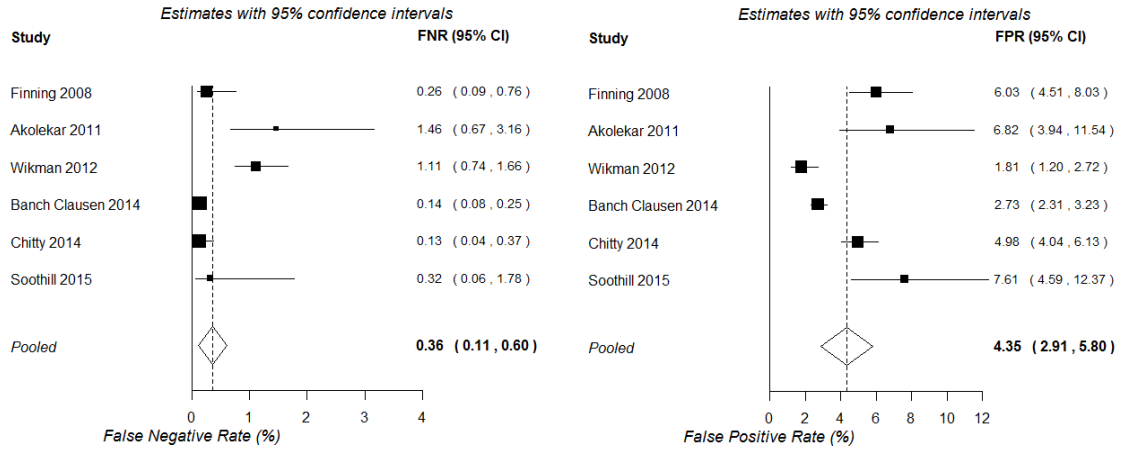
| **Short Title** | **Is the reference standard likely to correctly classify the target condition?** | **Were the reference standard results interpreted without knowledge of the results of the index test?** | **RoB: Could the reference standard, its conduct, or its interpretation have introduced bias?** | **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 (2011) | Unclear. Method NR | Unclear (NR) | Unclear. Method NR | Unclear. Method NR | No |
| Banch-Clausen (2014) | Yes | Unclear (NR) | Low | Low | No |
| Chitty (2014) | Yes | Unclear (NR) | Low | Low | No |
| Finning (2008) | Yes | Yes | Low | Low | No |
| Grande (2013) | Yes | Unclear (NR) | Low | Low | No |
| Soothill (2015) | Yes | Unclear (NR) | Low | Low | No |
| Thurik (2015) | Yes | Unclear (NR) | Low | Low | No |
| Wikman (2012) | Yes | Unclear (NR) | Low | Low. Author contacted: Appropriate except 5% of samples processed in citrate tubes. | No |

### 10.5.4 **Flow and timing**

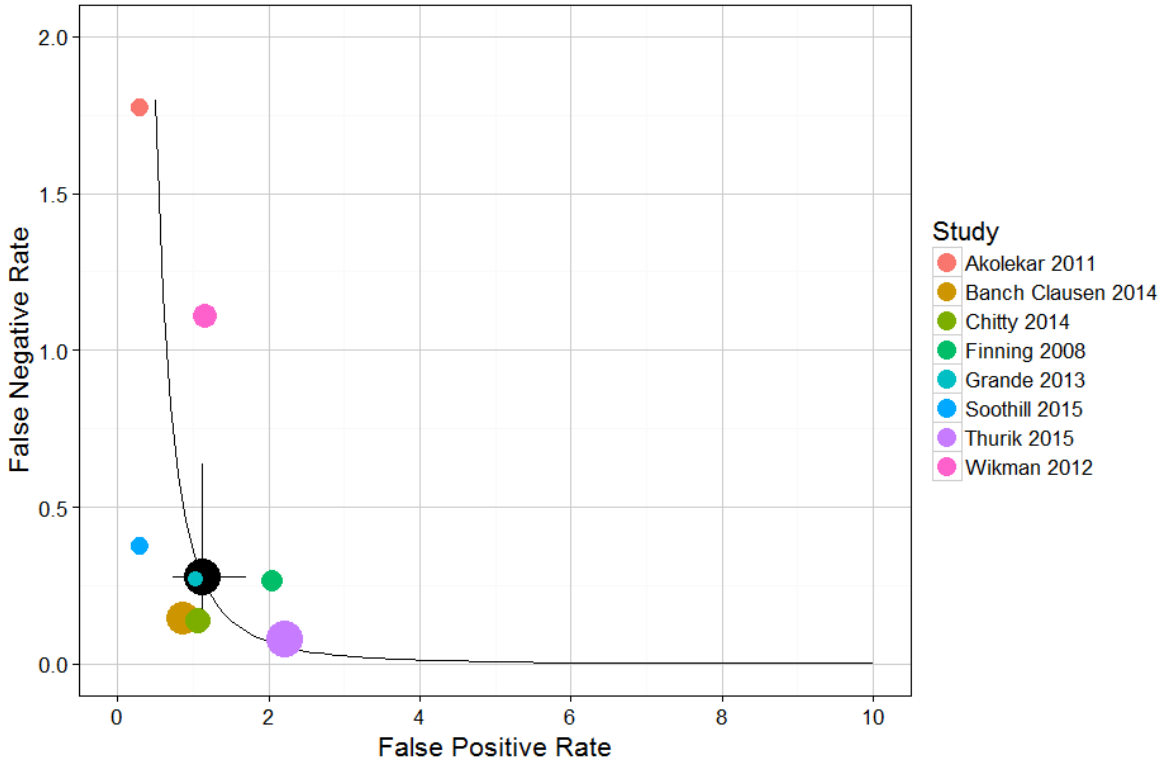
| **Short Title** | **Was there an appropriate interval between index test(s) and reference standard?** | **Did all patients (who provided data) receive a reference standard?** | **Did all patients receive the same reference standard?** | **Were all patients included in the analysis?** | **RoB: Could the patient flow have introduced bias?** |
|---|---|---|---|---|---|
| Akolekar (2011) | Yes | No<br>only those with ref std result and live birth were included in the study | Unclear | No<br>only those with ref std result and live birth were included in the study | Low |
| Banch-Clausen (2014) | Yes | No | Yes | Yes | Low |
| Chitty (2014) | Yes | No<br>185 without cord blood result, but | Yes | No<br>13% excluded for various reasons (all | Low |

| | | | | | |
|---|---|---|---|---|---|
| | | unlikely significant bias | | reported). | |
| Finning (2008) | Yes | No<br>4 did not due to fetal death. | Yes | No<br>128 fetal phenotypes were not available for paired analysis because 124 cord samples were untraceable and there were four fetal deaths. | Low |
| Grande (2013) | Yes | Yes<br>appears so | Yes | No<br>only 2 RhD positive mothers who underwent NIPT were excluded | Low |
| Soothill (2015) | Yes | No<br>5% did not have cord blood serology results | Yes | Yes | Low |
| Thurik (2015) | Yes | No<br>80% did. No reason provided for 20% not providing cord blood serology | Yes | No<br>20% samples received NIPT but not cord serology | High<br>20% samples received NIPT but not cord serology. No reasons provided |
| Wikman (2012) | Yes | No<br>11% pregnancies with no ref std measurement | No. 5% citrate samples (author contacted) | No<br>11% pregnancies with no ref std measurement | Low<br>despite limitations, risk of diagnostic accuracy results being significantly affected was not considered high |

## 10.6 Forest plot for analysis case 2




## 10.7 ROC plot for analysis case 3



## 10.8 Risk of bias assessment of clinical effectiveness studies

### 10.8.1 The ACROBAT-NRSI tool (1): At protocol stage

Specify the research question by defining a generic target randomized trial

| | |
|---|---|
| Participants | Rhesus negative pregnant women who are not known to be alloimmunised |
| Experimental intervention | High-throughput NIPT and targeted routine antenatal anti-d prophylaxis |
| Control intervention | Any |

Specify the nature of the target comparison (effect of interest)

e.g. effect of *initiating* intervention (as in an intention-to-treat analysis), or effect of *initiating and adhering to* intervention (as in a per-protocol analysis)

- Number of doses of anti-D immunoglobulin given (routine antenatal, following potential sensitising events and postnatal)
- Compliance with 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 in subsequent pregnancies
- Adverse effects of testing
- Health related quality of life including anxiety

List the confounding domains relevant to all or most studies

Gestational age at time of NIPT, ethnicity, potential sensitising event (pre-birth), compliance with antenatal anti-D prophylaxis, uptake of NIPT

List the possible co-interventions that could differ between intervention groups and could have an impact on study outcomes

| |
|---|
| Non-routine anti-D (due to potential sensitising event) |

### 10.8.2 **The ACROBAT-NRSI tool (2): Banch-Clausen et al. 2014**

Specify a target trial specific to the study.

| | | | |
|---|---|---|---|
| The protocol-specified target randomized trial fully applies | ☐ | *OR* | Participants: Rhesus negative pregnant women who have not developed anti-d antibodies |
| | | | Experimental intervention: High-throughput NIPT and targeted routine antenatal anti-d prophylaxis (RAADP) |
| | | | Control intervention: Routine management: post-natal anti-D prophylaxis only |

Specify which outcome is being assessed for risk of bias (typically from among those earmarked for the Summary of Findings table). Specify whether this is a proposed benefit or harm of intervention.

| |
|---|
| Compliance:<br>with prenatal anti-D<br>with postnatal anti-D<br>with RHD screening |

Specify the effect of interest

| |
|---|
| effect of *initiating and adhering to* intervention (for prenatal & postnatal anti-D compliance) |

Specify the specific result being assessed

| |
|---|
| compliance with antenatal anti-D: 93.2% vs. NA (not recommended in patients not receiving RHD screening)<br>compliance with postnatal anti-D: 99.7% vs. 95.7%<br>compliance with RHD screening: 84.2% |

Preliminary consideration of confounders

a. Within each confounding domain listed in the review protocol, list the relevant variables, if any, measured in this study.

| |
|---|
| Compliance with routine antenatal anti-D prophylaxis |

b List additional confounding domains, if any, specific to the setting of this particular study. Within each domain, list the relevant variables, if any, measured in this study.

| |
|---|
| Potential sensitising event |

c List additional domains and corresponding measured variables, if any, that the study authors identified as potential confounders that are not included in the above domains.

| |
|---|
| None |

Relationship between confounding domains and potential confounders.

| Confounding domain | Is the domain critically important?* | Measured Variable | Did the authors demonstrate that controlling for this variable was unnecessary?* | Is the domain measured validly and reliably by this variable (or these variables)? | OPTIONAL: Is adjusting for this variable (alone) expected to move the effect estimate up or down? ** |
|---|---|---|---|---|---|
| Potential sensitising event | Yes | No | No | No | Up: sensitising event likely to increase anti-d uptake |
| Anti-D prophylaxis compliance | Yes | Antenatal anti-D administered | No | Yes | Up: antenatal uptake may be associated with higher postnatal compliance |
| Gestational age at time of NIPT | No (same for both groups) | NA | NA | NA | NA |
| Ethnicity | No (unlikely) | NA | NA | NA | NA |

* In the context of a particular study, variables can be demonstrated not to be confounders and so not included in the analysis: (a) if they are not predictive of the outcome; (b) if they are not predictive of intervention; or (c) because adjustment makes no or minimal difference to the estimated effect of the primary parameter. Note that "no statistically significant association" is not the same as "not predictive".

** For example, if the crude effect estimate is 1.3, adjustment to 1.6 is up, while adjustment to 0.7 is down. If the effect estimate is 0.7, adjustment to 1.1 is up while adjustment to 0.4 is down.

Preliminary consideration of co-interventions

a. Are the (pre-specified) co-interventions likely to be administered in the context of this study?

| |
|---|
| Anti-D due to potential sensitising event (it appears that no distinction was made between routine and non-routine prenatal anti-D) |

b List additional co-interventions, if any, specific to the setting of this particular study.

| |
|---|
| None |

Co-interventions

In the table below, "critically important" co-interventions are those for which, in the context of this study, adjustment is expected to lead to a clinically important change in the estimated effect of the intervention. "Validity" refers to whether the variables fully measure the co-intervention, while "reliability" refers to the precision of the measurement (more measurement error means less reliability).

| Co-intervention | Is the co-intervention critically important?* | Did the authors demonstrate that controlling for this co-intervention was unnecessary? | Is the co-intervention measured validly and reliably? | Is presence of this co-intervention likely to favour outcomes in the experimental or the control group |
|---|---|---|---|---|
| (Non-routine) anti-D due to potential sensitising event | yes | no | no information | either |

Risk of bias assessment (cohort-type studies)

| | | | |
|---|---|---|---|
| Bias due to confounding | 1.1 Is confounding of the effect of intervention unlikely in this study?<br>If Y or PY to 1.1: the study can be considered to be at low risk of bias due to confounding and no further signalling questions need be considered | PN | Unadjusted analyses |
| | If N or PN to 1.1: | | |
| | 1.2. Were participants analysed according to their initial intervention group throughout follow up?<br>If Y or PY to 1.2, answer questions 1.4 to 1.6, which relate to baseline confounding | PY | |
| | 1.3. If N or PN to 1.2: Were intervention discontinuations or switches unlikely to be related to factors that are prognostic for the outcome?<br>If Y or PY to 1.3, answer questions 1.4 to 1.6, which relate to baseline confounding<br>If N or PN to 1.1 and 1.2 and 1.3, answer questions 1.7 and 1.8, which relate to time-varying confounding | NA | |
| | If Y or PY to 1.2, or Y or PY to 1.3 | | |
| | 1.4. Did the authors use an appropriate analysis method that adjusted for all the critically important confounding domains? | N | Unadjusted analyses |
| | 1.5. If Y or PY to 1.4: Were confounding domains that were adjusted for measured validly and reliably by the variables available in this study? | NA | |
| | 1.6. Did the authors avoid adjusting for post-intervention variables? | NA | |
| | If N or PN to 1.2 and 1.3 | | |
| | 1.7. Did the authors use an appropriate analysis method that adjusted for all the critically important confounding domains and for time-varying confounding? | NA | |
| | 1.8. If Y or PY to 1.7: Were confounding domains that were adjusted for measured validly and reliably by the variables available in this study? | NA | |
| | Risk of bias judgement | Critical | Analyses not adjusted for several potential confounders (see more details above) |
| | Optional: What is the predicted direction of bias due to confounding? | Unpredictable | |
| Bias in selection of participants into the study | 2.1. Was selection into the study unrelated to intervention or unrelated to outcome? | N | |
| | 2.2. Do start of follow-up and start of intervention coincide for most subjects? | Y | |
| | 2.3. If N or PN to 2.1 or 2.2: Were adjustment techniques used that are likely to correct for the presence of selection biases? | N | |
| | Risk of bias judgement | NI | Only participants from one of the five regions over a year (690/12,668) were included. Reasons were not provided |

| | | | |
|---|---|---|---|
| | Optional: What is the predicted direction of bias due to selection of participants into the study? | Unpredictable | |
| Bias in measurement of interventions | 3.1 Is intervention status well defined? | Y | RHD screening |
| | 3.2 Was information on intervention status recorded at the time of intervention? | PY | |
| | 3.3 Was information on intervention status unaffected by knowledge of the outcome or risk of the outcome? | Y | |
| | Risk of bias judgement | Low | |
| | Optional: 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 | 4.1. Were the critical co-interventions balanced across intervention groups? | NI | No information on non-routine anti-D, and whether it was measured as separate from routine administration |
| | 4.2. Were numbers of switches to other interventions low? | Y | NA |
| | 4.3. Was implementation failure minor? | PY | No information but unlikely |
| | 4.4. If N or PN to 4,1, 4.2 or 4.3: Were adjustment techniques used that are likely to correct for these issues? | N | |
| | Risk of bias judgement | Low | |
| | Optional: 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 | 5.1 Are outcome data reasonably complete? | NI | No information on missing data |
| | 5.2 Was intervention status reasonably complete for those in whom it was sought? | NI | |
| | 5.3 Are data reasonably complete for other variables in the analysis? | N | Lack of reported data on confounders |
| | 5.4 If N or PN to 5.1, 5.2 or 5.3: Are the proportion of participants and reasons for missing data similar across interventions? | NI | No information on missing data |
| | 5.5 If N or PN to 5.1, 5.2 or 5.3: Were appropriate statistical methods used to account for missing data? | NA | |
| | Risk of bias judgement | NI | |
| | Optional: What is the predicted direction of bias due to missing data? | Unpredictable | |
| Bias in measurement of outcomes | 6.1 Was the outcome measure objective? | Y | |
| | 6.2 Were outcome assessors unaware of the intervention received by study participants? | NI | |

| | | | |
|---|---|---|---|
| | 6.3 Were the methods of outcome assessment comparable across intervention groups? | PY | |
| | 6.4 Were any systematic errors in measurement of the outcome unrelated to intervention received? | NI | |
| | Risk of bias judgement | Low | No information to suggest otherwise |
| | Optional: 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...<br>7.1. ... multiple outcome *measurements* within the outcome domain? | PY | |
| | 7.2 ... multiple *analyses* of the intervention-outcome relationship? | PY | |
| | 7.3 ... different *subgroups*? | NI | Only participants from one of the five regions over a year (690/12,668) were included. Reasons were not provided |
| | Risk of bias judgement | NI | |
| | Optional: 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 a year (690/12,668) were included.<br><br>Analyses were not adjusted for any potential confounders |
| | Optional:<br>What is the overall predicted direction of bias for this outcome? | Unpredictable | Unpredictable due to insufficient information, although may be more likely to favour the intervention |

### 10.8.3 **The ACROBAT-NRSI tool (2): Tiblad et al. 2013**

Specify a target trial specific to the study.

| The protocol-specified target randomized trial fully applies | | | |
|---|---|---|---|
| | | Participants | Rhesus negative pregnant women who have not developed anti-d antibodies |
| ☐ | *OR* | Experimental intervention | High-throughput NIPT and targeted routine antenatal anti-d prophylaxis (RAADP) |

| | |
|---|---|
| Control intervention | Routine management: post-natal anti-D prophylaxis only |

Specify the outcome

| |
|---|
| Sensitisation (measured as development of anti-D antibodies after the 1st trimester of pregnancy or postpartum) |

Specify the effect of interest

| |
|---|
| effect of *initiating* intervention |

Specify the specific result being assessed

In case of multiple alternative analyses being presented, specify the numeric result (e.g. RR = 1.52 (95% CI 0.83 to 2.77) and/or a reference (e.g. to a table, figure or paragraph) that uniquely defines the result being assessed.

| |
|---|
| adjusted odds ratio 0.41 (95% CI 0.22–0.78), 0.19 versus 0.46% (from Neovius 2015[58]) |

Preliminary consideration of confounders

a. Within each confounding domain listed in the review protocol, list the relevant variables, if any, measured in this study.

| |
|---|
| Gestational age, sensitising event (pre-birth), compliance with routine antenatal anti-D prophylaxis (RAADP) |

b List additional confounding domains, if any, specific to the setting of this particular study. Within each domain, list the relevant variables, if any, measured in this study.

| |
|---|
| Gestational age at RAADP<br>Nulliparous pregnancies<br>NIPT sensitivity |

c List additional domains and corresponding measured variables, if any, that the study authors identified as potential confounders that are not included in the above domains.

| |
|---|
| None |

Relationship between confounding domains and potential confounders.

| Confounding domain | Is the domain critically important?* | Measured Variable | Did the authors demonstrate that controlling for this variable was unnecessary?* | Is the domain measured validly and reliably by this variable (or these variables)? | OPTIONAL: Is adjusting for this variable (alone) expected to move the effect estimate *within each arm* up or down? ** |
|---|---|---|---|---|---|
| Gestational age | Yes | Gestational age at time of NIPT | No | Yes | Up / Down / No information<br>Down<br>The later, the higher the risk of immunization pre-RAADP and at follow-up |
| | | Gestational age at RAADP | No | Yes | Down<br>The later, the higher the risk of immunization pre-RAADP and at follow-up |
| Potential sensitising event | Yes | Immunised | No | Yes | Down<br>The higher the rate, the higher the rate of immunisations at follow-up |
| | | Immunisation event before RAADP | No | Yes | Down<br>The higher the rate, the higher the rate of immunisations at follow-up |
| Compliance with antenatal anti-D | Yes | Received RAADP | No | Yes | Up<br>The higher the compliance, the lower |

| | | | | | |
|---|---|---|---|---|---|
| prophylaxis | | | | | the rate of immunisations at follow-up |

* In the context of a particular study, variables can be demonstrated not to be confounders and so not included in the analysis: (a) if they are not predictive of the outcome; (b) if they are not predictive of intervention; or (c) because adjustment makes no or minimal difference to the estimated effect of the primary parameter. Note that "no statistically significant association" is not the same as "not predictive".

** For example, if the crude effect estimate is 1.3, adjustment to 1.6 is up, while adjustment to 0.7 is down. If the effect estimate is 0.7, adjustment to 1.1 is up while adjustment to 0.4 is down.

Preliminary consideration of co-interventions

a. Are the (pre-specified) co-interventions likely to be administered in the context of this study?

| |
|---|
| Anti-D due to potential sensitising event (due to sensitising event) |

b List additional co-interventions, if any, specific to the setting of this particular study.

| |
|---|
| Routine post-partum antibody testing performed<br>Routine antibody testing at 25 weeks in routine management group |

Co-interventions

| Co-intervention | Is the co-intervention critically important?* | Did the authors demonstrate that controlling for this co-intervention was unnecessary? | Is the co-intervention measured validly and reliably? | Is presence of this co-intervention likely to favour outcomes in the experimental or the control group |
|---|---|---|---|---|
| (Non-routine) anti-D due to potential sensitising event | Yes<br>e.g. intervention with risk of fetal-maternal haemorrhage (FMH) | No (data not reported) | No | No information (could be either way) |

Risk of bias assessment (cohort-type studies)

| | | | |
|---|---|---|---|
| Bias due to confounding | 1.1 Is confounding of the effect of intervention unlikely in this study?<br>**If Y or PY to 1.1:** the study can be considered to be at low risk of bias due to confounding and no further signalling questions need be considered | N | Study with historical control and unadjusted analysis |
| | **If N or PN to 1.1**: | | |
| | 1.2. Were participants analysed according to their initial intervention group throughout follow up?<br>**If Y or PY to 1.2**, answer questions 1.4 to 1.6, which relate to baseline confounding | N | In the reference group, no routine post partum antibody testing was performed. The outcome was measured in the first trimester of the subsequent pregnancy. |
| | 1.3. **If N or PN to 1.2**: Were intervention discontinuations or switches unlikely to be related to factors that are prognostic for the outcome?<br>**If Y or PY to 1.3**, answer questions 1.4 to 1.6, which relate to baseline confounding<br>**If N or PN to 1.1 and 1.2 and 1.3**, answer questions 1.7 and 1.8, which relate to time-varying confounding | PN | |
| | **If Y or PY to 1.2, or Y or PY to 1.3** | | |
| | 1.4. Did the authors use an appropriate analysis method that adjusted for all the critically important confounding domains? | NA | |
| | 1.5. **If Y or PY to 1.4**: Were confounding domains that were adjusted for measured validly and reliably by the variables available in this study? | NA | |
| | 1.6. Did the authors avoid adjusting for post-intervention variables? | NA | |
| | **If N or PN to 1.2 and 1.3** | | |
| | 1.7. 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. |

| | | | |
|---|---|---|---|
| | 1.8. **If Y or PY to 1.7**: Were confounding domains that were adjusted for measured validly and reliably by the variables available in this study? | NA | |
| | **Risk of bias judgement** | Serious | Study with historical control. No adjustment for RAADP compliance or sensitising event. |
| | Optional: What is the predicted direction of bias due to confounding? | Unpredictable | |
| Bias in selection of participants into the study | 2.1. Was selection into the study unrelated to intervention or unrelated to outcome? | N | The control group was historical, pre-targeted routine anti-D prophylaxis<br>In the reference group, immunisation after delivery was defined as presence of anti-D antibodies in the first trimester in the subsequent pregnancy.<br>Routine antibody testing at 25 weeks in nulliparous women in routine management group was not performed |
| | 2.2. 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 |
| | 2.3. **If N or PN to 2.1 or 2.2**: Were adjustment techniques used that are likely to correct for the presence of selection biases? | N | |
| | **Risk of bias judgement** | Serious | The control group was historical, pre-targeted routine anti-D prophylaxis<br>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. |
| | Optional: 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 | 3.1 Is intervention status well defined? | Y | |
| | 3.2 Was information on intervention status recorded at the time of intervention? | Y | |
| | 3.3 Was information on intervention status unaffected by knowledge of the outcome or risk of the outcome? | Y | "hard" outcome |
| | **Risk of bias judgement** | Low | |

| | | | |
|---|---|---|---|
| | Optional: What is the predicted direction of bias due to measurement of outcomes or interventions? | Towards null | |
| Bias due to departures from intended interventions | 4.1. Were the critical co-interventions balanced across intervention groups? | NI | |
| | 4.2. Were numbers of switches to other interventions low? | PY | |
| | 4.3. Was implementation failure minor? | NI | |
| | 4.4. **If N or PN to 4,1, 4.2 or 4.3**: Were adjustment techniques used that are likely to correct for these issues? | N | |
| | **Risk of bias judgement** | Low | |
| | Optional: What is the predicted direction of bias due to departures from the intended interventions? | Unpredictable | |
| Bias due to missing data | 5.1 Are outcome data reasonably complete? | NI | In the control group, it appears that any pregnant woman with no recorded subsequent pregnancy was excluded. |
| | 5.2 Was intervention status reasonably complete for those in whom it was sought? | NI | Insufficient information |
| | 5.3 Are data reasonably complete for other variables in the analysis? | N | Limited data on participants excluded from the analyses due to no recorded subsequent pregnancy in the reference group. |
| | 5.4 **If N or PN to 5.1, 5.2 or 5.3**: Are the proportion of participants and reasons for missing data similar across interventions? | NI | |
| | 5.5 **If N or PN to 5.1, 5.2 or 5.3**: Were appropriate statistical methods used to account for missing data? | N | |
| | **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 2013) |
| | Optional: What is the predicted direction of bias due to missing data? | Unpredictable | |
| Bias in measurement of outcomes | 6.1 Was the outcome measure objective? | Y | |
| | 6.2 Were outcome assessors unaware of the intervention received by study participants? | NI | No mention of blinding |

| | | | |
|---|---|---|---|
| | 6.3 Were the methods of outcome assessment comparable across intervention groups? | Y | |
| | 6.4 Were any systematic errors in measurement of the outcome unrelated to intervention received? | PN | |
| | **Risk of bias judgement** | Low | |
| | Optional: 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... | | |
| | 7.1. ... multiple outcome *measurements* within the outcome domain? | PN | |
| | 7.2 ... multiple *analyses* of the intervention-outcome relationship? | PN | |
| | 7.3 ... different *subgroups*? | PN | |
| | **Risk of bias judgement** | Low | |
| | Optional: What is the predicted direction of bias due to selection of the reported result? | Towards null | |
| Overall bias | **Risk of bias judgement** | Serious | Primarily due to risk of selection bias, confounding and missing data |
| | Optional:<br>What is the overall predicted direction of bias for this outcome? | Unpredictable | Unpredictable due to insufficient information.<br>Note: the generalisability of the study findings to the UK is limited given that RAADP is recommended as part of routine care. |

## 10.9 Summary of anti-D reviews

| | Review details | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Review** | **N. studies** | **Anti-D group** | **Control** | **Outcome** | **Anti-D group** | **Control group** | **Relative risk** | **lower CI** | **upper CI** |
| | | | | | | | | | |
| McBain (2015) | 2 | Anti-D after 28 weeks | No treatment (standard care) | Alloimmunisation in pregnancy or postpartum | 5 | 13 | 0.42 | 0.15 | 1.17 |
| | 2 | | | Alloimmunisation within one year | 6 | 16 | 0.39 | 0.1 | 1.62 |
| | 1 | | | Positive Kleihauer at birth | 73 | 119 | 0.6 | 0.46 | 0.79 |
| | 1 | | | Jaundice | 1 | 4 | 0.26 | 0.03 | 2.3 |
| | | | | | | | | | |
| Turner (2012) | 10 | Anti-D (500IU) 28-34 wks | Standard postpartum or at sensitisation | Postpartum sensitisation | | | 0.31 | 0.17 | 0.56 |
| | | | | | | | | | |
| Pilgrim (2009) | 8 (total) | Anti-D (various doses) 28-34 weeks | No antenatal anti-D | Sensitisation | | | | | |
| | 4 | | | 500 IU | 0.30 % | 0.89 % | 0.33 | 0.2 | 0.55 |
| | 3 | | | 1500 IU | 0.34 % | 1.60 % | 0.2 | 0.13 | 0.29 |
| | 2 | | | 500IU community | 0.35 % | 0.95 % | 0.37 | 0.21 | 0.65 |
| | 1 | | | Compliance | | | 90% dose 1, 79% dose 2 | | |
| | | | | | | | | | |
| Fyfe (2014) | 8 | Not described | None | Compliance | | | 80% to 90% | | |

## 10.10 Existing cost-effectiveness evidence: list of excluded papers

1. Bernhofen DM: The Empirics of Comparative Advantage: Overcoming the Tyranny of Nonrefutability. Review of International Economics 2005, 13(5):1017-1023.

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 Proceedings of Rijeka School of Economics: Journal of Economics and Business 2002, 20(2):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. American Journal of Obstetrics and Gynecology 2006, 195(6):S119-S119.

4. Duan Q, Liao TW: Optimization of Blood Supply Chain with Shortened Shelf Lives and ABO Compatibility. International Journal of Production Economics 2014, 153:113-129.

5. Leistikow EA, Collin MF, Savastano GD, Desierra TM, Leistikow BN: Wasted health care dollars: routine cord blood type and Coombs' testing. Archives of Pediatrics and Adolescent Medicine 1995, 149(10):1147-1151.

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 2015:1-5.

7. Moise Jr KJ: Costs and Clinical Outcomes of Noninvasive Fetal RhD Typing for Targeted Prophylaxis...Obstet Gynecol. 2013 Sep;122(3):579-85. Obstetrics & Gynecology 2013, 122(6):1306-1306 1301p.

8. Roque H: Fetal RhD genotyping by maternal serum analysis: A two-year experience [7]. American Journal of Obstetrics and Gynecology 2006, 194(3):905-906.

9. Szczepura A, Bonsel G, Krauth C, Osipenko L, Haverkamp A: Is fetal RHD typing in all RhD negative women cost effective? Bmj 2008, 336(7650):906.

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(11):165A-165A.

## 10.11 Previous NICE Technology appraisals

Two previous technology appraisals were done on RAADP. The more recent appraisal (NICE TA 156) concluded that, compared to having no RAADP, RAADP reduces the incidence of sensitisation and, consequently, of haemolytic disease of the newborn. 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 A11.1). The total cost of providing RAADP to RhD-negative multigravidae in England and Wales was estimated to be around £2–£2.6 million per year (2008 values). Table A10.1 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 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 post-partum anti-D immunoglobulin to post-partum 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 (inc. phlebotomy, feto-maternal haemorrhage test and anti-D immunoglobulin treatment);
- We included the costs relating to post-partum treatment (inc. cord serology, phlebotomy, feto-maternal haemorrhage test and anti-D immunoglobulin treatment)

Table A11.1. Incremental cost-effectiveness outcomes associated with RAADP *vs* no RAADP (multigravidae) – NICE TA 156 (2008)

| Strategies | Incr. cost | Number of sensitisations avoided | Number of affected pregnancies avoided | Number of fetal losses avoided | Life Years gained | Incr. QALYS | Cost per sensitisation avoided | Cost per affected pregnancy avoided | Cost per fetal loss avoided | Cost per LY gained | ICER, cost per QALY gained |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **No RAADP *** | £1,796,546 | 630.5 | 353.4 | 14.1 | 2,878,648 | 2,533,240 | --- | --- | --- | --- | --- |
| **2x 500IU 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 |
| **1x 1500 IU RAADP (multi)** | £2,010,568 | 232.9 | 72.1 | 2.9 | 120.4 | 100.0 | £8,634 | £27,880 | £697,002 | £16,705 | £20,108 |

Table A11.2. Incremental cost-effectiveness outcomes associated with RAADP *vs* no RAADP in the current diagnostic assessment (2016) – deterministic and probabilistic results

| Strategies** | Incr. cost | Number of sensitisations avoided | Number of affected pregnancies avoided | Number of fetal losses avoided | Life Years gained | Incr. QALYS | Cost per sensitisation avoided | Cost per affected pregnancy avoided | Cost per fetal loss avoided | Cost per LY gained | ICER, cost per QALY gained |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Deterministic results** | | | | | | | | | | | |
| **No RAADP *** | £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 *** | £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 |

* No RAADP is absolute amounts

** For both strategies prophylactic anti-D immunoglobulin after a potentially sensitising event is considered together with further post-partum anti-D immunoglobulin administration to RhD-negative women whose baby RhD status is confirmed to be positive after cord serology; For the RAADP strategy, treatment is delivered to all RhD-negative pregnant women, either under single- or two-dose regimens.

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 lowest BNF price available. As a result of the amendments, 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 A11.2). The total cost of RAADP is estimated to be £16.7m and the total QALYs 2.4m. 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 to no RAADP. This is lower than the previous estimate of £20,108, largely due to the reduced unit cost of anti-D immunoglobulin based on updated BNF prices and the increased birth rate.