Australian Government
Department of Health

RATIFIED PICO

Application 1574:

Non-invasive prenatal testing for Rhesus D

Summary of PICO/PPICO criteria to define the question(s) to be addressed in an Assessment Report to the Medical Services Advisory Committee (MSAC)

Component	Description
Patients	Pregnant women who are Rhesus D (RhD)-negative . *This may require further restrictions.*
Prior tests	Serological testing is routinely conducted in all pregnant women to ascertain RhD status and presence of anti-D antibodies (MBS 65096).
Intervention	Non-invasive prenatal test (NIPT) to screen fetal *RhD* status (i.e. *RhD*-NIPT test).
Comparator	No testing for the *RhD* genotype of the fetus (prior testing alone)
Outcomes	**Safety:** Incidence of injection site infections and systemic adverse events from anti-D immunoglobulin (e.g. infections and reactions) **Assessment of diagnostic accuracy:** Sensitivity; Specificity; Positive and negative predictive values of the test. Additional relevant outcomes: Rate of fetal RhD true negatives identified; False-negative rates (FNRs) indicating the proportion of women at risk of sensitisation; Rate of fetal RhD true positives identified; False-positive rates (FPRs) indicating the proportion of women who received anti-D immunoglobulin unnecessarily; Rate of uninterpretable/inconclusive tests with reasons (e.g. insufficient DNA; *RhD* variant). Healthcare resource consequences: • Only RhD-negative pregnant women who test *RhD*-positive for the fetus (and those who have not been tested or have an indeterminate result) would be administered targeted anti-D immunoglobulin as prophylaxis against haemolytic disease of the fetus and newborn (HDFN). • RhD-negative pregnant women who test *RhD*-negative for the fetus would not be administered anti-D immunoglobulin • RdD-negative pregnant women (who are anti-D antibody negative) who do not receive the *RhD*-NIPT test would be administered standard of care (non-targeted) anti-D immunoglobulin (all RhD-negative pregnant women at 28 and 34 weeks' gestation, and within 72 hours of the delivery of an RhD-positive infant, or following other obstetric events associated with a risk of fetal-to-maternal haemorrhage). **Compliance outcomes:** Rate of RhD-negative pregnant women (who are not known to be sensitised to the RhD-antigen) who accepted RhD non-invasive prenatal testing (NIPT); Rate of uptake of anti-D (antenatal and postnatal) immunoglobulin in women identified as RhD-negative with fetuses identified as RhD-positive; Number of doses per woman of anti-D immunoglobulin given (routine antenatal, following potentially sensitising events and postnatal). **Intermediate outcomes:** Rate of RhD immunisation (sensitisation) occurring due to (i) forming anti-D antibodies in RhD-negative pregnant women due to false

Component	Description
	negative *RhD* results of the test, or (ii) non-compliance to undertake *RhD*-NIPT and/or anti-D (antenatal or postnatal) immunoglobulin. **Final patient outcomes:** Number of cases of HDFN in subsequent pregnancies (per 1,000 live births). **Health care resources:** Cost of testing (capital outlay for setting up a high-throughput NIPT + medical procedure + laboratory investigation); Cost of anti-D immunoglobulin; Cost of future immunised pregnancies; Cost of HDFN pregnancies. **Cost-effectiveness**: Test - incremental cost per fetal RhD true negative status detected; Intermediate - Incremental cost per alloimmunisation avoided; Final - Incremental cost per HDFN avoided **Total Australian Government healthcare costs**: Total cost to the Medical Benefits Schedule (MBS).

PICO or PPICO rationale for therapeutic and investigative medical services only

Population

The patient population for whom public funding of the proposed medical service is intended in the application includes Rhesus-D (RhD) -negative pregnant women. While not specified in the proposed item descriptor or explicitly stated in the application, the request is further limited to those who are not sensitised to the RhD-antigen, based on the current and proposed clinical management algorithms provided. PASC accepted that the population is all RhD-negative pregnant women, regardless of whether or not they have previously been sensitised. The Australian Red Cross Lifeblood (Lifeblood November 2019) limits testing to those not carrying dizygotic (fraternal) twins (due to the inability to ensure that DNA from both twins has been tested). This would presumably also apply to higher-order multiple pregnancies (e.g., triplets).

The application (p16) notes that "Although the Rh system comprises 61 antigens, the D antigen is the most immunogenic and important, with routine Rh typing only testing for the presence or absence of the D antigen on red cells. The presence of RhD antigen confers Rh positivity; while people who lack RhD antigen are Rh negative".

The Australian Red Cross Lifeblood (Lifeblood) estimates that approximately 17% - 19% of Australian women who become pregnant are RhD-negative.[1] The prevalence of RhD-negative females in the total Australian population of pregnant women would vary with respect to the ethnic origin of the women, see Table 1.

Table 1: Prevalence of RhD-negative blood type by ethnicity

Ethnicity	Prevalence of RhD negative blood type	Source
European	15-17%	Fisher, 1946
African	3-5%	NICE 2016
Eastern Asian	Very rare	NICE 2016
Chinese	0.3-0.5%	Peng 2003
Pakistan, District of Swat	12.22% (of 5,723 females)	Khattak 2008
Northern Pakistan	4.06% (of 850 females)	Mahmood 2018
Indian	5.74-7.75%	Sarkar 2012, Das 201

Among those of European family origin, most RhD-negative people have an *RhD* gene deletion while less than 1% have *RhD* gene variants.

Australians of British heritage represent the majority (67.4%) of the RhD-negative population. This is followed by other European ethnicities: Irish (8.7%), Italian (3.8%), and German (3.7%). Those of Chinese ethnicity and the Aboriginal, and Native Australians represent 3.6% and 3% of the population, respectively. Other ethnicities represented in smaller numbers: Indian (1.7%), Greek (1.6%), Dutch (1.2%), and Other (5.3%). The "Other" ethnicity includes individuals from many countries, particularly European and Asian[2].

[1] Australian Red Cross Lifeblood https://www.donateblood.com.au/anti-d-program
[2] https://www.worldatlas.com/articles/ethnic-background-of-australians.html (last updated on June 13, 2018).

RhD genotyping performed on DNA from 1,997 RhD-negative pregnant women in UK identified 36.06% RhD-negative fetuses (35.9% correctly identified + 0.16% false negatives) (Finning 2008). This is consistent with information received from the Lifeblood, which showed that 37.2% of RhD-negative pregnant women carried an RhD-negative fetus (Health PACT 2017).

Rationale

Approximately one in seven (i.e. about 14%) women has a Rhesus (Rh) D-negative blood type (RANZCOG 2015). RhD-negative women carrying an *RhD*-positive fetus are at risk of becoming sensitised (alloimmunised). Alloimmunisation occurs when fetal cells enter the maternal circulation (an event called feto-maternal haemorrhage or FMH) and the mother's immune system is exposed to the fetus' RhD-positive red blood cells. Upon exposure, the mother's immune system recognises the RhD-antigen as "foreign" and starts producing antibodies against the RhD antigen. The developing antibodies then fight and destroy these foreign cells placing the fetus at risk of haemolytic disease of the fetus and newborn (HDFN). PASC noted that HDFN is usually mild to moderate, but more severe cases can result in haemolysis and anaemia, and lead to heart failure or death. Management of a fetus affected by HDFN includes in utero blood transfusion (risking premature delivery), post-natal exchange transfusion and phototherapy to prevent kernicterus.

Although sensitisation can occur at any time during gestation, it usually occurs in the third trimester or during labour. Additionally, sensitisation can result from medical interventions (e.g. chorionic villus sampling, amniocentesis or external cephalic version), terminations, late miscarriages, antepartum haemorrhage and abdominal trauma. While alloimmunisation largely affects subsequent pregnancies with an *RhD*-positive fetus, there is potential for the first pregnancy with an *RhD*-positive fetus to be affected (dependent on the severity and timing of FMH). The incidence of HDFN depends on the proportion of the population that is RhD-negative. This proportion varies between ethnic groups and is highest in the white population (NICE 2008). PASC noted that RhD-negative women with RhD-positive fetuses, with only postpartum RhD-Ig have about a 2% risk of sensitisation.

Currently, all pregnant women who are RhD-negative and who are not known to be sensitised to the RhD-antigen are universally administered antenatal anti-D prophylaxis, without the knowledge of the *RhD* status of the fetus (the presence of an *RhD* gene suggests an RhD-positive fetus). Currently in Australia, Anti-D prophylaxis is given as two doses at weeks 28 and 34 of pregnancy (dose 625IU). It is also administered following other obstetric events associated with a risk of fetal-to-maternal haemorrhage (e.g. external cephalic version, terminations, late miscarriages and abdominal trauma). Anti-D is also administered postpartum within 72 hours if the baby is found to be RhD-positive based on a cord blood serology test (RANZCOG Guidelines 2015). Anti-D prophylaxis is not necessary for RhD-negative women who are carrying an *RhD*-negative fetus.

RhD-negative pregnant women who are sensitised to the RhD-antigen are currently tested under an arrangement between Lifeblood and the National Blood Authority (NBA), see below. This is because expert advice indicated that utilisation of the non-invasive prenatal test (NIPT) avoids invasive tests like amniocentesis to determine the *RhD* status of the fetus. Expert advice also indicated that management of women carrying an *RhD*-positive fetus is in part dependent on obstetric history, but would typically require monthly quantification of antibody load, serial measurement of mid cerebral artery velocities using Doppler ultrasound (first four weekly then fortnightly later in pregnancy –

depending on previous obstetric history) +/- fetal blood sampling if direct assessment of fetal haemoglobin was needed. These scans are typically done in tertiary units and patients frequently travel long distances to be assessed. Intensive monitoring is not necessary for RhD-negative women who are sensitised to the RhD-antigen and carrying an *RhD*-negative fetus.

The Lifeblood information sheet (Lifeblood November 2019) states that high-throughput *RhD* non-invasive prenatal testing (HT-NIPT) is a laboratory-developed test currently offered by the Lifeblood, under a contract with the NBA, for a limited number of clinical indications:

1) RhD-negative pregnant women who are Rh(D) alloimmunised.
2) RhD-negative pregnant women with obstetric indications such as severe feto-maternal haemorrhage during pregnancy, or intrauterine fetal death.
3) Other scenarios in non-sensitised RhD-negative pregnant women with a relative contraindication to routine antenatal anti-D prophylaxis, such that the fetal *RhD* genotype result assists in the risk-benefit assessment to guide anti-D management decisions (for example prior allergic reaction to RhD-immunoglobulin (RhD-Ig), or cultural/religious beliefs).

PASC noted that women with high-risk pregnancies are already funded for *RhD* NIPT under the national blood arrangements, approved by the Jurisdictional Blood Committee. However, PASC advised that potential MBS funding should not exclude this group of women, as equity must be considered, especially if current funding is time-limited.

The population requested by the applicant (pregnant women who are RhD-negative and are not sensitised to the RhD-antigen) are not eligible for testing under the current arrangements between the Lifeblood and NBA. PASC confirmed its advice that the population for the application should comprise all RhD-negative pregnant women, regardless of whether they are sensitised to the RhD-antigen or not.

Prior test (investigative services only - if prior tests are to be included)

Serological testing is routinely conducted in all pregnant women to ascertain RhD status and identification and quantitation of any antibodies detected (including anti-D antibodies) (MBS Item 65096).

Intervention

High-throughput NIPT for fetal *RhD* genotyping involves analysing cell-free fetal DNA (cffDNA) to detect fetal *RhD* DNA circulating in maternal blood. The test requires a venepuncture to be performed on a pregnant woman for the collection of 2 x 6mL dedicated anti-coagulated whole blood samples that are referred to a pathology laboratory for genetic analysis, where the plasma has to be separated from the cells within 72 hours of collection. After extracting cffDNA from the maternal plasma, a quantitative polymerase chain reaction (qPCR) assay is used to amplify the *RhD* gene. The time to complete the test from sample receipt to report generation is 5 to 6 hours.

The application refers to *RhD* NIPT as a 'high-throughput' test. No specific definition for 'high-throughput' was provided. A recent systematic review and meta-analysis (Saramago 2018a) was conducted to inform NICE on high-throughput NIPT cell-free fetal DNA tests of maternal plasma used to determine fetal *RhD* status. The authors noted that 'high-throughput' is a subjective concept and there is no clear consensus on its definition. For pragmatic reasons the authors considered any NIPT tests which were conducted using an automated robotic platform (including automated DNA

extraction and liquid handling) that were able to process large numbers of samples rapidly for large scale screening purposes, as high-throughput. PASC confirmed the intervention is HT-NIPT of cffDNA by PCR.

The applicant's clinical experts indicated that the platforms used in Australian laboratories would likely vary across laboratories where a variety of techniques could be used including: quantitative real time polymerase chain reaction (qRTPCR), qRT digital PCR, array based platforms and sequencing. The experts also noted that there are no Therapeutic Goods Administration (TGA) licenced tests currently available, so this would require laboratories to validate and register their test as an in-house *in-vitro* diagnostic (IVD). PASC noted that most current *RhD* testing is centralised through the Lifeblood. PASC advised it would be appropriate to maintain this (because some women would already be in the system, and the Lifeblood laboratory provides proven, robust test results.

The application does not specify details regarding the nature of the DNA amplification, however it is noted that this varies in the literature. Some studies cited by the application report amplification of exons 4, 5 and 10 of the *RhD* gene, while others limit this to exons 7 and 10 or 10 alone. Many of the studies also report that testing is conducted in replicates (up to quadruplicate). The studies cited by the application also varied with respect to how the results were interpreted (e.g., all replicates need to be negative to be considered negative versus 'x' of 'y' replicates need to be negative to be considered negative). Expert advice provided by the applicant's clinical experts indicated that the nature of the DNA amplification would be variable depending on the specific test each laboratory employs and validates, but that the tests should be run, at a minimum, in duplicate.

Given the RhD-negative blood type is largely associated with a deletion of the *RhD* gene, no PCR product is amplified in those who are *RhD*-negative. As no product is amplified, it is not possible to be certain whether: (i) the fetus is *RhD*-negative; (ii) there was sufficient cffDNA present in the sample to allow for detection; or (iii) there was a failure of the PCR reaction.

Amplification of the male-associated *SRY* gene can serve as an internal control for the presence of fetal DNA, and a chemokine receptor gene, *CCR5*, can serve as a measure of sample integrity. In the event that the *RhD* gene is not detected from the sample, the *SRY* gene (a Y-chromosome specific gene) is useful in male fetuses to ensure the samples contain cffDNA. If the *SRY* gene is not detected a supplemental quantitative polymerase chain reaction (qPCR) assay for hypermethylated *RASSF1A* can be used to confirm the presence of fetal DNA sequences in the plasma DNA sample. *RASSF1A* can be used to distinguish between maternal and fetal DNA as *RASSF1A* is hypermethylated in the placenta but hypomethylated in adult tissues. Expert advice indicated that the testing of hypermethylated *RASSF1A* is a complex and expensive test so is generally not included in high-throughput tests implemented on a national level.

Although there are potential internal controls that can be used to check for the quantity and integrity of cfDNA, the expert also indicated that many high-throughput screening programs do not use any control for the presence of fetal DNA, there is often no further testing beyond the *RhD* exons and if *RhD* is not detected the result is called negative. This is in recognition that if the test is done beyond a certain point in gestation, using a test method with very high sensitivity, that the false negative rate is very low (acceptably low).

The Lifeblood information sheet (Lifeblood November 2019) states that the results are interpreted as follows (remembering that the Lifeblood currently only performs *RhD*-NIPT for certain clinical indications):

- Positive results for all three exons (4, 5 and 10) are interpreted as *RhD* detected predicting the fetus is RhD-positive.
- Negative results for all three exons (4, 5 and 10) with either the *SRY* gene positive or the *RASSF1A* hypermethylated are interpreted as *RhD* NOT detected predicting the fetus is RhD-negative. A follow-up sample will be requested on all predicted RhD-negative fetuses to confirm these results.
- All other result combinations will be reported as inconclusive and further samples requested.

The Lifeblood (November 2019) indicates that a follow-up sample is requested on (i) all predicted *RhD*-negative fetuses to confirm the results and (ii) inconclusive results (*RhD*-negative and *SRY*-negative and no *RASSF1A* hypermethylated). Expert advice indicated that for high-throughput national screening programs, often a repeat test is not requested – if *RhD* is not detected they are just reported as *RhD* not detected, and no repeat sample is requested. The experts also indicated that if this testing is offered by multiple laboratories, then the individual laboratories would have to determine their protocols for testing / reporting / requesting repeat samples.

While *RhD*-NIPT should be conducted prior to 28 weeks' gestation (the time at which the first anti-D immunoglobulin dose is administered), there is no apparent consensus about the best timing for *RhD*-NIPT that would maximise diagnostic accuracy, which may vary according to different gestational ages at the time of sampling (NICE 2016). Although concentrations of cffDNA in maternal blood increase throughout pregnancy, suggesting that tests will not be as accurate early in pregnancy as they are at 26-28 weeks' gestation, the application states (p17) two meta-analyses found that the diagnostic accuracy of *RhD*-NIPT was higher in the first trimester than in the second and third. However, one subsequent cohort study in 2288 women, generating 4913 assessable fetal results found that fetal *RhD* genotyping was more accurate for the prediction of *RhD* status if it was performed after, rather than before, 11 weeks' gestation (Chitty 2014). Similarly, analysis of the data collected in the systematic review by Saramago (2018a) suggested that high-throughput NIPT was less accurate before around 11 weeks' gestation (i.e. in first trimester), but diagnostic accuracy was consistent at any time after 11 weeks' gestation.

This is consistent with the Lifeblood statement, which indicates that "the gestational age must be at least 12 weeks. The concentration of fetal DNA in the mother's blood increases with the progression of the pregnancy. Any sample collected before 12 weeks gestation can lead to inconclusive results and will not be tested." (Lifeblood, November 2019). Expert advice obtained in the process of preparing the PICO Confirmation was that there is sufficient cffDNA in maternal blood for conducting *RhD*-NIPT from 9 weeks' gestation. The experts also indicated that for compliance, it would be sensible for sample collection for *RhD*-NIPT to align with another routine obstetric test or visit. PASC noted that the earliest NIPT could be performed to detect fetal DNA in the maternal circulation is 6–8 weeks' gestation. However, PASC noted the clinical expert's advice that, while testing can be done as early as 6-8 weeks' gestation, 11 weeks' gestation is the optimal timing for this test. The Department has advised that test timing is one of the issues being considered in the NBA's systematic review of Rhesus D Guidelines.

Although there is no consensus regarding the minimum gestational age to conduct the test, an expert suggested that testing after 34 weeks of gestation would not be of any real benefit. This is because a dose of anti-D at ≥34 weeks should last through to delivery, unless women had an obstetric indication like recurrent placental bleeding, intrauterine fetal death or massive feto-maternal haemorrhage where the NIPT for *RhD* result could guide whether additional doses of anti-D were necessary.

The application indicated that *RhD*-NIPT is a "Once off diagnostic test for each pregnancy of an RhD-negative woman with the possibility of repeat testing in some instances where results are **inconclusive**". Based on expert advice, "inconclusive" here refers to instances where there is no reliable reproduction of results across replicates, rather than uncertainty regarding sufficient cffDNA quantity or integrity (given testing will likely not extend beyond *RhD* exons, see above). The application also suggests that women with an inconclusive result are usually treated as if positive and are administered anti-D prophylaxis. This practice is likely due to the results of diagnostic accuracy studies which suggest that the probability of an RhD-positive baby is higher among women in whom the *RhD*-NIPT is inconclusive (70.7%) compared with the probability across all RhD-negative women (Saramago 2018a).

The application indicated that TGA approval is not required for *RhD*-NIPT to be rolled out to all RhD-negative pregnant women. Expert advice suggested that as there are currently no commercially available tests registered with TGA as IVDs for this purpose, each laboratory would have to validate and register its own assay with TGA as an in-house IVD regardless of their experience in testing DNA. Each laboratory will be required to assess the assay against the Australian classification rules for IVD's described by TGA. The classification rules are based on a risk based approach to regulation, and IVDs are classified according to the health risk (either to the public or an individual) that may arise from an incorrect result. The expert noted that this test would be either class 3 or class 4.PASC noted there are currently no TGA–approved commercial tests available for *RhD* NIPT.

Healthcare resource consequences

The *RhD*-NIPT result would be reported to the treating medical practitioner/obstetrician who would advise the patient of the result and whether or not anti-D prophylaxis should be administered. The results of the test would allow a targeted administration of anti-D immunoglobulin, which is unnecessary with an *RhD*-negative fetus.

Comparator

PASC confirmed the comparator is no testing for the *RhD* genotype of the fetus.

Healthcare resource consequences

Following routine serological testing of all pregnant women for RhD status and presence of anti-D antibodies (MBS 65096), the current standard of care is routine administration anti-D immunoglobulin prophylaxis (dose 625 IU) to all RhD-negative and anti-RhD antibody-negative pregnant women at 28 and 34 weeks' gestation, or following other obstetric events associated with a risk of fetal-to-maternal haemorrhage (e.g. external cephalic version, late miscarriages and abdominal trauma). Currently, cord blood is taken at the time of delivery to serologically determine the baby's RhD status for all births from RhD-negative mothers and postpartum anti-RhD is

administered within 72 hours (usually 625 IU, but could be adjusted depending on the results of feto-maternal haemorrhage test (FMH) only if the baby is RhD-positive (RANZCOG Guidelines 2015).

In certain clinical situations all RhD-negative and antibody-negative women should be offered anti-D (dose 250 IU) during the first trimester, these include:

- chorionic villus sampling;
- miscarriage;
- termination of pregnancy (either medical or surgical);
- ectopic pregnancy.

Table 2 in the Appendix summarises Rh(D) immunoglobulin dosage recommendations for Rh(D)-negative women (Lifeblood FAQs). Women should be appropriately informed about all risks and benefits of treatment with anti-D so they are able to give an informed consent. It has been reported that the fetal *RhD* test acceptance exceeds 95% (Health PACT 2017, citing private correspondence). This is consistent with the observed compliance rates with antenatal anti-D prophylaxis estimated in the range of 86% to 96.1% (four studies; n=23,993 women approximately) and compliance rates with postpartum anti-D estimated in the range of 92% to 99.7% (three studies; n=18,889 women approximately) in women who undertook NIPT and the results were *RhD*-positive (Saramago 2018a).

The introduction of anti-RhD immunoglobulin prophylaxis for RhD-negative mothers has successfully decreased rates of maternal alloimmunisation from 10–15% to 0.8–1.5% after initial postpartum use in the 1970s, with a further reduction to 0.18–0.35% with routine antenatal anti-RhD prophylaxis (Allard 2018). This led to a decrease in mortality associated with HDFN from 46 in 100,000 births before 1969 to 1.6 in 100,000 births by 1991 (Saramago 2018a).

Anti-D immunoglobulin products available in Australia are manufactured from the domestically donated and imported plasma by:

- CSL Behring, Australia (plasma derived - domestic) ($29.79 for 250 IU and $74.44 for 625 IU); (NBA 2018)[3];
- Rhophylac[4] (plasma derived- imported) ($411.22 for 1500 IU) (NBA 2018).

[3] The price does not include the starting plasma provided to CSL Behring (Australia) Pty Ltd by the Australian Red Cross Lifeblood (Lifeblood report) or costs associated with distribution, storage or administration.
[4] The only product that can be delivered both intramuscularly and intravenously.

Reference standard

PASC confirmed the reference standard was RhD status, assessed by:

- amniocentesis;
- chorionic villus sampling; or
- cord blood sampling after birth.

Outcomes

PASC confirmed the following patient-related outcomes:

- Safety
  - incidence of injection site adverse effects (e.g. infections and reactions)
  - systemic adverse effects (e.g. infections and reactions) from anti-D Ig

- Assessment of diagnostic accuracy
  - analytical sensitivity and specificity
  - positive and negative predictive values
  - rate of fetal RhD true negatives identified
  - false-negative rates, indicating the proportion of women at risk of sensitisation
  - rate of fetal RhD true positives identified
  - false positive rates, indicating the proportion of women who will receive anti-D Ig unnecessarily
  - rate of uninterpretable/inconclusive tests with reasons (e.g. insufficient DNA, *RhD* variant).

PASC confirmed the following healthcare system, resource and cost outcomes:

- Healthcare resources
  - cost of testing (capital outlay for setting up HT-NIPT + medical procedure + laboratory investigation)
  - cost of anti-D Ig
  - cost of future immunised pregnancies
  - cost of HDFN pregnancies

- Healthcare resource consequences
  - RhD-negative pregnant women who test *RhD*-positive for the fetus will be administered targeted anti-D Ig only as prophylaxis against HDFN
  - RhD-negative pregnant women who test *RhD*-negative for the fetus will not be administered anti-D Ig
  - RhD-negative pregnant women (who have not developed anti-D antibodies) who do not receive the *RhD*-NIPT test would be administered standard of care (non-targeted) anti-D Ig

- Compliance outcomes
  - rate of RhD-negative pregnant women (who are not known to be sensitised to the RhD-antigen) who accepted NIPT
  - rate of uptake of anti-D Ig (antenatal and postnatal) in women identified as RhD-negative with fetuses identified as *RhD*-positive
  - number of doses per woman of anti-D Ig given (routine antenatal, following potentially sensitising events and postnatal)

- Intermediate outcomes
  - rate of RhD immunisation (sensitisation) occurring due to formation of anti-D antibodies in RhD-negative pregnant women due to false negative *RhD* results of the test
  - the estimated absolute number of women per year for whom sensitisation occurs as a result of false negative *RhD* results with *RhD* NIPT
  - need for serial ultrasound assessment for fetal well-being and treatment of an affected fetus with in utero blood transfusion, in such an isoimmunised pregnancy
  - risk of premature delivery, including from in utero blood transfusion, in such an isoimmunised pregnancy
  - non-compliance to undertake *RhD*-NIPT and/or anti-D (antenatal or postnatal) Ig

- Final patient outcomes
  - number of HDFN cases in subsequent pregnancies (per 1000 live births), including the excess number of HDFN occurring as a result of false negative *RhD* results with *RhD* NIPT

- Cost-effectiveness
  - test – incremental cost per fetal RhD true negative status detected
  - intermediate – incremental cost per alloimmunisation avoided
  - final – incremental cost per HDFN avoided

- Total Australian Government healthcare costs:
  - total cost to the MBS.

Rationale

A systematic review (Saramago 2018a) identified eight studies (n=54,477 women approximately) on the diagnostic accuracy of high-throughput NIPT. These were conducted in five European countries. There were three high-quality studies in which NIPT was performed by the UK NHS Blood and Transplant International Blood Group Reference Laboratory (Bristol, UK). The reference standard in all studies was cord blood serology at birth. The majority of the studies were assessed as having a low risk of bias. Two studies were judged as having a high risk of bias. Meta-analyses included women mostly at or post 11 weeks' gestation and showed very high diagnostic accuracy of high-throughput NIPT. In the primary analyses, where women with inconclusive/uninterpretable test results were treated as having tested positive, the pooled false negative rate (i.e. women at risk of sensitisation) was 0.34% (95% CI: 0.15%, 0.76%) and the pooled false positive rate (i.e. women receiving anti-D unnecessarily) was 3.86% (95% CI: 2.54%, 5.82%).

The NICE review committee noted that although the *RhD*-NIPT test has demonstrated a high degree of sensitivity, the rates of false negative results ranged from 0.21% to 0.38% (Table 1 of NICE 2016). The committee also noted that, based exclusively on the [high quality] UK data there was a small increase in the false-negative rate for high-throughput NIPT to determine fetal *RhD* genotype (0.21%; 95% CI: 0.09, 0.48) compared with postpartum cord blood typing. This means that some women with an *RhD*-positive fetus would be incorrectly identified as having an *RhD*-negative fetus and would not be offered routine antenatal anti-D prophylaxis or anti-D immunoglobulin after potentially sensitising events.

The applicability of the results from meta-analysis (Saramago 2018a) to Australian practice is uncertain with respect to both (i) the algorithm for PCR (some studies targeted two rather than three exons, generally exons 5 and 7) and (ii) the type of robotic platforms that varied across the studies. Also, in the primary analysis inconclusive/uninterpretable test results were treated as having tested positive rather than adjusting the final results of the test using a repeated sample. The sensitivity analysis excluded inconclusive/uninterpretable test results from the estimates of diagnostic accuracy, which did not affect the rate of false negatives but significantly reduced the rate of false positive results.

Citing the results of Saramago (2018a), it appears that "around 0.3 to 0.4 per cent of women undergoing NIPT will have a false negative test, which would mean three or four per 1,000 women with an *RhD*-positive fetus would not receive antenatal anti-D when they potentially need it, leaving them at risk of sensitisation. This is on the significantly higher background rate of around 0.2% of women who become sensitised even with full and appropriate anti-D prophylaxis. At the same time, around four per cent of a false positive NIPT would indicate that around one in 25 women carrying an *RhD*-negative fetus would receive unnecessary anti-D, compared to almost all women in current clinical practice (Health PACT 2017).

There is a possibility for the health care system to save on the cost of unnecessary administration of anti-D immunoglobulin, however savings come at the increased risk of sensitisation in RhD-negative women whose fetuses were falsely identified as *RhD*-negative. The comparator (existing) test does not have perfect sensitivity and specificity as there are also diagnostic errors in serologic testing of both the mother's RhD status and the newborn RhD status on cord blood testing. The specificity of the existing test methodology is lower than for NIPT for RhD, which impacts the interpretation and management depending on which methodology is methodology used. In comparison to universal administration of anti-D immunoglobulin to all *RhD*-negative women in current practice, this would translate into additional health care resources for anti-D sensitised pregnancies due to the small proportion of false negative NIPT results, along with cost of short- and long-term treatment of surviving babies with HDFN. The magnitude of this increase should be compared to the known failure rate of appropriate prophylaxis, and the rate of incomplete compliance with universal prophylaxis. A modelled economic evaluation would establish a break-even price (threshold) of *RhD*-NIPT that would just offset the additional costs and below which there is cost-saving to the health care system. The amount of cost-saving would also depend on whether cord blood testing is continued (e.g. in women with a negative, absent or inconclusive NIPT result) as the basis for administering postpartum anti-D (Saramago 2018a). (*See economic evaluation section below*).

Current clinical management algorithm for identified population

Figure 1 shows the current care pathway for the management of pregnant women on the basis of RhD and anti-D antibody status. This differs to the algorithm presented in the application, with the purpose of providing more detailed information. Two sources were utilised in designing the algorithm:

- RhD negative pregnant women & RhD immunoglobulin-VF: anti-D prophylaxis pathway in the community (Peninsula health, Victoria) Anti-D Prophylaxis Pathway in the community
- Rhesus (D) status in pregnant women: Care Pathway (NSW Health) (reproduced in the application)

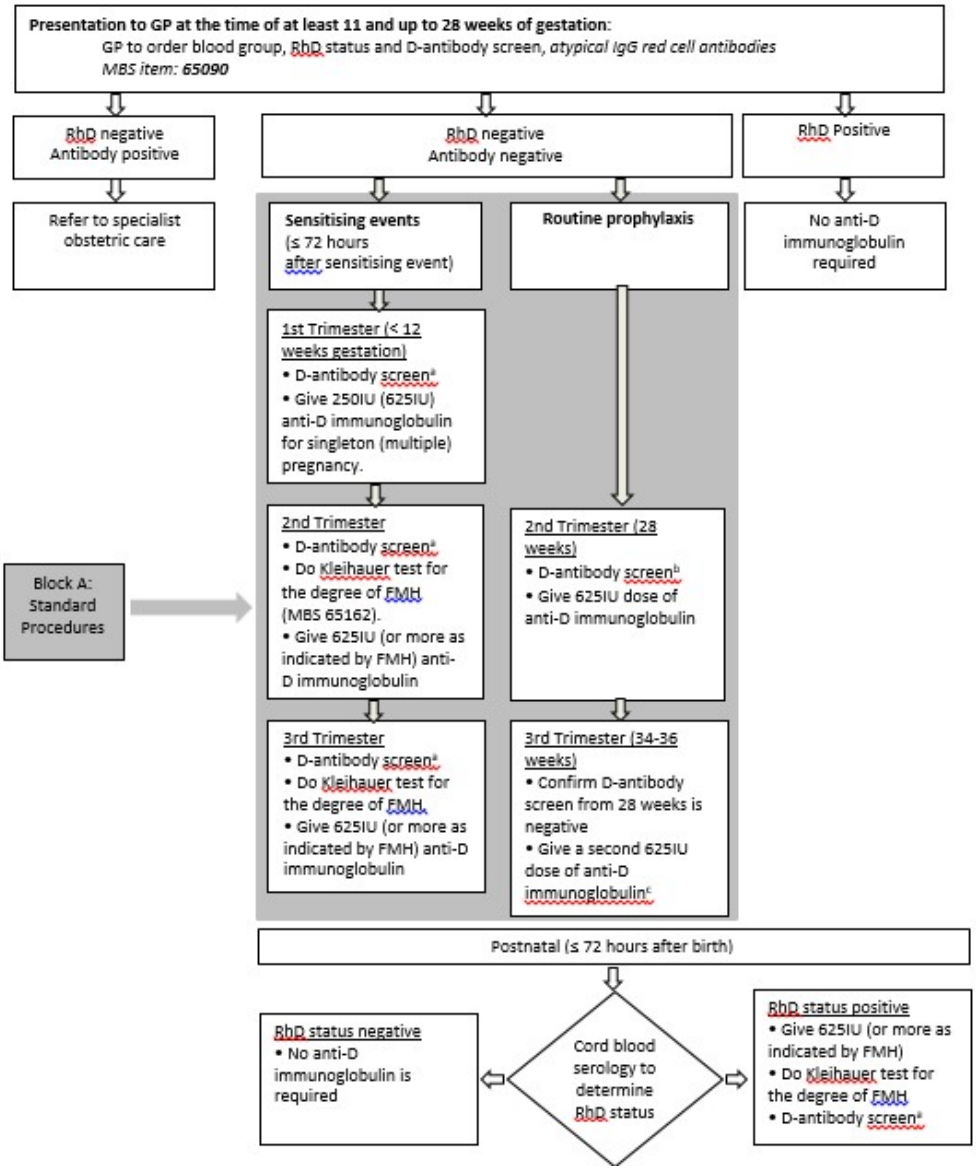

Figure 1: Current clinical management algorithm

[a] positive D-antibody screen leads to further investigation of red cell antibodies and possible referral to obstetrician.

[b] It is not necessary to wait for the D-antibody screen results to give anti-D immunoglobulin.

[c] New D-antibody screen test is not required prior to the second dose of anti-D immunoglobulin.

Proposed clinical management algorithm for identified population

Figure 2 shows the proposed clinical management algorithm with *RhD*-NIPT conducted on all women identified as RhD-negative and anti-D antibody negative (Source: application). Upon receiving results of the test, anti-D Immunoglobulin is administered as prophylaxis (as well as in case of a sensitising event) only to women whose fetus is identified as *RhD*-positive. Expert advice indicated that if the test results are either non-reportable or inconclusive, the *RhD* status of the fetus is treated as positive and women follow the standard procedure for sensitising event and prophylaxis as depicted in Block A of Figure 1.

Cord blood serology is currently conducted to determine the RhD status of a baby and used to determine whether administration of postpartum anti-D immunoglobulin is necessary (Figure 1), the function of the cord blood serology in the proposed care pathway is to additionally validate the *RhD*-NIPT results, in particular to identify the rate of false negatives.

In the Netherlands, cord blood serology for RhD typing to guide post-partum anti-D continued for about 2 years after the introduction of their national non-invasive fetal *RhD* testing and targeted anti-D program until analysis of the false negative rates of the screening program provided a high degree of confidence in the outcomes. However, the applicant's expert warned that a similar nation-wide program of monitoring false negatives in Australia will be almost impossible if different laboratories have different testing assays, and there is no central haemo-vigilance reporting system to collate reported cases of false negatives.

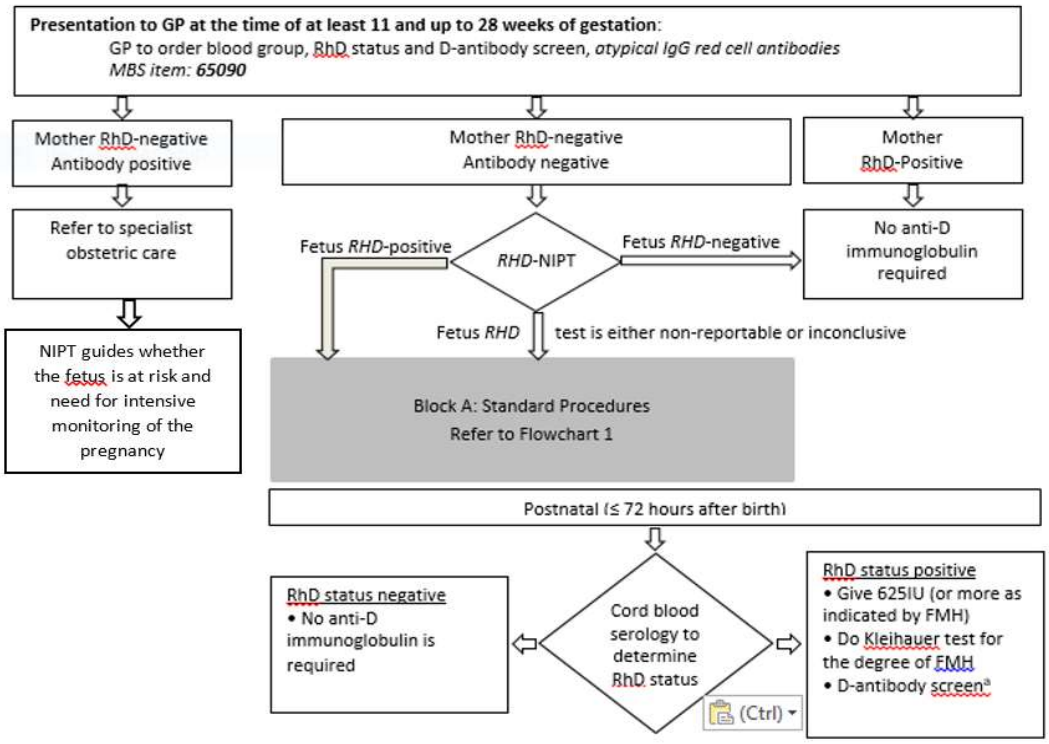

Figure 2: Proposed clinical management algorithm

[a] positive D-antibody screen leads to further investigation of red cell antibodies and referral to obstetrician

Proposed economic evaluation

PASC noted the claim of superiority over the comparator (current scenario):

- unnecessary use of anti-D Ig avoided
- potentially safer
- potentially more cost-effective.

PASC confirmed the appropriate economic evaluation would be a cost-effectiveness or cost-utility analysis.

The appropriate type of economic evaluation for test specific outcomes (sensitivity, specificity, and negative and positive predictive values) of NIPT for fetal *RhD* status would be a cost-effectiveness analysis that would estimate an incremental cost per true *RhD*-negative fetus detected (taking into account the rates of indeterminate results, false negatives and false positives).

With respect to the patient outcomes, the cost-effectiveness analyses estimating incremental cost per avoided incident of alloimmunisation and HDFN would likely result in the comparator dominating *RhD*-NIPT as current practice applies a universal administration of anti-D prophylaxis, while the proposed test of fetal *RhD* is associated with extra costs and a small, but not negligible, rate of false negatives. Cost-offsets favouring the proposed test include (i) reduction in use of anti-D, (ii) reduction in risk associated with blood-product infections, but these costs are reportedly zero since there were no recent infections reported in the developed world since 1999 (NBA 2003), (iii) reduction in the number of injection site reactions and (iv) costs associated with attending the appointments and the administration of the product. Given the possibility of false-negatives with *RhD*-NIPT, a proportion of undetected *RhD*-positive fetuses may result in alloimunisation of the mother, with the potential for subsequent *RhD*-positive fetuses to develop HDFN, which will result in additional costs to the health care system in comparison to the application of a universal anti-D prophylaxis to all RhD-negative pregnant women. In one published cost-effectiveness analysis, the risk of immunisation in an RhD-negative woman with an *RhD*-positive fetus, where the screening test was false negative and no anti-D prophylaxis was received antenatally or after delivery, was set to 15% (Neovius 2015, Bowman 1983). This analysis also included the consequences of HDFN in terms of mild or severe disability of the surviving child or his/her death. The corresponding costs varied with the degree of disability. However, as noted by the PASC above (page 5), the likelihood of sensitisation in an at-risk pregnancy (RhD-negative women with RhD-positive foetuses), who only receive postpartum RhD-Ig is about 2%.

To account for the long-term consequences of alloimmunisation a cost-utility analysis could be considered. It would combine a decision tree model for the nine months of a gestational period with a Markov extension covering future pregnancies. An example of such a model was produced for the UK (NICE 2008) with utility values reflecting the loss of quality-adjusted life years (QALYs) from the parental perspective. Death of a child was arbitrarily assumed to be equal to 10 QALYs, however the disutility of caring for a child with a disability was not included in the model. The advantage of a cost-utility analysis would be in estimating the comparative costs and benefits of *RhD*-NIPT over a longer time horizon and whether the corresponding ICER fell under the accepted value-for-money threshold (in the UK, it is currently at £20K to £30K per QALY gained). However, in comparison to cost-effectiveness analyses would introduce additional sources of uncertainty associated with the

choice of utility values, distribution of HDFN by the degree of severity and the associated cost of care accumulated over the chosen time horizon.

Most of the published economic evaluations of high-throughput NIPT for determining fetal *RhD* status are short-term cost minimisation analyses conducted from health-services' perspective (Saramago 2018a) including an example for the Australian health care system (Gordon 2017). This study tested a hypothesis of whether *RhD*-NIPT would deliver savings to the Australian health system from a reduction in unnecessary use of anti-D administered to RhD-negative pregnant women carrying an *RhD*-negative fetus. Another cost-offset comes from reduction in the number of referrals to reference laboratories to elucidate whether the antibody detected is passive or immune, since the proportion of RhD-negative women receiving the anti-D prophylaxis would be limited to RhD-negative women with *RhD*-positive fetuses. The additional benefits were reducing the potential reliance on an overseas source for anti-D, and reduction of risk of infection through the use of blood products. Results of cost-minimisation analyses are expressed in terms of the incremental costs (savings) to health care system due to reduction in anti-D immunoglobulin use. Results of these publications are shown to be sensitive to a number of factors, including the cost of the test. A threshold analysis estimating the price per *RhD*-NIPT corresponding to the break-even point where additional costs are equal to savings is a necessary component of cost-minimisation analyses conducted from the health system perspective.

All types of economic evaluation would be able to estimate costs with respect to the following health-related outcomes:

- Cost per alloimmunisation; and
- Cost per HDFN.

Sensitivity analysis (discussion)

The prevalence of RhD-negative pregnant women in the total Australian population of pregnant women is potentially a variable factor that should be subjected to the sensitivity analysis in the modelled economic evaluations. As the ethnic composition of Australian population changes, so would the future demand for fetal *RhD* genotyping.

Since administration of anti-D immunoglobulin is required after any potentially sensitising event, including those occurring earlier than those received during a routine anti-D prophylaxis regimen (currently recommended from 28 weeks), implementation of fetal *RhD* testing earlier in pregnancy (e.g. at 9 weeks rather than the currently required 12 weeks) would be associated with some reduction in demand for anti-D immunoglobulin. Scenario analyses varying the gestational age at which *RhD*-NIPT is performed would show how sensitive economic evaluation outcomes are to the variations in this parameter.

Another area of uncertainty relates to the current fee associated with *RhD*-NIPT conducted by the Lifeblood. In particular, whether expanding NIPT to a larger population for fetal *RhD* status screening would lower the cost of testing despite the possible additional capital outlay and staff training.

The only published estimate of a cost per *RhD*-NIPT is $45.58, reported in the recent cost-effectiveness analysis conducted from the Australian health care perspective (Gordon, 2017). All laboratory equipment, consumables, and setup costs for the estimated 46,000 high-throughput NIPTs were estimated from records at the Lifeblood's testing laboratory. In addition, costs of RhD

donor program management were estimated at $20.64 per vial for anti-D production. The estimate seems to relate to the expected throughput if *RhD*-NIPT becomes a routine screening test but does not include the opportunity cost of donated plasma, therefore does not reflect the true value of this resource. By not including the opportunity cost of donated plasma, the economic and financial calculations treat this resource as an infinite and available at zero production cost, an assumption which would not withstand elementary theoretical scrutiny. It also appears incongruent to the admission that in Australia, anti-D immunoglobulin is manufactured from plasma collected from a small and shrinking pool (less than 200) of RhD-negative male plasma donors who have had a transfusion of RhD-positive red cells to stimulate the production of anti-D antibodies.

It is outside the scope of this PICO confirmation to estimate the risks to the blood/plasma donors (who are exposed to a risk of anaphylaxis) and sustainability of producing anti-D immunoglobulin from finite resource as indicated by the shortages in the past (Dean, 2000). However, it is likely that the current price (per IU) of domestically produced anti-D immunoglobulin does not correspond to the real value (utility) of the product, if assessed from the societal, or even the health system perspective. One way of addressing this concern is to subject the current Australian prices for anti-D immunoglobulin to sensitivity analysis using prices for internationally sourced product.

Proposed item descriptor

The proposed item descriptor presented in the application is provided below. PASC accepted the proposed item descriptor.

Category 6 (Group P7 Genetics) - Pathology Services
Non-invasive prenatal testing of blood from a Rhesus D negative pregnant woman for the detection of Rhesus D fetal DNA circulating in maternal blood. Fee: To be determined

PASC noted the proposed fee for earlier (different) Application 1492 (NIPT for common trisomies) was $500. For that application, MSAC considered $400 was more appropriate, because the cost of NIPT has decreased (and this trend is expected to continue, particularly if MBS listing increases uptake of these tests). PASC noted other real-time tests currently cost between $36 and $260, depending on test complexity. PASC recommended that costings from the Lifeblood and National Blood Authority (NBA) should guide appropriate fees.

Consultation feedback

PASC noted that Lifeblood and the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (ANZCOG) were both supportive of the application. However, ANZCOG advised that:

MSAC should consider the estimated rate of false negative results leading to Rh negative women at risk missing out on prophylactic anti-D. The Danish national experience of [false negatives] was 0.087% and false positives of 0.32% (1 in 300) which they considered acceptable (Clausen 2014). Other studies observed rates of 0.1–0.2%.

Other issues

PASC noted the NBA Guidelines for administration of anti-D Ig are currently under review; specifically, anti-D Ig treatment as a single or double dose, as well as the sensitising event for prophylaxis. PASC considered it would be appropriate to wait at least until release of the draft guidelines in July 2019 before proceeding with the Evaluation Sub-Committee assessment. Variables in the guidelines are pivotal to the HTA evaluation.

PASC noted that most current *RhD* testing is centralised through the Lifeblood). PASC advised it would be appropriate to maintain this (because some women would already be in the system, and the Lifeblood laboratory provides proven, robust test results).

Depending on outcomes of the Guidelines review, the final service model should maintain the current high sensitivity and specificity of test results, which a central (as opposed to dispersed) model may facilitate. The Lifeblood has stated it could rapidly accommodate increased testing required for routine NIPT *RhD* of all RhD negative pregnant women. Access could be a problem (in a central model) if the central laboratory could not guarantee a rapid turnaround of test results.

Summary of discussion

PASC accepted the proposed PICO, as detailed above. PASC reinforced that the population NOT be limited to non-sensitised women, as access equity was important.

PASC discussed the false-negative rates of NIPT (which might vary across laboratories). PASC concluded that false negative *RhD* NIPT results may lead to excess cases of sensitisation and HDFN, and the likelihood of these should be clarified.

PASC stated the current NBA Guidelines review is highly relevant to this application. Review outcomes could affect the application, so PASC recommended application 1574 be put on hold until outcomes from the review are released.

Next steps

PASC recommended the application be put on hold until the NBA releases its interim outcomes from its Guidelines review. Following this and the Departmental review of the Draft guidelines for the *Prophylactic used of Rh D immunoglobulin in maternity care*[5]*,* it was confirmed with PASC that the PICO including clinical management algorithms sufficiently aligned with the NBA's Expert Reference Group (ERG) recommendations within these guidelines, and thus the application can progress to a DCAR (Department-contracted assessment report). *Please note: DCAR is the new name for a 'contracted assessment' (CA).*

[5] Public consultation draft guideline. Prophylactic use of Rh D immunoglobulin in maternity care. National Blood Authority, 2019

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APPENDIX

Table 2: Rh(D) immunoglobulin dosage recommendations for Rh(D)-negative women

Rh(D) immunoglobulin dosage recommendations for Rh(D) negative women	Dose
Obstetric conditions	
Sensitising events in the first trimester (up to and including 12 weeks gestation) for every Rh(D) negative woman with no preformed anti-D. Including: • miscarriage; • termination of pregnancy; • ectopic pregnancy; • chorionic villus sampling; and • hydatidiform mole	**250 IU (50 µg)**
Sensitising events beyond the first trimester for every Rh(D) negative woman with no preformed anti-D. Including: • chorionic villus sampling, amniocentesis, cordocentesis and fetoscopy; • abdominal trauma considered sufficient to cause FMH; • each occasion of revealed or concealed antepartum haemorrhage (where the patient suffers unexplained uterine pain, the possibility of concealed antepartum haemorrhage should be considered, with a view to immunoprophylaxis); • external cephalic version (performed or attempted); and • miscarriage or termination of pregnancy.	**625 IU (125 µg)**
Pregnancy	
Antenatal prophylaxis (at 28 and 34 weeks[1]) for all Rh(D) negative women (Primigravid and Multigravid)	**625 IU (125 µg)**
Postpartum	
For every Rh(D) negative woman following delivery of an Rh(D) positive baby. Rh(D) immunoglobulin should not be given to women with preformed anti-D, except where the preformed anti-D is due to the antenatal administration of Rh(D) immunoglobulin. If it is unclear whether the anti-D detected in the mother's blood is passive or preformed, the treating clinician should be consulted. If there is continuing doubt, Rh(D) immunoglobulin should be administered. The magnitude of the FMH should be assessed by a method capable of quantifying a haemorrhage of ≥6 mL of fetal red cells. Further doses should be administered sufficient to prevent maternal immunisation.	**625 IU (125 µg)**