Australian Red Cross Lifeblood

Non-Invasive Prenatal Analysis (NIPA) for RhD

November 2019 Version 2.0

What is NIPA?

Non-Invasive Prenatal Analysis (NIPA) for fetal RHD is a molecular blood group genotyping assay used to predict the RhD status of the fetus in pregnancies where the mother is RhD negative and the fetus is at risk of being affected by Haemolytic Disease of the Fetus and Newborn (HDFN) due to anti-D. NIPA utilises a maternal peripheral whole blood sample for the extraction of cell-free fetal DNA (cffDNA), which is analysed for the presence of the RHD gene.

This technique replaces the requirement for invasive direct sampling methods for fetal DNA, such as amniocentesis or chorionic villus sampling (CVS) sampling.

What do we test for?

After extracting cffDNA from the maternal plasma, a quantitative Polymerase Chain Reaction (qPCR) assay is used to amplify exons 4, 5 and 10 of the RHD gene in quadruplicate. The combination of these exons ensures that fetuses with RhD variant genes are not missed and called RhD negative. The assay also amplifies the male-associated SRY gene, and a chemokine receptor gene, CCR5, which serve as internal controls for the presence of fetal DNA, and 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 our samples contain cffDNA. If the SRY gene is not detected a supplemental qPCR assay for hypermethylated RASSF1A is used to confirm the presence of fetal DNA sequences in the plasma DNA sample. In the placenta RASSF1A is hypermethylated but in adult tissues RASSF1A is hypomethylated allowing us to distinguish between fetal and maternal derived RASSF1A.

Sensitivity and specificity

The NIPA for fetal RHD assay has demonstrated specificity of >98% and a sensitivity of >99%.

A proportion of samples will be reported as inconclusive, as explained below, and a repeat sample will be requested

How are the results interpreted?

- 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.

In a small number of seemingly RhD negative pregnant women the results with the three exons (4, 5 and 10) will indicate a possible maternal RHD variant gene and will be further investigated with the RHD BeadChip test. We are unable to predict the fetal RhD status when the mother is a weak or variant D type.

How early in the pregnancy can samples be tested?

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.

Multiple Pregnancy?

We are unable to test dizygotic (fraternal) twins as it is not possible to confirm that DNA from both twins has been tested.

Clinical Indications

The Australian Red Cross Lifeblood currently offers NIPA for RHD for the following clinical indications:

1. RhD negative pregnant women who are RhD alloimmunised
2. RhD negative pregnant women with obstetric indications such as severe fetomaternal 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-Ig, or cultural/religious beliefs)

Sample Requirements

- A minimum of 2 x 6mL dedicated EDTA whole blood samples.
- Samples must be received and processed by the Red Cell Reference Laboratory within 72 hours of collection.
- Samples should be stored at 2-8°C.
- Samples must be labelled with a minimum of 2 identifiers (prefer at least 3)
- Samples must be provided with completed request form with a minimum of 3 identifiers and include the tests requested, referring organisation's contact details and any relevant clinical information.
- The identifiers on the sample must match the request form exactly.

Note: Identifiers must include full name and at least one of either date of birth or unique medical record number.

Samples should be sent packaged with a cold ice-brick to the QLD Red Cell Reference Laboratory, via the address below as soon as possible following collection.

QLD Red Cell Reference Laboratory
Australian Red Cross Lifeblood
44 Musk Avenue (delivery via Blamey Street)
Kelvin Grove, Queensland AUSTRALIA 4059

Phone:+61 7 3838 9493 Fax: +61 7 3838 9410

Reporting

It is expected that results will be reported within 5 working days from receipt of the sample.
A report containing predicted RhD status of the fetus will be provided by email via Secure Send only to the referring laboratory.

Enquiries

Please direct all enquiries to the Red Cell Reference Laboratory on +61 7 3838 9493.