NIPA for Clinically Significant Fetal Antigens

Updated July 2024 Version 4.0

What is NIPA?

Non-Invasive Prenatal Analysis (NIPA) for Clinically Significant Fetal Antigens is a molecular blood group genotyping assay used to predict the blood group phenotype of the fetus. It is performed for cases where the mother is antigen negative and has a corresponding allo-antibody placing the fetus at risk of being affected by Haemolytic Disease of the Fetus and Newborn (HDFN). NIPA utilises maternal peripheral whole blood EDTA samples. Cell-free DNA (cfDNA), which contains cell-free fetal DNA (cffDNA) is extracted from maternal plasma. The DNA is analysed for the presence of the gene or molecular variation responsible for (or associated with) the fetal blood group phenotype under investigation. This technique replaces the previous requirement for invasive direct sampling methods for fetal DNA, such as amniocentesis or chorionic villus sampling (CVS) which pose a risk to the pregnancy.

Clinical Indications

The Australian Red Cross Lifeblood currently offers NIPA for the following clinical indications in high risk pregnant women:

Fetal antigen	Clinical Indications
D	1. Rh D negative pregnant women who are RhD alloimmunised; 2. Rh D negative pregnant women with obstetric indications such as severe fetomaternal haemorrhage during pregnancy, or intrauterine death; or 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 the RhD immunoglobulin, or cultural/religious beliefs). For 2 and 3 please discuss with a Lifeblood Transfusion Specialist prior to collection or shipping.
K or k	Alloimmunised (anti-K or anti-k detected, regardless of titre)
C, c, E, Fya, Fyb	Clinically significant level of alloantibody (titre ≥ 32) or history of HDFN

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. Samples collected before 12 weeks gestation can produce inconclusive or false negative results and will not be tested.

Reporting

It is expected that results will be reported within 10 working days from receipt of the sample. A report containing predicted blood group phenotype of the fetus will be provided by email via Secure Send only to the referring laboratory.

Requests and Sample Requirements

- A minimum of 3 x 6mL dedicated EDTA whole blood samples.
- Sample collection should be planned to ensure they are received by the Red Cell Reference Laboratory for processing within 72 hours of collection.
- Samples are only able to be processed Monday – Thursday.
- Samples should be stored at 2-8°C.
- Samples must be provided with completed Red Cell Reference Laboratory request form with a minimum of 3 identifiers and include the tests requested, referring organisation's contact details and any relevant clinical information, including antibody titre.
- The identifiers on the sample must match the request form exactly.

The Red Cell Reference Laboratory request form can be downloaded from Lifeblood's website using this link Red Cell Reference Forms.

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 38389493 ***Fax: +61 7 38389410***

Enquiries

Please direct sample collection and shipment enquiries to the Red Cell Reference Laboratory on +61 7 3838 9493.

Please direct all clinical enquiries to the Transfusion Specialist for your state. The contact details are available using this link Contact | Lifeblood.

How do we test?

The assay is performed using the Bio-Rad Droplet Digital PCR system (ddPCR). This system uses custom designed primer and probe sets for each target, which are combined with a Mastermix and divided into approximately 20,000 individual droplets or 'partitions' using the Automated Droplet Generator (AutoDG). Each patient cfDNA sample is tested in five replicates for the fetal antigen of interest, therefore up to 100,000 droplets are potentially generated. Maternal genomic DNA (gDNA) is also tested. Partitioned droplets are amplified using the C1000 Touch thermal cycler. Amplified partitions are measured for fluorescence using the ddPCR QX200 Reader and analysed using QuantaSoft Pro software to determine if fetal antigen signals are present.

Performance

The assay has a positive predictive value (PPV) ≥98% and negative predictive value (NPV) ≥ 98%. The accuracy based on the validation and study data is 100% with a 95% Confidence Interval of 97.2% to 100%.

A repeat collection and test is advised to confirm negative results in order to minimise the risk of false negatives.

A proportion of samples will be reported as inconclusive. In this instance a repeat sample will also be requested.

How are the results interpreted?

Final patient cfDNA interpretation always takes into account the combination of results obtained for patient cfDNA and maternal gDNA (blood group allele and a control target) according to the following criteria.

Result	Criteria:
Not Detected	• cfDNA: o Target has ≤ 2 positive droplets o control has a concentration of 7-1000 copies/µL • gDNA o Target has ≤ 1 positive droplets o control has a concentration of 0.5-500 copies/µL Repeat collection and testing is recommended when the assay target is interpreted as 'Not Detected' and negative antigen prediction is reported.
Detected	• cfDNA: o Target has ≥ 8 positive droplets o control has a concentration of 7-1000 copies/µL • gDNA o Target has ≤ 1 positive droplets o control has a concentration of 0.5-500 copies/µL
Inconclusive	• cfDNA: o Target has 3-7 positive droplets • and/or o control has a concentration of <7 or >1000 copies/µL • gDNA o Does not meet any of the criteria for gDNA listed for valid samples • All cfDNA results will be considered inconclusive if the gDNA sample does not meet the validity criteria.

What do we test for?

After extracting cfDNA from the maternal plasma, a droplet digital Polymerase Chain Reaction (ddPCR) assay is used to predict the fetal blood group phenotype for the antigens of interest – D, C, c, E, K, k, Fy^a, Fy^b.

Table 1: ddPCR assays to predict fetal antigen status

Assay name	**Gene**	**Allele/s**	**Target**	**Ag prediction**
RHD	*RHD*	*RHD*01*/*RHD*01N*	Exon 5 and Exon 10	D+/D-
KEL	*KEL*	*KEL*01.01/KEL*02*	rs8176058 578C>G	K+/- or k +/-
FY	*ACKR1*	*FY*01/FY*02*	rs12075 125A>G	Fy^a or Fy^b
RHCE*c	*RHCE*	*RHCE*c*	rs676785 307C	c+/-
RHCE*E	*RHCE*	*RHCE*E*	rs609320 676G>C	E+/-
RHCE*C	*RHCE*	*RHCE*C*	*RHCE* intron 2 109bp insert associated with C antigen expression	C+/-