

Molecular Genotyping Platform Frequently Asked Questions

Updated November 2019 Version 3.0

1. What request form is to be used for requesting testing?

The **Red Cell Reference Laboratory Request Form** includes genotyping and can be downloaded from our website transfusion.com.au/red_cell_reference

2. What sample tube is to be used to send samples for testing?

A dedicated EDTA whole blood sample with at least 4 mL is required for genotyping. (Lithium Heparin samples are not suitable.)

Fetal phenotype can be predicted from a minimum of 10mL of amniotic fluid or cultured amniocytes (harvested, trypsin treated and suspended in 10mL of PBS). Cultured samples are preferred as native amniotic fluid may not yield sufficient DNA to perform the genotyping assay.

3. Do samples require 2 or 3 identifiers as a minimum?

The patient identification requirements for the request form and samples must meet the National Pathology Accreditation Advisory Council Requirements for Medical Pathology Services, Third Edition 2018.

- When identifying the patient, three identifiers must be used on the request form. This
 also applies to unidentifiable and unconscious patients who will need a unique medical
 record number and two other descriptors e.g. head injury and motorbike accident.
 CA8.5(iii)
- When labelling the patient's specimen, two identifiers must be used or three identifiers where practicable. *CA8.5(iv)*

4. Is patient consent required?

Ensuring appropriate patient consent to perform pathology testing is the responsibility of the requesting clinician.

5. Are there any restrictions on how old the sample can be?

The manufacturers of the Immucor BioArray BeadChip assays and the QIAGEN QIAamp DSP DNA blood mini kit used for DNA extraction have no documented sample age restrictions. However, advice received from Immucor recommends that DNA extraction be performed within 14 days of collection for optimal results.

6. Where should we send our samples for testing?

Where genotyping only is required, the sample should be referred **directly** to the laboratory in Brisbane for testing.

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

If the requirement to perform genotyping is dependent on the outcome of additional serology testing, then the sample should be referred to your regional Red Cell Reference Laboratory in the first instance.

7. What is the best time to send samples?

Ideally, it is best to send samples during the normal operational hours of the laboratory (7:00am to 5:30pm Monday to Friday). However, Lifeblood is able to receive samples at any time.

8. What is the testing turnaround time?

Genotyping is performed in batches and we would expect to be able to provide results within 5 working days from receipt of the sample. The provision of urgent results may be facilitated if there is a significant clinical need for the results. Where a delay in result release is anticipated, the customer will be notified of the reason for the delay.

9. How much does the test cost?

Australian governments fund Lifeblood to provide blood, blood products and services to the Australian community.

10. How are the results conveyed to the referring lab?

The results obtained using the HEA BeadChip TM kit will be reported as a predicted phenotype. For example, the results may look like:

Blood Group System	Antigen	*Predicted Phenotype
Rhesus	С	+
	С	0
	E	0
	е	+
	V	0
	VS	0

^{*} The predicted phenotypes were determined based on the molecular genotyping using the Immucor BioArray HEA Precise BeadChip.

11. Is sequencing performed and how is the result conveyed to the referring lab?

The service provided by the Australian Red Cross Lifeblood is not that of sequencing the relevant gene. Lifeblood utilises the BioArray BeadChipTM Technology for the simultaneous detection of a wide range of single nucleotide polymorphisms (SNPs) affecting red cell antigen expression.

The results obtained using the RHD BeadChip $^{\text{TM}}$ kit will be reported as the Rh Phenotypic variant e.g. weak D type 11

While it is recommended to use the ISBT nomenclature to report genotypes, we believe a more user friendly format for ease of understanding the report is more appropriate.

12. Does the RHD BeadChip[™] assay provide zygosity determination?

No, the RHD BeadChip[™] assay does not provide an indication of the RHD zygosity.

13. How is the HEA BeadChip[™] able to provide a phenotype in recently transfused patients?

The BeadChip assays utilise the DNA obtained from the buffy coat of the Whole Blood EDTA sample for analysis. The presence of transfused red cells does not interfere with the extraction of the patient's own DNA from the sample.

14. Which cases should we send to Lifeblood for genotyping?

Where partial expression of the RhD antigen is suspected, or where an apparent RhD positive patient has an antibody that appears to be anti-D, these cases should be referred to your **regional** Red Cell Reference Laboratory for serological investigation. Subsequently, these laboratories will arrange RHD genotyping when necessary.

For patients who are unsuitable for phenotyping, e.g. recently transfused or positive DAT, or where the phenotyping results are ambiguous, or require confirmation e.g. Fy(a-b-) for GATA, should be referred for HEA genotyping.

Fetal phenotype can be predicted from amniotic fluid for determining the risk of Haemolytic Disease of the Foetus and Newborn (HDFN) in isoimmunised pregnancy.

Otherwise, please still send all other investigations that you normally would, directly to the regional Red Cell Reference Laboratory.

15. Whom do I contact for urgent queries?

The Laboratory in Brisbane should be contacted for specific patient enquiries.

QLD Red Cell Reference Laboratory Phone: +61 7 3838 9493

For general enquiries please contact your regional Red Cell Reference Laboratory.