

Preparation of plasma to be sent to IBGRL for fetal genotyping

Sample requirements:

16 mL EDTA blood from mother **per test requested**.

3 mL EDTA blood from father if available: **only for RhD typing** (buffy coat only needed).

Request form requirements:

[FRM4674](#) Request for Fetal Blood Group Genotyping.

[FRM4739](#) Request for Fetal Sex Typing from Maternal Blood.

Health and Safety:

- Gloves and laboratory coat must be worn when handling samples.
- Use filtered pipette tips when preparing the plasma aliquots.
- Process the blood in a clean DNA free area of the laboratory. Non-invasive testing is extremely sensitive and contamination of fetal samples with any other DNA can lead to false positive results.

Procedure:

- 1.1 Maternal EDTA blood must be processed within **48 hours** of venepuncture for fetal Kell genotyping, within **72 hours** of venepuncture for fetal RhD/C/c/E genotyping and within **7 days** of venepuncture for fetal sex typing.
- 1.2 Transfer blood sample to labelled 15 mL plastic tube and centrifuge at 1942-2103 x g for 10 minutes with the brake off.
- 1.3 Visually assess the appearance of the plasma layer and note if haemolysis has occurred. Haemolysed samples will not be tested.
- 1.4 Without disturbing the buffy coat, transfer plasma into a clean labelled 15mL plastic tube. Mix the plasma briefly by inversion, then transfer 1.4mL aliquots of plasma into labelled eppendorfs (minimum of 4x eppendorfs per patient). Centrifuge the plasma aliquots at 4700 x g for 10 minutes.
- 1.5 Remove 700µl buffy coat from the 15mL tube in section 1.4 to a clean eppendorf. Label the buffy coat aliquot with three points of identification (**i.e. full name, DOB and hospital number/unique identifier**) and **date separated**. Store at -20°C to -30°C.
- 1.6 Remove 1.2mL aliquots of plasma from section 1.4 to new eppendorfs, without disturbing pellet (minimum of 4x eppendorfs per patient). **Label the plasma aliquots with three points of identification (i.e. full name, DOB and hospital number/unique identifier)** and **date separated**. Store at -20°C to -30°C.
- 1.7 Send the request form, the frozen plasma aliquots and buffy coat on dry ice to Molecular Diagnostics, IBGRL, NHSBT, 500 North Bristol Park, Northway. Filton, Bristol, United Kingdom, BS34 7QH.

Pack with sufficient dry ice to ensure the samples remain frozen for the duration of transport and that they arrive between 09:00-17:00, Monday to Friday. Samples must be received within 5 days of shipment. Samples that do not arrive frozen **will not** be tested.

The **name of the person who prepared the plasma samples** and the **date of the plasma preparation** must be included on the request form.

The **requestor must sign** the request form to acknowledge that the NHSBT Terms and Conditions apply to this referral unless they have signed a contract with NHSBT.

Samples/ request forms that do not meet the acceptance criteria listed above will not be tested.