Guidelines for the Blood Transfusion Services

13.5: Fetomaternal haemorrhage estimation by flow cytometry

http://www.transfusionguidelines.org/red-book/chapter-13-patient-testing-red-cell-immunohaematology/13-5-fetomaternal-haemorrhage-estimation-by-flow-cytometry

13.5: Fetomaternal haemorrhage estimation by flow cytometry

13.5.1 Indications for testing

For any D negative pregnant mother where the screening test indicates a fetomaternal haemorrhage which is >2mL of suspected D positive fetal red cells, a sample can be referred to RCI for confirmation by flow cytometry. Results are used to support the management of prophylactic anti-D dose recommendation to mitigate against maternal sensitisation.

13.5.2 Procedures for testing in the RCI laboratory

As per BSH guideline recommendations, referred samples must not have been used for determination of an ABO blood group due to the risk of foetal cells being removed at the RBC/plasma interface and therefore possible underestimation of FMH.

Flow Cytometry procedure

- RCI laboratories should procure and maintain fully validated and supported flow cytometers
- Direct staining is recommended using a commercially available fluorochrome conjugated IgG monoclonal anti-D known to have high avidity for the D antigen
- Testing must include a suitable range of control cells and negative control antibodies in line with BSH guidelines. Laboratories must ensure operational consistency by defining acceptance ranges for control cell populations and ensuring that they are met with each patient investigation.
- White cell populations have been demonstrated to cause interference in the testing of delivery samples for fetomaternal haemorrhage volume by flowcytometry. A fluorochrome conjugated antibody, raised against a white cell specific marker, can be used in combination with the anti-D and negative antibody control reagents to remove interference from testing e.g. PE conjugated anti-CD66b or PE conjugated anti-CD45.
- The Mollison formula should be used to calculate the packed fetomaternal haemorrhage cell volume, which considers the patient to be 75Kg in weight, have a red cell volume of 1800mL and that fetal cells are 22% larger than adult cells
- The uncertainty of measurement (UoM) associated with the test should be calculated and applied to ensure the correct dose of prophylactic anti-D is used to mitigate against sensitisation. Laboratory teams should undertake an annual exercise to determine the UoM for the test.

- Laboratories should participate in an external quality assessment scheme, regularly review the results and act on the findings