



# Estimation of Feto-Maternal Haemorrhage

## IBGRL Research Products

The International Blood Group Reference Laboratory (IBGRL) markets a range of monoclonal antibodies which are sold worldwide as IBGRL Research Products. Currently, 83 monoclonal antibodies are available, three of which are CE marked for the determination of the volume of feto-maternal haemorrhage (FMH). These are BRAD 3 FITC (anti-D), AEVZ 5.3 FITC (isotype matched negative control) and a novel reagent, BIRMA 17C PE (anti-CD66b), which works in combination with BRAD 3 and the negative control to reduce assay interference.

Details of other IBGRL Research Products and their prices can be found using the following hyperlinks: [IBGRL Research Products](#); [Protein Development and Production Unit PDPU](#); [Price List](#).

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## Background

Measurement of FMH in a D negative (-ve) pregnant women is performed to determine the dose of prophylactic anti-D (RhIG) required to prevent immunisation against red blood cells (RBCs) from a D positive (+ve) fetus. Several methods are used; Kleihauer-Betke/acid elution test (KBT/AE), serological rosetting, fetal haemoglobin (HbF) flow cytometry (FC) and anti-D FC.

## Kleihauer-Betke test/Acid Elution

The KBT/AE is used in the USA and UK and is based on the resistance of HbF to acid elution. RBCs containing HbF are stained whereas adult RBCs are not. Interpretation of results can be troublesome due to inter-operator variability and in cases of maternal hereditary persistence of HbF.

## Serological rosetting using Immucor FMH Rapid Screen

This assay can detect the presence of high levels of D +ve cells in the maternal circulation (>30mL) but cannot be used to quantitate FMH (Immucor., 2014).

## Anti-HbF Flow Cytometry

Anti-HbF reagents are used in FC for FMH quantitation in the USA. Although the result is quantitative, it can be difficult to interpret in cases of maternal hereditary persistence of HbF.

## Anti-D Flow Cytometry

An anti-D reagent is used in FC for FMH quantitation in the UK. Results are unaffected in cases of maternal hereditary persistence of HbF as the test is specific for RhD.

## Advantages of anti-D Flow Cytometry

Although both FC assays give accurate estimations of FMH, anti-HbF testing is laborious as it involves fixation and permeabilisation steps. Anti-D testing has the advantage of not requiring the RBCs to be pre-treated. Also, results are unaffected in cases of maternal hereditary persistence of HbF.

## Anti-D UK Guidelines

In the UK, the British Committee for Standards in Haematology (BCSH) recommend confirmatory testing by anti-D FC in all cases where the KBT estimates an FMH of greater than 2mL, to check whether additional RhIG is needed.

## CE Marked Reagents for the Determination of FMH by Flow Cytometry

At the end of 2006, BRAD 3 FITC and the -ve control (AEVZ 5.3 FITC) were CE marked and together they comprise the IBGRL 'FMH Classic'. The FMH Classic Plus (BRAD 3 FITC, AEVZ 5.3 FITC and BIRMA 17C PE) was introduced in 2015 to improve accuracy in samples with a high neutrophil count post-partum. FMH reagents can be purchased individually (9433FI/9442FI/9453PE) or together for a discounted price.

Product Code	Antibody	Specificity
9433FI	BRAD 3 FITC	RhD
9442FI	AEVZ 5.3 FITC	Negative Control
9453PE	BIRMA 17C PE	Granulocytes (CD66b)

## Antibody Components of the FMH

### BRAD 3 FITC (anti-D)

BRAD 3 is a human monoclonal antibody with specificity for RhD (D). It reacts as an indirect agglutinin with all D +ve red cells tested, except those of the rare D<sup>VI</sup> or R<sub>0</sub><sup>har</sup> types. BRAD 3 can also discriminate weak D in FMH where RhD site numbers exceed 1000/cell.

### AEVZ 5.3 FITC (negative control)

AEVZ 5.3 is a recombinant human monoclonal antibody used as an isotype matched -ve control for BRAD 3.

### BIRMA 17C PE (anti-CD66b/Granulocyte)

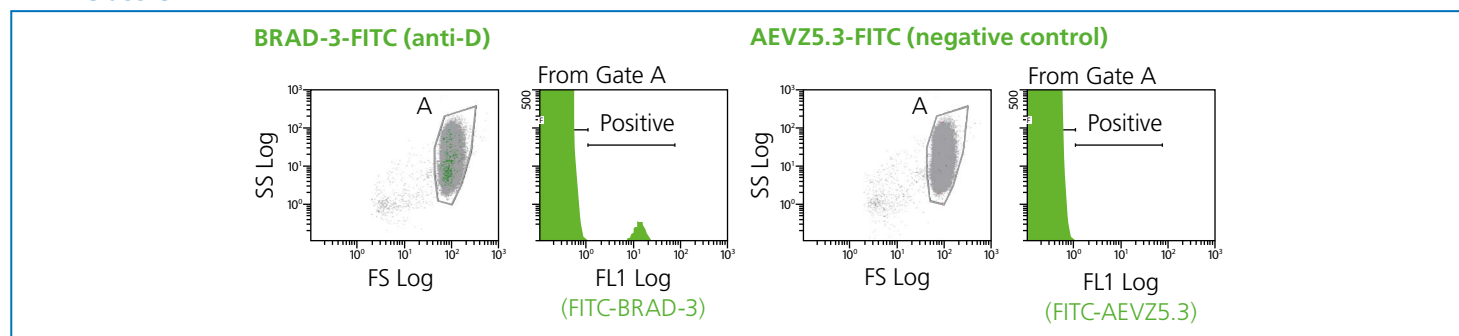
BIRMA 17C is a mouse monoclonal anti-CD66b antibody that reacts with granulocytes and has no reactivity with erythrocytes, platelets, lymphocytes or monocytes. BIRMA 17C PE can be used to exclude interfering granulocytes in those samples with high numbers of neutrophils to allow for a more accurate measurement of FMH by FC.

## Methodology FMH Classic

BRAD 3 FITC and the -ve control are supplied ready for use at 5 µl per test (alternatively, 50µl of a 1 in 10 dilution of the conjugate in PBS can be used). To a pellet of 10<sup>7</sup> washed packed erythrocytes add 45 µl of PBS pH 7.2-7.4. Add 5 µl of BRAD 3 FITC. Mix. Incubate at 37°C in the dark for 30 min. Wash the cells once in PBS. Analyse by FC.

During analysis, cells are displayed in a dot plot (Forward Scatter v Side Scatter) and a gate is drawn around the RBCs from which subsequent plots are generated (Gate A in Figure 1). The % of D +ve cells is calculated from the FL1 histogram which differentiates between labelled and unlabelled cells. Background events can be subtracted from the D +ve gate using the -ve control. The FMH is calculated using the Mollison calculation (1972).

### FMH Classic



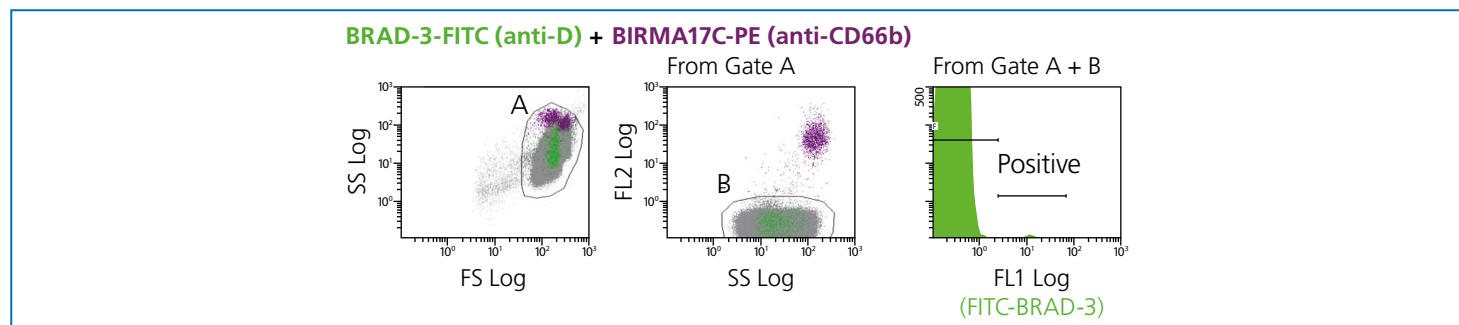
**Figure 1.** Results of labelling with the FMH Classic.

## Methodology FMH Classic Plus

BIRMA 17C PE is supplied at x 10 final working concentration for use at 5 µl per test. To a pellet of 10<sup>7</sup> washed packed erythrocytes (or 20 µl of 3% erythrocytes) add 40 µl of PBS pH 7.2-7.4. Add 5 µl of BRAD 3 FITC and 5 µl of BIRMA 17C PE. Mix. Incubate at 37°C in the dark for 30 min. Wash the cells once in PBS. Analyse by FC.

Combining BRAD 3 FITC and BIRMA 17C PE in the same assay allows granulocytes to be excluded thus improving the accuracy of results in samples with a high neutrophil count (Figure 2). The cells in gate A are displayed in a dot plot of Side Scatter v FL2. Gate B is placed around the unlabelled cells. The cells within gate B are displayed in a FL1 histogram and gates are placed above the +ve and -ve peaks (Figure 2). The procedure for calculating the FMH is the same as for the FMH Classic.

### FMH Classic Plus



**Figure 2.** Results of labelling with the FMH Classic Plus.



## Advantages of using Classic Plus

- The elimination of discrepancies caused by neutrophil contamination.
- Same assay time as the FMH Classic.
- Large discount when all 3 reagents purchased together (FMH Classic Plus).

## Mollison Calculation for Flow Cytometry (taken from BCSH Guidelines; 2009)

This formula assumes that:

- The maternal red cells would have a packed volume of 1800mL
- Fetal cells are 22% larger than maternal cells

The fetal bleed should be calculated as follows:

$$\frac{\text{Percentage Fetal Cells}}{100} \times 1800 \times \frac{122}{100}$$

Or can be simplified to: percentage fetal cells x 1800 x 1.22 e.g. where 0.5% of the red cells are fetal, the FMH will be calculated as  $0.5 \times 18 \times 1.22 = 10.98\text{mL}$  packed fetal red cells.

## Customer Support

For a free trial or technical advice please contact us (details provided below).

## UK NEQAS

UK National External Quality Assessment Service (UK NEQAS) is a registered charity offering external quality assessment (EQA) services across all pathology disciplines including measurement of FMH. The primary aim of UK NEQAS is to maintain and improve the performance of diagnostic testing. Participation in EQA is an established part of Quality Assurance and is actively encouraged by professional bodies. Many users of the FMH Classic kit take part in this scheme including IBGRL and NHSBT.

## References

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