CE 1434 IVD

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Product Code	Product Name
9442FI	AEVZ 5.3 FITC Conjugated Control Reagent
9433FI	BRAD 3 FITC Conjugated Anti-RhD Reagent
9453PE	BIRMA 17C PE Conjugated Control Reagent

Amendments from the previous version of this IFU are in purple text

Intended use
For professional use as an IVD device to give quantitative data to determine the size of a Feto-Maternal Haemorrhage (FMH). BRAD 3 FITC is intended to be used to quantitate accurately the number of RhD positive cells in a mixture of RhD positive and negative cells and thereby estimate the size of an FMH by analysis of the maternal blood sample. AEVZ 5.3 FITC is intended to be used as a negative control. BIRMA 17C PE is intended to be used to remove granulocytes during flow cytometry which may otherwise interfere with test results.

Principles of the examination method
RhD positive infants born to RhD negative women may suffer from haemolytic disease of the new-born. The disease can be prevented by administration of anti-D post-partum or antenatally. The dosage of anti-D required depends on the size of the FMH.

When red cells from a RhD negative maternal sample, with a suspected fetal bleed from a RhD positive fetus, are incubated with a fluorochrome conjugated IgG monoclonal anti-D reagent, the minor population of RhD positive fetal cells are bound by the anti-D antibodies through an immunohaematological reaction. The sample is then tested using flow cytometry. Flow cytometry works on the principle of light scattering, as cells pass through a laser beam in single file. The RhD positive cells, which have been bound by the fluorochrome conjugated IgG monoclonal anti-D reagent, can be counted due to their emission of fluorescence.

By flow cytometry, BRAD 3 FITC conjugated anti-D reagent can be used to quantitate accurately the number of RhD positive cells in a mixture of RhD positive and negative cells, and thereby estimate the size of a FMH. The variability of fluorescence of rr cells (RhD negative) when labelled with FITC anti-D means that a negative control antibody, such as AEVZ 5.3 FITC, should be used to gain accurate results. This FITC conjugated control reagent is unreactive with human red cells irrespective of their RhD type.

Most FMH samples have elevated numbers of maternal granulocytes, especially neutrophils. These granulocytes stain with BRAD 3 and other IgG antibodies non-specifically, giving FITC fluorescence that may overlap RhD negative and positive red cells. Labelling with BIRMA 17C PE enables the PE-labelled granulocytes to be eliminated from analysis by excluding cells with PE fluorescence.

Components
- AEVZ 5.3 is a human IgG3 engineered monoclonal antibody. Each vial contains 0.51mL +/- 0.05mL AEVZ 5.3 FITC conjugate in an amber bottle. The reagent has been prepared using a diluent containing 1% BSA and 0.099% sodium azide.

- BRAD 3 is a human IgG3 anti-RhD monoclonal antibody. Each vial contains 0.51mL +/- 0.05mL BRAD 3 FITC conjugate in an amber bottle. The reagent has been prepared using a diluent containing 1% BSA and 0.099% sodium azide.
- BIRMA 17C is a mouse IgG1 anti-granulocyte antibody. Each vial contains 0.51 mL +/- 0.05mL BIRMA 17C R-Phycoerythrin (PE) conjugated reagent in an amber bottle. The reagent has been prepared using a diluent containing 1% BSA and 0.099% sodium azide.

Special materials and equipment required but not supplied

- Calibrated volumetric pipettes.
- Flow cytometer
- Centrifuge
- Phosphate Buffered Saline Solution (PBS)
- Water bath or dry heat incubators
- Test tubes which are demonstrated to be non-adherent for red cells, e.g. polyethylene

Reagent Preparation

Allow to reach room temperature, mix before use.

Storage and shelf life after first opening

Store at 2-8ºC in the dark.
Once opened the device can be used until stated expiry date.
Keep stored in the amber bottle.
Do not use beyond the expiry date.
After use, the vial should be capped and returned to the correct storage conditions and temperature.

Warnings and precautions

The device and any contaminated packaging should be disposed in accordance with local state or national legislation.
For healthcare professional use only.
The reagent should not be used if turbid or if a precipitate, gel or particles are present.
This device is not provided sterile.
Do not use if the reagent vial is cracked or leaking.
It is imperative to use accurate, properly calibrated volumetric pipettes to avoid variations which may affect the test outcome.
When used in accordance with the Instructions for Use and Good Laboratory Practices, there is limited potential for carryover.
The fluorescent activity of FITC and PE conjugated reagents is degraded by exposure to light. Minimise the exposure of these reagents to light where possible.
Do not freeze.

Primary sample collection, handling, and storage

Use EDTA plasma samples according to the current edition of the BSH Guidelines for The Estimation of Fetomaternal Haemorrhage.

Examination procedure

1. **Procedure for use of BRAD 3 FITC and AEVZ 5.3 FITC without BIRMA 17C PE**

BRAD 3 FITC is supplied ready for use at 5 µl per test.

- Sample cells should be resuspended in a final volume of 50 µl PBS pH 7.2-7.4 prior to testing. This can be achieved by adding 50 µl PBS to a pellet of 10^7 washed packed erythrocytes. Alternatively, dispense 20 µl of 3-5% erythrocytes into a tube and add 30 µl of PBS.

- Add 5 µl of BRAD 3 FITC.
- Mix and incubate at 37°C in the dark for 30 min.
- Wash the cells once in PBS.
- Analyse by flow cytometry.

AEVZ 5.3 FITC is supplied ready for use at 5 µl per test as a negative control alongside BRAD 3 FITC.

- Sample cells should be resuspended in a final volume of 50 µl PBS pH 7.2-7.4 prior to testing. This can be achieved by adding 50 µl PBS to a pellet of 10^7 washed packed erythrocytes. Alternatively, dispense 20 µl of 3-5% erythrocytes into a tube and add 30 µl of PBS.
- Add 5 µl of AEVZ5.3 FITC.
- Mix and incubate at 37°C in the dark for 30 min.
- Wash the cells once in PBS.
- Analyse by flow cytometry.

2. Procedure for use of BRAD 3 FITC and AEVZ 5.3 FITC with BIRMA 17C PE

BIRMA 17C PE is ready for use at 5 µl per test.

- Sample cells should be resuspended in a final volume of 60 µl PBS pH 7.2–7.4 prior to testing. This can be achieved by adding 60 µl PBS to a pellet of 10^7 washed packed erythrocytes. Alternatively, dispense 20 µl of 3-5% erythrocytes into a tube and add 40 µl of PBS. Add 5 µl of either BRAD 3 FITC or AEVZ 5.3 FITC and 5 µl of BIRMA 17C PE.
- Mix and incubate at 37ºC in the dark for 30 min.
- Wash the cells once in PBS.
- Analyse by flow cytometry.

Control procedure

Users are responsible for determining the appropriate quality control procedures for their laboratory and for complying with applicable laboratory regulations. If controls set up with the batch of tests fail to give required results, then all tests must be repeated.
A negative control such as AEVZ 5.3 FITC reagent should be included to control for variability in fluorescence of rr (RhD negative) cells. PE conjugated BIRMA 17C can be used in conjunction with FITC BRAD 3 in a dual colour assay for FMH quantitation.

Interpretation of results

The analysis of the results should be according to the Guidelines on the Estimation of Feto-Maternal Haemorrhage, which are published by the Working party of the British Committee for Standards in Haematology, Transfusion Taskforce (BCSH FMH guidelines 2009). For NHSBT centres please refer to the Management Process Description MPD444. See Figure 1 for identification of R1r cells stained with BRAD 3 FITC and R1r and rr cells stained with AEVZ5.3 FITC. Figure 2 shows how granulocytes stained with BIRMA 17C PE can be excluded from analysis when the dual colour assay is employed.

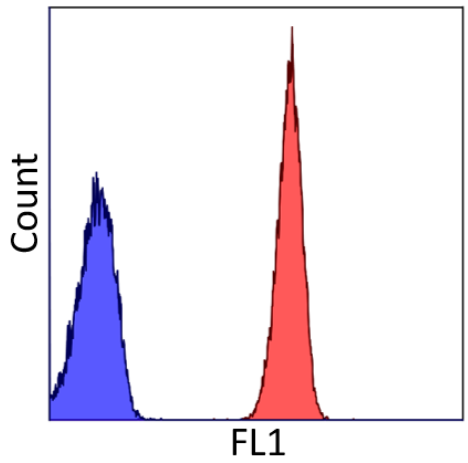

Figure 1. AEVZ 5.3 FITC with R1r and rr cells (blue peak) and BRAD 3 FITC with R1r cells (red peak)

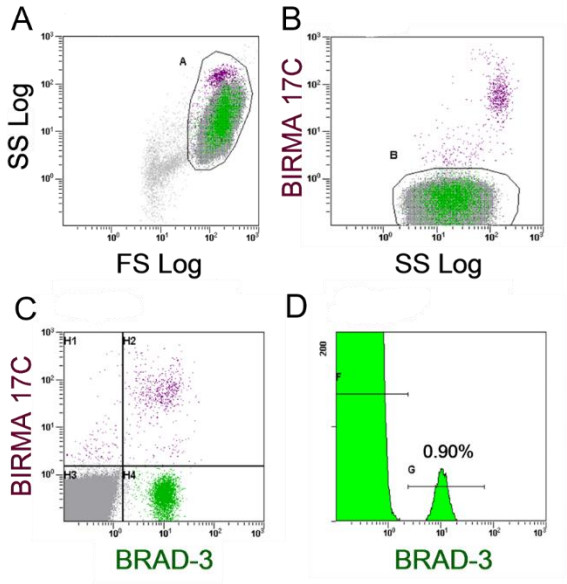

Figure 2. **A**: gate A excludes debris (with low FS and SS) and the cells within gate A are displayed in a SS vs. FL2 dot plot (**B**) where gate B excludes PE-positive cells stained with BIRMA 17C PE. Cells in gate B are displayed as a histogram (**D**) where a marker can be applied to cover the range of fluorescence of D-positive cells and their percentage is calculated by the flow cytometer; example shown 0.90%. **C**: dot plot of BRAD 3 FITC vs. BIRMA 17C PE shows D-positive cells (green/FITC) and granulocytes (purple/PE). A gate to exclude FL2-positive cells may alternatively be placed on a dot plot to generate the histogram, or a quadrant may be applied as shown in C and the percentage of cells in the lower right quadrant determined.

See BSH website for Guidelines on the Estimation of Feto-Maternal Haemorrhage, 2009.

Performance characteristics

Controls are prepared containing RhD positive cells at 100%, 1.0% and 0.20% of the total red cell population. When the fluorescently labelled antibodies for FMH investigation are used together to test these controls for RhD positive cells, results are given within the following limits.

Nominal % of RhD positive cells	Result range from FMH antibody investigation
100%	>99.2%
1.0%	0.91%-1.15%
0.20%	0.16%-0.23%

Limitations of the examination procedure

This reagent is not for use as a blood grouping reagent.
BRAD 3 reacts as an indirect agglutinin with all RhD positive red cells tested except those of the rare DVI or RoHar types.
Most FMH samples have elevated numbers of maternal granulocytes, especially neutrophils. These granulocytes stain with BRAD 3 and other IgG antibodies non-specifically, giving FITC fluorescence that may overlap RhD negative and RhD positive red cells. Labelling with BIRMA 17C PE enables the PE-labelled granulocytes to be eliminated from analysis by excluding cells with PE (FL-2) fluorescence.
False positive or false negative results may occur due to contamination of test material, improper storage, incorrect incubation time or temperature, improper or excessive centrifugation or deviation from the recommended technique.
Visual evidence of hyperlipidaemia or haemolysis and age of specimen may affect the interpretation of test results.

Literature references

Directive 98/79/EC on In vitro diagnostic medical devices.
BSH guidelines for the estimation of fetomaternal haemorrhage (FMH)
Lloyd-Evans P, Austin EB, Gilmour JEM, Scott ML (1999) Use of a negative control antibody in the quantitation of feto-maternal haemorrhage by flow cytometry. Transfusion Medicine 9 suppl. 1:33. 9.

Manufacture

These reagents are manufactured by IBGRL at NHSBT 500 North Bristol Park, Northway, Filton, Bristol BS34 7QH. Great Britain. Phone +44 (0)117 921 7500 on behalf of NHSBT Reagents.

Note – Any serious incident that has occurred in relation to using this reagent should be reported to the manufacturer and the competent authority in which the user and/or the patient is established.

EC REP
Quality First International OÜ,Laki 30,12915 Tallinn, Estonia

Symbols used on NHSBT Reagents labels.

Note - not all symbols listed are applicable for this product - please refer to product labels.

Detail	Label details
Batch code symbol	LOT
Use by date symbol	
Expiry date format	YYYY.MM.DD
In Vitro Diagnostic medical device symbol	IVD
Instructions for use symbol (with website - electronic IFU)	 blood.co.uk/reagents
Negative control symbol	CONTROL –
Positive control symbol	CONTROL +
EC Rep symbol	EC REP

Detail	Label details
2-8°C temperature range symbol	
Below -20°C symbol	
CE Mark symbol	CE
UKCA symbol	UK CA
Manufacturer's symbol	
Keep Away from Sunlight symbol	
Contains human blood or plasma derivatives symbol	
Unique Device Identifier symbol	UDI

Lot number Format

Lot numbers are in the following format:

For BRAD 3 FITC (9433FI) – FBr3.XX

For AEVZ 5.3 FITC (9442FI) – FAEVZ5.3-XX

For BIRMA 17C PE (9453PE) – PBM17C.XX

XX Lot number