

**International Blood Group
Reference Laboratory**500 North Bristol Park
Northway
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Antigen	RhD Negative control
Clone	AEVZ 5.3
Product Code	9442
Immunoglobulin Class	Human IgG3, lambda light chain

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and Production Unit**Tel: +44 (0)117 921 7500
Fax: +44 (0)117 912 5796Website: <http://ibgri.blood.co.uk>Email: enquiries.IBGRL@nhsbt.nhs.uk**Clone**

AEVZ 5.3 is produced from a recombinant cell line which has been made by the transfection of NSO cells with a mammalian expression vector containing light and heavy chain cDNA's of VAZO 5 anti-Varicella Zoster human IgG3 lambda antibody engineered onto the heavy chain constant domains of BRAD-3 IgG anti-D. This antibody has been used as a negative control for the measurement of feto-maternal haemorrhage (FMH) together with BRAD 3 anti-D monoclonal antibody^{1,2}. BRAD 3 can also discriminate weak D in feto-maternal bleeds where the site/cell numbers are above 1000 RhD sites³. Guidelines for estimation of FMH have been published^{4,5}. When measuring the variance of rr cells in terms of background binding of FITC conjugated IgG, the use of a negative control FITC-labelled antibody should be used in parallel with FITC anti-D on clinical samples⁶. Background events are subtracted from positive events recorded for the clinical sample before calculating the FMH.

For estimation of FMH, introduction of the use of BIRMA 17C conjugated to R-Phycoerythrin (PE) in 2014 showed that the removal of granulocytes during flow cytometry which may otherwise interfere in the assay and thus affect the final calculated bleed, gives a more accurate result. PE conjugated BIRMA 17C is used in conjunction with FITC conjugated BRAD 3 as a two (dual) colour reagent used for FMH quantitation^{16,17} as well as AEVZ 5.3 FITC negative control^{7,8}.

References

1. Lloyd-Evans *et al*, (1995) *Transfusion Medicine* **5**, suppl 1, 23.
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3. Lloyd-Evans *et al*, (1999) *Transfusion Medicine* **9** 155-160.
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5. Austin E. *et al*. (2009) Guidelines for the estimation of fetomaternal haemorrhage. Working party of the BCSH Transfusion Taskforce. BCSH FMH Guidelines 2009 p1-23.
6. Lloyd-Evans P, Austin EB, Gilmour JEM, Scott ML (1999) *Transfusion Medicine* **9** suppl 1:33. 9.
7. Kumpel B, Hazell M, Guest A, Mushens R. (2012). A novel reagent for rapid and accurate fetomaternal haemorrhage quantitation by flow cytometry that eliminates leucocytes from analysis. *Transfusion Medicine*, **22** (suppl. 1), 22.
8. Belinda Kumpel, Matthew Hazell, Alan Guest, Jonathan Dixey, Rosey Mushens, Debbie Bishop, Tim Wreford-Bush and Edmond Lee (2014) Accurate quantitation of D+ fetomaternal hemorrhage by flow cytometry using a novel reagent to eliminate granulocytes from analysis. *Transfusion*, **54**, 1305–1316.