Bristol Institute for Transfusion Sciences
Incorporating the
International Blood Group Reference Laboratory

NHS Blood and Transplant

IBGRL RESEARCH PRODUCTS

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Antigen	RhD Negative control
Clone	AEVZ 5.3
Product Code	9442
Immunoglobulin Class	Human IgG3, lambda light chain

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. This antibody has been used as a negative control for the measurment of feto-maternal haemorrhage (FMH) together with BRAD 3 anti-D monoclonal antobody[1,2]. BRAD 3 can also discriminate weak D in feto-maternal bleeds where the site/cell numbers are above 1000 RhD sites[3]. BCSH Guidelines for 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 samples[6].

Transfusion Medicine 9 suppl 1:33. 9 Abstract 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.

Quantitation of feto-maternal haemorrhage (FMH) by flow cytometry is increasing, and BCSH Guidelines have been recently published[4]. Maternal samples are labelled with fluorochrome-conjugated monoclonal IgG anti-D. Background binding is assessed by labelling a known D-negative control sample in parallel. Background events are then subtracted from positive events recorded for the clinical sample before calculating the FMH. A more appropriate control would be the use of a non-red cell reactive, matched FITC-conjugated antibody, labelling the clinical sample in parallel with the anti-D. The heavy chain variable domain of a human monoclonal antibody specific for varicella-zoster virus (VZV) was engineered onto the heavy chain constant domains of BRAD-3 IgG anti-D, co-expressed with the anti-VZV light chain in NS0 cells and conjugated with FITC. This conjugate was tested in parallel with FITC-BRAD-3 with 5 different rr samples. There was no significant difference between the two reagents in events observed in the positive region. However, the range of background events varied from 0.009% to 0.072%. This would result in a range of 0.2 to 1.58 ml being deducted from any FMH assays, dependent upon which rr cells were selected as the negative control. Given this variance of rr cells in terms of background binding of FITC conjugated IgG, we advocate the use of a negative control FITC-labelled antibody in parallel with FITC anti-D on all clinical samples.

References

1. Lloyd-Evans *et al*, (1995) Transfusion Medicine **5**, suppl 1, 23.
2. Lloyd-Evans *et al*, (1996) Transfusion, **36**, 432-437.
3. Lloyd-Evans *et al*, (1999) Transfusion Medicine **9** 155-160.
4. Chapman JF (1999) Working party of the BCSH Blood Transfusion and General Haematology Task Forces (1999) Transfusion Medicine **9**, 87-92.
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.