

# ONE-TUBE HLA-B27 TYPING BY FLOW CYTOMETRY USING THREE MONOCLONAL ANTIBODIES



WELSH BLOOD SERVICE  
GWASANAETH GWAED CYMRU

WELSH TRANSPLANTATION AND  
IMMUNOGENETICS LABORATORY



E. COATES AND C. DARKE

## Introduction

We have established a single tube flow cytometry-based method for HLA-B27/B2708 typing using the murine monoclonal antibodies (moab): Com-B27 (Chemicon Australia), consisting of moab ABC-m3 to B27/B2708 PE conjugated and a non conjugated anti-B7 moab that blocks 'cross-reactivity' to B7; FD705 (One Lambda), FITC conjugated anti-B27, and UCHT 1 (Beckman Coulter), phycoerythrin-Cy5 conjugated anti-CD3, to analyse T-cells only.

## Materials and Methods

100  $\mu$ l of EDTA blood or lymphocytes ( $2 \times 10^6$  mL); moab 'cocktail' (5  $\mu$ l each of Com-B27, FD705, CD3), vortex mixed, incubated in the dark at 4°C for 15 minutes. Two mL of Puregene RBC lysis solution (Gentra Systems) added, vortexed and incubated in the dark at 22°C for 15 minutes. After a single wash (2 mL of 4°C PBS in a Diacent 2000 Cell Washer [DiaMed]), 300  $\mu$ l of 4°C PBS added, the cells vortexed and analysed on a Coulter Epics XL-MCL flow cytometer (System II [ver. 3] software) and the FITC and PE median channel fluorescent intensities (MCFI) of the gated T-cells measured using a four decade log amplifier.

Assay verification included testing 50 PCR-SSP and serologically typed reference cells possessing: B7, B13, B27 (B\*2702 and B\*2705), B37, B42, B55, B56, B73, B2708; B7 with B\*2705, B55; B7 only, and 12 donors lacking these specificities; 4-6 donors were used for each specificity (except B73 [n=2]).

## Results

Com-B27 reacted with the 22 B27 and B2708 cells (MCFI range 5.1-17.9 [mean 8.65]). The highest MCFI against the 9 B7 positive cells was 1.9 (B7,B22); against the 4 B42 cells was 2.3. The highest value against all other cells was 1.4. Thus, this reagent clearly differentiated B27/B2708 from B7 and the other specificities known to react with the ABC-m3 moab. FD705 reacted with the 12 B\*2705 cells (range 8.6-38.0 [mean 16.5]); gave weak results against the 4 B\*2702 cells (range 1.2-1.7 [mean 1.5]) and was negative against the 6 B\*2708 cells (mean 0.4) - the highest MCFI value against all other cells was 1.3.

Figures 1 and 2 show the reactivity of Com-B27 and FD705 tested together in a single tube assay against B27 specificities and antigens of the B7/B27 CREG.

Figure 1. Reactivity of Com-B27 and FD705 against B27 and B2708.

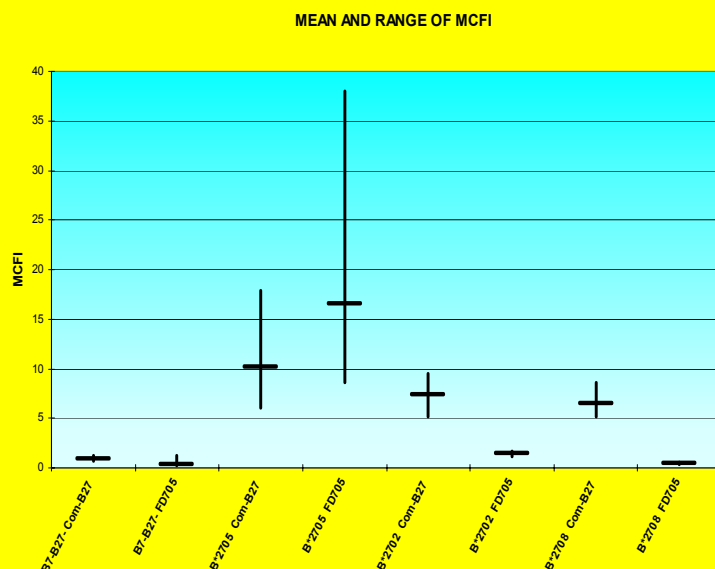
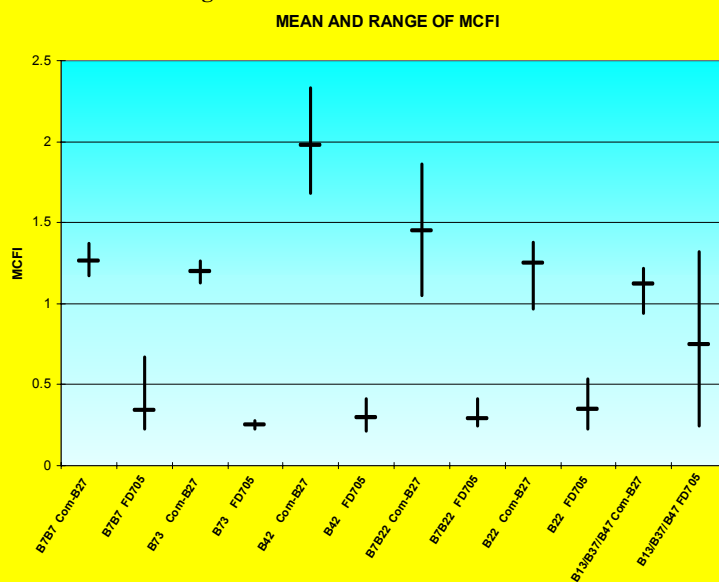


Figure 2. Reactivity of Com-B27 and FD705 with B7/B27 CREG antigens.



## Comments

Using this single tube assay ensures robust detection of the common B\*2705 product using two moab. Cells consistently reacting with one moab only must be "DNA typed" to identify the allele involved. A large 'side by side' comparison with our standard flow cytometry technique is now underway.