

# AN EVALUATION OF "COM-B27" A FLUORESCCEIN CONJUGATED MOUSE ANTI-HLA-B27 REAGENT

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FS 27925



28-JAN-2000 TO 28-JAN-2001

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## Introduction

Accurate determination of patient HLA-B27 status is an important routine service commitment with flow cytometry and DNA-based assays being the current methods of choice.

However, flow cytometry testing can be bedeviled by the "cross-reactivity" of anti-B27 mouse monoclonal antibodies, particularly with the ubiquitous HLA-B7 antigen. For example, the commonly used B27 antibody ABC-m3 (1) reacts with B7, B42, B73, B22, B37 and B44 specificities to various degrees (2).

However, a new formulation of ABC-m3 (Com-B27-mouse anti-HLA-B27 reagent fluorescein conjugated-product code 21HHLA08E, Amrad Biotech, Boronia, Victoria, Australia) is claimed to demonstrate less cross-reactivity to HLA-B7.

## Methods

We evaluated the Com-B27 reagent in three concentrations (undiluted, PBS diluted 1:2, 1:4), using our standard flow cytometry method (2), against 54 reference cells, including our B27 typing control material (2). These covered: HLA-B7, B\*2702, B\*2705, B2708, B37, B42, B44, B55, B56, B47 and B73 with non-confounding specificities; homozygous B7 cells and negative controls.

## Results

Figure 1

- Using the undiluted reagent all B27 and B2708 cells gave a median channel fluorescence intensity (MCFI) of  $>5$ .
- All homozygous B7 cells gave an MCFI of  $<2$ .
- All cells possessing B7 and a non-confounding, i.e. B7 non-crossreacting, specificity gave an MCFI of  $<2$ .
- HLA-B42 and B73 positive cells and those possessing both B7 and B55 or B56 gave MCFIs of  $>2 < 5$ .

Figure 2

- In all assays the test:negative control ratio did not differ significantly between the three reagent concentrations.

## References

1. Trapani et al. (1983) Hum Immunol, 7, 205.
2. Darke & Coates (1998) Eur J Immunogenet, 25, 29.

Figure 1. Reactivity of Com-B27

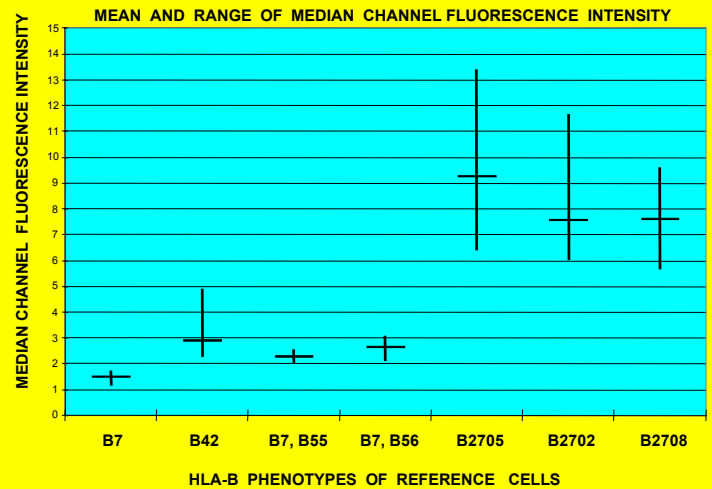
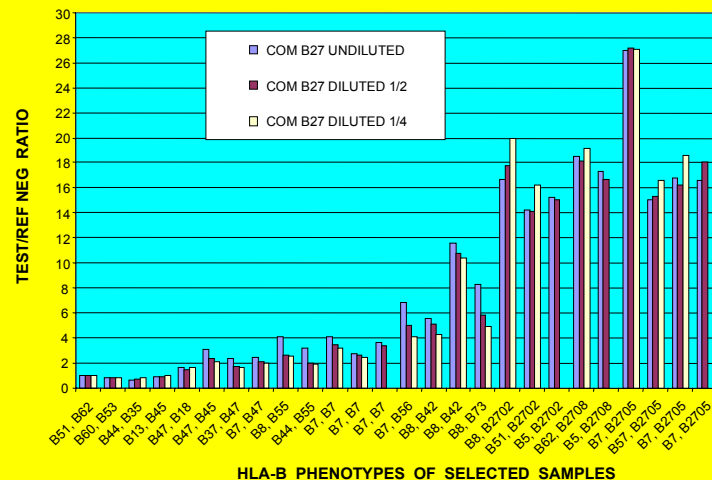


Figure 2. Dilutions of Com-B27



## Comment

Compared with our previous experience with ABC-m3 (2), where B7 positive, B27 negative cells gave an MCFI of up to 5 and B27/B2708 gave an MCFI of  $>4$ , the discrimination between B27/B2708 and B7, using the Com-B27-mouse anti-HLA-B27 reagent, was significantly improved.

However, although Com-B27 provides good discrimination between B27/B2708 and B7 caution still needs to be exercised in its use. Thus, cells possessing a combination of B7 and a B7 cross-reactive specificity, e.g. B42 or B22, could give a borderline B27 positive reaction.

Therefore, to ensure dependable HLA-B27/B2708 antigen assignment we still recommended the careful selection of several complementary monoclonal antibody preparations (2).