

# Materials and Methods

## The flow cytometric T cell crossmatch in solid organ transplantation

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### Introduction

Prior to organ transplantation, the flow cytometric crossmatch is performed to detect antibodies that may be detrimental to the performance of the organ.

The flow cytometric crossmatch (FCXM) assay involves the incubation of an organ donor's lymphocytes with serum from potential recipients of the graft. The principle of the test is shown in Fig. 1. Excess serum and unbound immunoglobulins are removed by washing the cells several times. Bound immunoglobulins on the cell surface are detected by incubation with a FITC conjugated anti-human IgG antibody and any free conjugate is removed by washing. The T cell population is identified using a PE monoclonal anti-human CD3. After incubation the cells are washed and resuspended in a small volume of diluent.

### Specimen required

Peripheral blood lymphocytes or lymphocytes separated from the donor spleen and 10ml of pre-transplant blood taken from potential transplant recipients into a plain glass tube as the source of test serum.

### Reagents

- 1 Phosphate-buffered saline with Fetal Calf Serum (PBS/FCS)
- 2 Anti-human IgG (FITC)
- 3 Anti-human CD3 (PE)
- 4 Negative control - AB non-transfused male serum
- 5 Positive control - Mixed pool of patient sera known to contain multispecific HLA antibodies.

### Method

- 1 50 $\mu$ l of cells ( $10^6$  ml<sup>-1</sup>) and 50 $\mu$ l of serum are mixed and incubated in 75  $\times$  12mm flow cytometer tubes for 30 minutes in a waterbath at 37°C.
- 2 The non-bound IgG in the serum is removed by washing the cells three times using 4ml of PBS/FCS for each wash.
- 3 50 $\mu$ l of antihuman IgG FITC and 5 $\mu$ l of the anti-CD3 PE are added to the cell pellet, mixed and incubated for 30 min at 4°C.
- 4 Non-bound conjugates are removed by washing the cells once with 4ml PBS/FCS.
- 5 The cells are resuspended in 200 $\mu$ l of PBS/FCS.

### Acquiring and analysing data on the flow cytometer

The samples are then processed on an optimally set up flow cytometer, and the data are analysed by identifying the lymphocyte population by its forward and side light scatter properties (Fig. 2). The lymphocytes are then analysed by gating (R1) in Fig. 2) and generating a FL1 versus FL2 dot-plot from which the positively stained T cells can be identified (Fig. 3). Finally, a histogram of the T cell peak is displayed to show the degree of anti-IgG binding (Fig. 4). The average of the median T cell peak for the negative controls is compared with the recipient test samples and the difference between the two is calculated and expressed as a 'positive' or a 'negative' result.

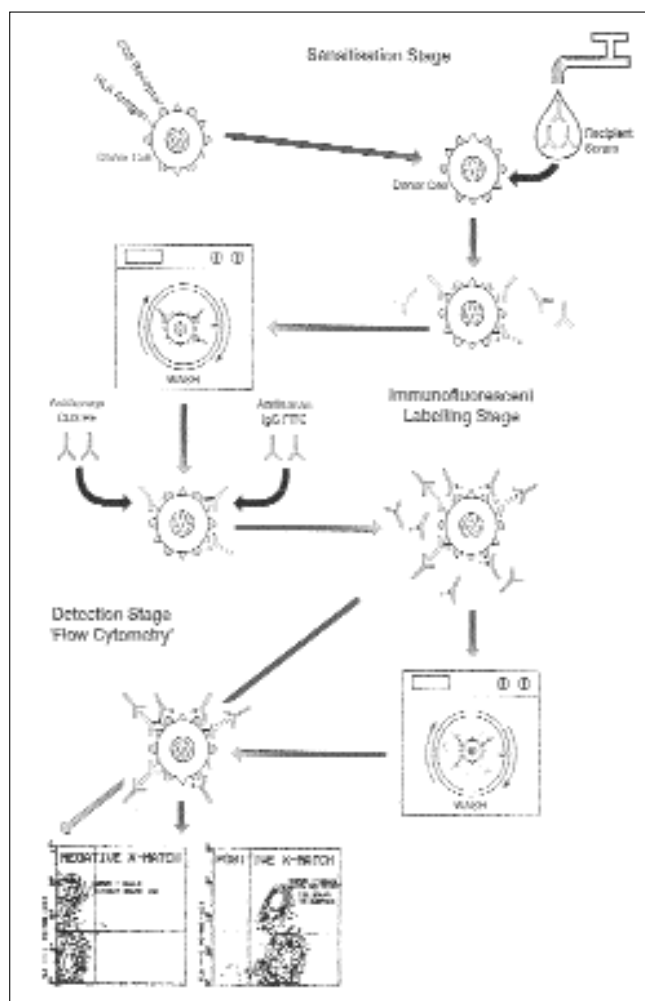
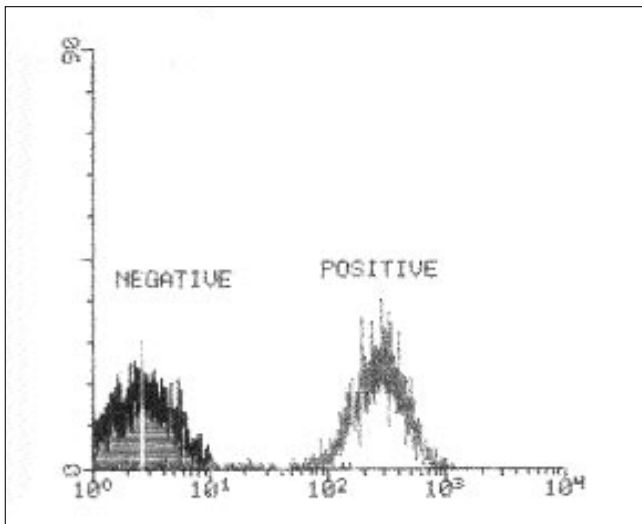
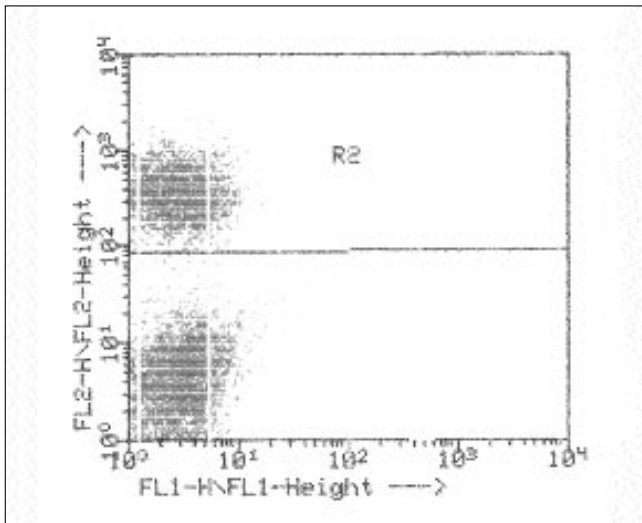
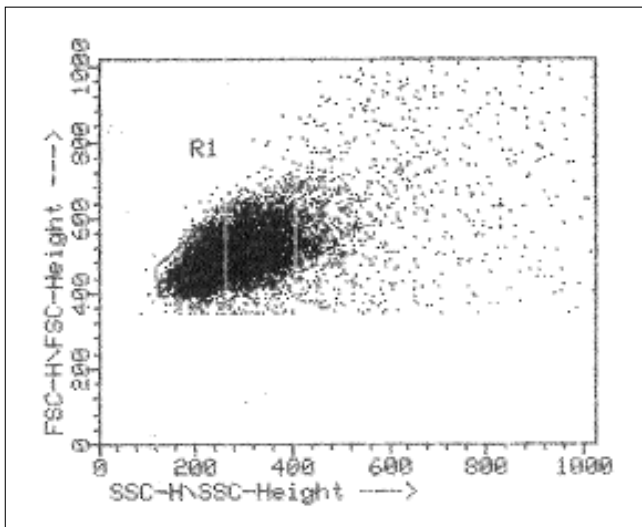


Fig. 1. Assay protocol for the flow cytometric T cell crossmatch.



**Figs. 2-4.** The analysis of flow cytometric crossmatch data.

### Special notes

Harmer *et al.* (1996) have published the preliminary finding of a multi-centre study organised by the flow cytometry special interest group of The British Society for Histocompatibility and Immunogenetics. The study identified the great variation in methodology between centres, which could have accounted for variations reported in the correlation between flow crossmatch results and the performance of transplants in different centres. This was later supported by a sec-

ond publication from the group, by Shenton *et al.* (1997), which showed that differences in results of the flow cytometric crossmatch were due not only to methodology but also to differences in the determination of criteria for defining positivity of test samples. The study showed that when both unified methodology and standard cut-off criteria were employed, 95% agreement was found between the different centres. However, this study was unable to determine the relationship between flow crossmatch test results and the clinical outcome of the transplants. This has many variables as the patient management and recipient selection procedures differ in many of the transplant units.

The clinical value of this assay can be addressed only by individual studies performed by each centre. Retrospective studies should be carried out using an optimised method and standardised criteria for determining positivity established in each individual centre. It will be clear from the above that each individual laboratory has to decide upon its own crossmatch protocol. However, for guidance, a method has been given here. This is not intended to be a definitive method; it is more of a starting point from which workers may develop their own protocol. Although the importance of selection and standardisation of a method has been mentioned, it is essential to realise that selection of control sera is fundamental to the crossmatch, as they are the negative controls on which the definition of positivity is based.

### References

- Harmer, A.W., Garner, S., Bell, A. E. *et al.* (1996) Evaluation of the flow cytometric crossmatch. Preliminary results of a multicentre study. *Transplantation* **61**, 1108 -1111.
- Shenton, B.K., Bell, A.E., Harmer, A.W. *et al.* (1997) Importance of methodology in the flow cytometric crossmatch : a multicentre study. *Transplantation Proceedings* **29**, 1454 -14 55.

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