16.7: Leucocyte crossmatching in blood transfusion

Crossmatching may be used in the diagnosis of TRALI and the treatment of HLA- or HNA-sensitised patients with granulocyte transfusions. Unusually it may also be used in the management of patients refractory to random donor platelet transfusion.

A patient’s serum should be comprehensively screened for HLA-specific antibodies prior to the crossmatch being performed. The chosen crossmatch technique should be of similar or greater sensitivity than the screening technique.

The presence of HLA-specific antibodies in a current patient serum sample that gives rise to a positive crossmatch excludes that donor providing platelets or leucocytes for that particular patient.

16.7.1: Lymphocytotoxic crossmatch

Assessment of leucocyte crossmatches must comply with EFI Standards within F6.000 and the standards for serological investigation given above.

16.7.1.1: Cytotoxic crossmatch requirements

A policy to determine which sera should be crossmatched should be established and based on local clinical data, where possible, before a crossmatch service is provided.

A negative control serum derived from a pool of sera that has been previously shown not to react with lymphocytes by complement-dependent cytotoxicity (CDC) should be used.

At least one positive control serum reacting with all lymphocytes or a mixture of anti-HLA-Bw4 and anti-HLA-Bw6-specific reagents should be used to confirm the activity of complement and HLA expression on the cell surface.

The crossmatch should be performed with and without dithiothreitol (DTT) to distinguish between IgM and IgG antibodies. An IgM control reagent should be included in the crossmatch test as a control for DTT activity.

Each patient’s serum should be tested in triplicate to control for unusual reactions in individual wells of the microplate.

16.7.2: Flow cytometric crossmatch

The flow cytometric crossmatch (FCXM) offers greater sensitivity than the microlymphocytotoxicity test for the detection of HLA-specific antibodies in patients receiving blood products. The FCXM may be performed with platelets, lymphocytes and/or granulocytes from the donor.
A two- or three-colour FCXM should be used with one antibody directed against human IgG conjugated to a fluorochrome (e.g. fluorescein isothiocyanate (FITC)). Antibody conjugated to different fluorochromes (e.g. anti-CD3 (T cells) and phycoerythrin (PE) and anti-CD19 (B cells) and allophycocyanin (APC)), should be used to identify the cell lineage under investigation, unless a purified cell population is used, to distinguish between anti-HLA Class I and II reactivity. Testing must be in compliance with EFI Standards M4.000.

16.7.2.1: FCXM requirements

A policy to determine which sera should be crossmatched should be established and based on local clinical data, where possible, before a crossmatch service is provided.

A negative control serum derived from a pool of sera that has been previously shown not to react with lymphocytes by flow cytometry should be used.

At least one positive control serum reacting with all lymphocytes or a mixture of anti-HLA-Bw4 and anti-HLA-Bw6-specific reagents should be used to confirm the activity of complement and HLA expression on the cell surface.

Each patient’s serum should be tested in duplicate to control for unusual reactions.

An additional weak positive control, which gives a fluorescent intensity just greater than the cut-off point between positive and negative, may also be included to evaluate assay performance.