Guidelines for the Blood Transfusion Services

16.6: Testing for HLA-specific antibodies

http://www.transfusionguidelines.org/red-book/chapter-16-hla-typing-and-hla-serology/16-6-testing-for-hla-specific-antibodies

16.6: Testing for HLA-specific antibodies

HLA-specific antibody screening and characterisation must comply with the relevant EFI Standards.

Sera containing HLA-specific antibodies may be interpreted in terms of specific antigens (i.e. whole gene products), cross-reactive groups, single epitopes, or any combination of these as long as standard and unequivocal nomenclature is used. Specificity characterisation may be helped by computer analysis but a final result must involve manual interpretation.

Solid phase techniques have now superseded cellular based methods for HLA antibody detection and identification. Commercial kits are available which consist of beads impregnated with differing ratios of two fluorochromes resulting in a unique signal for each bead and which have one or several types of HLA molecules attached.

The assay involves:

- incubation of a patient's serum with the beads
- if the patient has HLA antibodies the serum will react with the bead expressing the appropriate HLA molecule
- after washing, the beads are incubated with a secondary antibody, usually with a phycoerythrin (PE)-labeled antihuman IgG

Three levels of testing are possible depending on requirements:

1. The first level provides a positive/negative result with respect to a patient's antibody status. In this instance, the beads are bound with a large number of HLA class I or class II molecules derived from lymphoblastoid cell lines.
2. Beads used in second level testing are bound with molecules derived from a single cell line and hence express two HLA molecules for each of the HLA loci (HLA-A, -B, -C for class I and HLA-DR, -DQ and -DP for class II).
3. The third level of testing involves the use of beads bound with single HLA molecules produced by recombinant technology, so called single antigen beads (SAB). These beads provide a real advantage of this technology as complex mixtures of antibodies can be characterized and HLA specificities accurately determined. This technology is now considered essential for the pretransplant testing of sensitized patients.

The composition of the panel should be sufficient to discriminate the specificities (Class I, Class II, or both as appropriate) given in Table 16.2. The full list of antigens comprising a panel should be supplied and typed to the higher level of resolution shown in Table 16.1.

The detector reagent should be able to identify IgG and discriminate between IgG, IgA and IgM. Cut-off values for HLA antibody detection should be set in accordance with manufacturer's instructions and local clinical evaluation.

For DNA typed reagents the types should be supplied at the four-digit (second field) level (e.g. HLA-A*02:01) and null alleles identified.

Table 16.2 Characterisation of HLA-specific antibodies

HLA-A broad specificities	Splits	HLA-B broad specificities	Splits	HLA-C broad specificities	Splits	HLA-DR broad specificities	Splits	HLA-DQ broad specificities	Splits
A1		B5	B51	Cw1		DR1		DQ1	DQ5
A2		B5	B52	Cw2		DR103		DQ1	DQ6
A3		B7		Cw3	Cw9	DR2	DR15	DQ2	
A9	A23	B8		Cw3	Cw10	DR2	DR16	DQ3	DQ7
A9	A24	B12	B44	Cw4		DR3	DR17	DQ3	DQ8
A10	A25	B12	B45	Cw5		DR3	DR18	DQ3	DQ9
A10	A26	B13		Cw6		DR4		DQ4	
A10	A34	B14	B64	Cw7		DR5	DR11		
A10	A66	B14	B65	Cw8		DR5	DR12		
A11		B15	B62	Cw12		DR6	DR13		
A19	A29	B15	B63	Cw14		DR6	DR14		
A19	A30	B15	B75	Cw15		DR7			
A19	A31	B15	B76	Cw16		DR8			
A19	A32	B15	B77	Cw17		DR9			
A19	A33	B16	B38	Cw18		DR10			

A19	A74	B16	B39						
A28	A68	B17	B57						
A28	A69	B17	B58			DR51			
A36		B18				DR52			
A43		B21	B49			DR53			
A80		B21	B50						
		B22	B54						
		B22	B55						
		B22	B56						
		B27							
		B35							
		B37							
		B40	B60						
		B40	B61						
		B41							
		B42							
		B46							
		B47							

		B48							
		B53							
		B59							
		B67							
		B70	B71						
		B70	B72						
		B73							
		B78							
		B81							
		Bw4							
		Bw6							