Chapter 16: HLA typing and HLA serology

16.1: Preamble

For the eighth edition we have made significant changes in both the structure and content to the previous version of this chapter concerning human leucocyte antigen (HLA) typing and antibody testing. This obviously reflects the continuing scientific and technical development in the field. DNA-based testing for HLA alleles now predominates over serological phenotyping and antibody detection/characterisation mostly involves non-cell-based methods. This HLA section is constructed of three main parts, concerning reagents (section 16.4), testing (sections 16.5, 16.6 and 16.7), and application to donor and patient investigations (section 16.8). For certain patient or donor investigations there is, of course, an overlap with the guidance given in this and the granulocyte or platelet immunology chapters (Chapters 17 and 18 respectively). This is particularly relevant to the laboratory investigations of platelet refractoriness and transfusion-related acute lung injury (TRALI), so diagrams are included (Figures 16.1 and 16.2) to indicate how the different guidelines relate to each other.

16.2: Introduction

The transfusion or transplantation of blood components bearing allogeneic HLA can stimulate clinically significant immunological responses. All cellular components except erythrocytes express HLA and any plasma-containing product may include HLA-specific antibodies which are potentially harmful to the recipient.

Prospective HLA typing of platelet donors is undertaken for transfusion of immune refractory patients and those with disorders of platelet function and structure. Potential haematopoietic progenitor cell (HPC) donors are HLA typed to be placed on one of the national donor registries.

HLA typing or antibody investigations may be undertaken for diagnostic purposes or to investigate harmful consequences of transfusion. Thus the diagnosis of immune refractoriness requires the demonstration of HLA-specific antibodies (or other platelet-specific antibodies) in the patient. As part of the investigation of TRALI, implicated donors are screened for HLA (and human neutrophil antigen, HNA)-specific antibodies and the patient is HLA typed if possible.

The European Federation for Immunogenetics (EFI) has established standards\(^1\) (at the time of writing, version 5.7) for histocompatibility testing and where appropriate these guidelines will refer to the relevant EFI Standard, which will be stated in the text. In general, guidance for practice is indicated by the term ‘should’. The use of the term ‘must’ is mostly limited to circumstances where an EFI Standard applies.

16.3: Terminology and nomenclature
All HLA assignments, irrespective of the method, must comply with the current WHO Nomenclature Committee for Factors of the HLA System Report and Nomenclature for Factors of the HLA System, 2010 (and see EFI Standards D1.000–D1.320, inclusive). Examples of acceptable HLA assignments are as follows: HLA-B12, HLA-B44, HLA-B-44(12), HLA-B*44, HLA-B*44:09.

HLA may be serologically typed (to determine the phenotype) or typed by DNA molecular analysis. The term genotype is properly used to describe the genetic (DNA) constitution determined by the pattern of inheritance (EFI Standard D1.230).

HLA typing by DNA-based molecular techniques, which employ either DNA-based probes or primers, type for the presence or absence of sequence motifs. Kits using this technology are able to define the HLA alleles present in an individual to a variable level of resolution dependent on a number of factors. These include the number of probes or primers employed, the number of alleles defined for a given locus and the HLA alleles present in the individual. Although it is possible to achieve a high resolution or allele level typing using molecular methods, it is not a clinical requirement in transfusion practice. Therefore, patients and donors are typed to a low or medium level of resolution, and may present HLA typing results that include some ambiguity in interpretation.

Each serologically defined HLA antigenic specificity may be encoded by a number of different HLA alleles. Conversely many HLA alleles have no determined serologically defined antigen. Thus it is not always possible to assign a serological equivalent to each HLA allele. A consequence of this is that it is not practical to subject serological and DNA-based typing to the same standard as this would need to be unacceptably low (i.e. the lowest common denominator). Both techniques are in general use, each having specific advantages and disadvantages, and under these circumstances professional judgement together with the following guidelines should be used to deliver an appropriate standard of HLA typing.

Caution should therefore be exercised if an HLA type assigned using DNA-based molecular techniques is converted into a serological equivalent and such conversion must always be avoided with alleles for which the phenotype has not been unequivocally defined.

16.4: Reagents

16.4.1: General guidelines

HLA reagents prepared from human source material should comply with the guidelines in section 11.1.4.10.

Exceptionally, reagents not tested at source as required in section 11.1.4.10, and for which no alternative exists, may be supplied for use with the expressed approval of the user and with the understanding that the reagent must be regarded as potentially infectious.

These reagents should be marked ‘Potentially infectious – not tested at source...’, as appropriate, both on the immediate container label or multi-well tray or reservoir, and the outer packaging.

The instructions for use of these reagents should indicate that the reagent(s) has not been tested at source as required in section 11.1.4.10, and that the reagents are to be considered as potentially infectious. In addition, the package insert should give information on the safe disposal of the material and the container, multi-well tray or reservoir.

16.4.1.1: Immediate container label
HLA reagents issued separately: The label should conform to the requirements of EN ISO 18113:2009. In addition, the body of a container presented in sealed bags or foiled pouches should be marked with a unique identifier to enable identification and traceability.

Multi-component test systems: In addition to the label information required above, a test system comprising multiple components should be marked to ensure identification and traceability of all components, for example multi-well trays or reservoirs, strips and pre-prepared membranes.

The instructions for use should contain the information required by EN ISO 18113:2009, and should comply, where applicable, with the requirements of section 11.1.4.12.

16.4.2: Serological typing reagents

The following information must be provided for each individual serological HLA typing reagent or HLA typing set:

- The claimed HLA specificity(ies) of the reagent, the percentage of specific reactions giving a cytotoxicity score of 80% to 100% cell death, the values of the correlation coefficient r obtained by the pre-testing of the reagent against a well-characterised cell panel, and the reaction score.

- The manufacturer should provide information of the incidence of equivocal cytotoxicity scores within the package insert.

- HLA typing sets should include a representation of the multi-well tray or reservoir layout indicating the position, HLA specificity(ies) and batch (or sub-batch) reference of the HLA typing reagent contained in each well.

- Monoclonal antibodies should be identified as such.

- An instruction that thawing and refreezing of the HLA typing reagents should be kept to the absolute minimum from the date of manufacture to the date of use. HLA typing sera frozen in micro-well trays should be used within 1 hour of thawing. Sera supplied freeze-dried in micro-well trays should be used within 1 hour of their reconstitution; unused trays should not be refrozen for later use.

- When reagents are supplied as an HLA typing set for the detection of a single antigen, the instructions for use should indicate which controls are appropriate to demonstrate specificity and cross-reactivity.

- For HLA typing sets, a list should be provided of those specificities that cannot be adequately defined in the presence of other specified specificities.

- Each HLA typing set for Class I or Class II phenotyping should contain at least one positive control antibody preparation, previously shown to react with all target cells, and should include at least one negative control preparation, previously shown to lack antibody activity or be from a male with no history of blood transfusion.

16.4.2.1: Preservation

HLA typing reagents may be preserved in the liquid or in the dried state. Reagents should be stored as recommended by the manufacturer.

HLA typing reagents, after being thawed or reconstituted, should be transparent and should not contain any sediment, gel or particles visible on microscopy (x 200).
16.4.2.2: Stability and expiry date

Manufacturers should ensure that HLA typing reagents have a shelf life of at least 1 year, when stored as recommended.

Any extension by the user of the expiry date stated by the manufacturer should be supported by documented test data.

Manufacturers should notify all primary users if an HLA typing reagent or a constituent reagent of an HLA typing set stored as recommended fails to perform satisfactorily within the expiry date allotted by the manufacturer.

16.4.3: Requirements for phenotype assignment

HLA Class I and Class II serological typing must comply with EFI Standards E1.000 to E2.740 inclusive. HLA reagents and kits to be used for phenotyping lymphocytes by cytotoxicity should comply with the following:

- HLA typing reagents, when used by all methods recommended by the producer, should react with all lymphocyte samples with the corresponding antigen(s) when tested against a panel of lymphocyte samples bearing those antigen(s) collected from at least 25 individuals. HLA typing reagents should not react with any lymphocyte samples when tested against a panel of lymphocyte samples known not to bear the corresponding antigen(s) collected from at least 100 individuals. Reagents that conform to the requirements of this paragraph are termed operationally monospecific.

- Not more than half of the HLA typing reagents used together to detect an antigen should have the same extra claimed specificity.

- None of the HLA typing reagents used together should have been shown to react with more than 5% of the separate samples of a lymphocyte panel which do not express any of the antigen(s) that the reagent is claimed to detect.

- Manufacturers should indicate in the instructions for use those specificities whose detection does not comply with the requirements of any of the above.

16.4.4: Rabbit complement for use in HLA serology

16.4.4.1: General guidelines

Rabbit complement supplied for use in HLA serology should be stored as recommended by the manufacturer.

Manufacturers should be aware that highly active complement can cause unwanted specificities to become apparent in HLA typing reagents that have been characterised on less active but adequate complement.

16.4.4.2: Immediate container label

The label of the immediate container of rabbit complement for use in HLA serology should conform to the specifications in section 11.1.4.11.

16.4.4.3: Instructions for use

The instructions for use supplied with rabbit complement for use in HLA serology should conform to the specifications in section 11.1.4.12.
The instructions for use should offer guidance on the method of thawing. In addition, they should contain an instruction that the complement, once thawed from the immediate container or reconstituted from the freeze-dried state, should not be refrozen.

The instructions for use should state whether the rabbit complement has been tested and found suitable for use with monoclonal HLA typing reagents.

16.4.4.4: Potency tests on rabbit complement for use in HLA serology

Rabbit complement should be tested prior to use, in accordance with EFI Standards E2.700–E2.740.

16.4.5: DNA typing reagents

Methods available for HLA typing of DNA samples rely on identification of polymorphic HLA gene sequence motifs. In all widely used methods, the polymerase chain reaction (PCR) is utilised, either through the use of sequence-specific primers as in PCR-SSP, or to produce a locus-specific DNA template (e.g. HLA-A) which can subsequently be typed using a panel of sequence-specific oligonucleotide probes (PCR-SSOP). The locus-specific template may also be directly sequenced using locus or allele group-specific sequencing primers.

DNA can be prepared from various tissues by a variety of methods. The laboratory should prepare DNA by a standard method that has been reported in the scientific literature and validated in the laboratory for the HLA typing method to be used.

16.4.5.1: Instructions for use

In addition to section 11.1.4.12 of these guidelines, the instructions for use must adhere to the EFI Standards for Nucleic Acid Analysis (Section L) and should include the following:

- a statement explaining the test and intended application of the kit
- the principle of the procedure
- reagents and equipment required to perform the test
- detailed instructions for all components of the test
- the gene targeted as a PCR amplification control (PCR-SSP)
- the specificity and nucleotide sequence of all primers and probes used in the HLA typing kit
- a table or diagram indicating the location of the probes and/or primers utilised in the test
- a list of ambiguous combinations of alleles defined for each test kit – this may also be given as part of interpretative software
- the HLA alleles which are claimed to be detected by the HLA typing kit, further divided into the following groups:
  - those HLA alleles which have been detected in appropriately controlled validation tests
  - those HLA alleles which have not been directly detected in validation tests but where the reactivity of the allele is expected to be detected
  - those HLA alleles which have not been directly detected in validation tests and whose reactivity cannot be assumed to be detected by the kit
• those HLA alleles that are known to produce weak or unreliable signals in the output systems
• the date and the source of the sequence information used in the kit design and a statement that new alleles described following the date of design may not be detected by the kit
• the control tests to be performed to check for contamination (negative control) of the test system
• the control DNA to be included to check for quality of sample DNA used
• the control test to be performed to generate a true positive signal
• acceptable limits of signal intensity should be specified for positive and negative results
• all computer software assisted interpretation of results should be validated on control DNA
• the chemical components of the kits should be listed and reference made to any toxic substances included in the kit with recommendations for their safe disposal. Reference to material safety data sheets should be given.

16.4.5.2: Requirements

Manufacturers should inform all primary users of a DNA-based HLA typing kit when any changes to a kit’s ability to perform are detected. All users of DNA-based HLA typing kits should report any kit-related problems directly to the manufacturer and maintain records of such events.

16.5: Testing of HLA genes and gene products

DNA-based methods must identify all HLA alleles included in the most recent WHO Nomenclature Committee for Factors of the HLA System Report and Nomenclature for Factors of the HLA System, 2010. Alleles should be reported either as individual alleles or as allele groups with two digits (first field). Definitions of allelic, high, intermediate and low resolution molecular HLA typing are available through EFI.

The minimum level of resolution by serological typing is given in Table 16.1. Typing to the level of broad specificities is acceptable but the higher level to include the split specificities, as indicated, is recommended. HLA-C types Cw12 and Cw14 to Cw18 have not been formally designated as recognised antigens and may not be identified serologically.

Table 16.1 HLA antigens that are defined by serological typing (with broad specificities shown in brackets)

<table>
<thead>
<tr>
<th>HLA-A</th>
<th>HLA-B</th>
<th>HLA-C</th>
<th>HLA-DR</th>
<th>HLA-DQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>B7</td>
<td>Cw1</td>
<td>DR1</td>
<td>DQ5 (1)</td>
</tr>
<tr>
<td>A2</td>
<td>B8</td>
<td>Cw2</td>
<td>DR15 (2)</td>
<td>DQ6 (1)</td>
</tr>
<tr>
<td>A3</td>
<td>B13</td>
<td>Cw9 (3)</td>
<td>DR16 (2)</td>
<td>DQ2</td>
</tr>
<tr>
<td>A11</td>
<td>B18</td>
<td>Cw10 (3)</td>
<td>DR17 (3)</td>
<td>DQ7 (3)</td>
</tr>
<tr>
<td>A23 (A9)</td>
<td>B27</td>
<td>Cw4</td>
<td>DR18 (3)</td>
<td>DQ8 (3)</td>
</tr>
<tr>
<td>A24 (A9)</td>
<td>B35</td>
<td>Cw5</td>
<td>DR4</td>
<td>DQ9 (3)</td>
</tr>
<tr>
<td>A25 (A10)</td>
<td>B37</td>
<td>Cw6</td>
<td>DR11 (5)</td>
<td>DQ4</td>
</tr>
<tr>
<td>A26 (A10)</td>
<td>B38 (B16)</td>
<td>Cw7</td>
<td>DR12 (5)</td>
<td></td>
</tr>
<tr>
<td>A29 (A19)</td>
<td>B39 (B16)</td>
<td>Cw8</td>
<td>DR13 (6)</td>
<td></td>
</tr>
<tr>
<td>A30 (A19)</td>
<td>B41</td>
<td>Cw12#</td>
<td>DR14 (6)</td>
<td></td>
</tr>
<tr>
<td>A31 (A19)</td>
<td>B42</td>
<td>Cw14#</td>
<td>DR7</td>
<td></td>
</tr>
<tr>
<td>A32 (A19)</td>
<td>B45</td>
<td>Cw15#</td>
<td>DR8</td>
<td></td>
</tr>
<tr>
<td>A33 (A19)</td>
<td>B44</td>
<td>Cw16#</td>
<td>DR9</td>
<td></td>
</tr>
<tr>
<td>A34 (A10)</td>
<td>B46</td>
<td>Cw17#</td>
<td>DR10</td>
<td></td>
</tr>
<tr>
<td>A36</td>
<td>B47</td>
<td>Cw18#</td>
<td>DR103</td>
<td></td>
</tr>
<tr>
<td>A43</td>
<td>B48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A66 (A10)</td>
<td>B49 (B21)</td>
<td></td>
<td>DR51</td>
<td></td>
</tr>
<tr>
<td>A68 (A28)</td>
<td>B50 (B21)</td>
<td></td>
<td>DR52</td>
<td></td>
</tr>
<tr>
<td>A69 (A28)</td>
<td>B51 (B5)</td>
<td></td>
<td>DR53</td>
<td></td>
</tr>
<tr>
<td>A74 (A19)</td>
<td>B52 (B5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A80</td>
<td>B53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B54 (B22)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B55 (B22)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B56 (B22)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B57 (B17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B58 (B17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B60 (B40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B61 (B40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B62 (B15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B63 (B15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B64 (B14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B65 (B14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B71 (B70)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B72 (B70)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B73</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# The products of the Cw12 and Cw14 to Cw18 genes have not been formally designated as recognised antigens and might not be identified serologically.

## 16.6: Testing for HLA-specific antibodies

### 16.6.1: General guidance

HLA-specific antibody screening and characterisation must comply with EFI Standards Section F (Antibody Screening and Crossmatching) and Section M (Flow Cytometry).

All commercial HLA antibody test kits should be CE and in vitro device (IVD) marked and validated for use. Each batch of commercial test kit or in-house panel should be evaluated against a minimum of three sera of known HLA specificity from different cross-reacting groups.

HLA-specific antibodies may be detected using reagent lymphocytes (or cell lines), solid-phase bound, purified HLA molecules, or particle bound, purified HLA molecules. If such techniques are used for screening (i.e. not characterisation of specificity) the following apply:

- There should be discrimination between HLA Class I and Class II-specific antibodies.
- Overall the target cells or molecules should cover either all the known HLA immunogenic epitopes or all HLA specificities (Class I, Class II, or both as appropriate) found in the population at over 0.5%.

### 16.6.2: Characterisation of antibody specificity

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.
Panels of HLA typed cells or purified HLA molecules are used for identification. 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.

There are many techniques available for the detection of HLA antibodies such as those developed for the detection and identification of HLA antibodies utilising Luminex microspheres. These assays are highly sensitive, leading to the detection of very low levels of HLA antibodies. 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

<table>
<thead>
<tr>
<th>HLA-A broad specificities</th>
<th>Splits</th>
<th>HLA-B broad specificities</th>
<th>Splits</th>
<th>HLA-C broad specificities</th>
<th>Splits</th>
<th>HLA-DR broad specificities</th>
<th>Splits</th>
<th>HLA-DQ broad specificities</th>
<th>Splits</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>B5</td>
<td>B51</td>
<td>Cw1</td>
<td>DR1</td>
<td>DQ1</td>
<td>DQ5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>B5</td>
<td>B52</td>
<td>Cw2</td>
<td>DR103</td>
<td>DQ1</td>
<td>DQ6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>B7</td>
<td></td>
<td>Cw3</td>
<td>Cw9</td>
<td>DR2</td>
<td>DR15</td>
<td>DQ2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A9</td>
<td>A23</td>
<td>B8</td>
<td>Cw3</td>
<td>Cw10</td>
<td>DR2</td>
<td>DR16</td>
<td>DQ3</td>
<td>DQ7</td>
<td></td>
</tr>
<tr>
<td>A9</td>
<td>A24</td>
<td>B12</td>
<td>B44</td>
<td>Cw4</td>
<td>DR3</td>
<td>DR17</td>
<td>DQ3</td>
<td>DQ8</td>
<td></td>
</tr>
<tr>
<td>A10</td>
<td>A25</td>
<td>B12</td>
<td>B45</td>
<td>Cw5</td>
<td>DR3</td>
<td>DR18</td>
<td>DQ3</td>
<td>DQ9</td>
<td></td>
</tr>
<tr>
<td>A10</td>
<td>A26</td>
<td>B13</td>
<td>Cw6</td>
<td>DR4</td>
<td></td>
<td></td>
<td>DQ4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A10</td>
<td>A34</td>
<td>B14</td>
<td>B64</td>
<td>Cw7</td>
<td>DR5</td>
<td>DR11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A10</td>
<td>A66</td>
<td>B14</td>
<td>B65</td>
<td>Cw8</td>
<td>DR5</td>
<td>DR12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A11</td>
<td>B15</td>
<td>B62</td>
<td>Cw12</td>
<td>DR6</td>
<td>DR13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A19</td>
<td>A29</td>
<td>B15</td>
<td>B63</td>
<td>Cw14</td>
<td>DR6</td>
<td>DR14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A19</td>
<td>A30</td>
<td>B15</td>
<td>B75</td>
<td>Cw15</td>
<td>DR7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A19</td>
<td>A31</td>
<td>B15</td>
<td>B76</td>
<td>Cw16</td>
<td>DR8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>------</td>
<td>-----</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A19</td>
<td>A32</td>
<td>B15</td>
<td>B77</td>
<td>Cw17</td>
<td>DR9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A19</td>
<td>A33</td>
<td>B16</td>
<td>B38</td>
<td>Cw18</td>
<td>DR10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A19</td>
<td>A74</td>
<td>B16</td>
<td>B39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A28</td>
<td>A68</td>
<td>B17</td>
<td>B57</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A28</td>
<td>A69</td>
<td>B17</td>
<td>B58</td>
<td></td>
<td>DR51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A36</td>
<td>B18</td>
<td></td>
<td></td>
<td></td>
<td>DR52</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A43</td>
<td>B21</td>
<td>B49</td>
<td></td>
<td></td>
<td>DR53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A80</td>
<td>B21</td>
<td>B50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B22</td>
<td>B54</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B22</td>
<td>B55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B22</td>
<td>B56</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B37</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B40</td>
<td>B60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B40</td>
<td>B61</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
16.6.2.1: HLA antibody characterisation by complement dependent cytotoxicity

*Rabbit complement*

Rabbit complement used for detection of HLA antibodies by complement dependent cytotoxicity should comply with guidelines in section 16.4.4.

*Instructions for use*
In addition to the information required in section 16.6.2, the instructions for use should include the following information on each individual preparation of HLA reagent lymphocytes or set of HLA reagent lymphocytes:

- the HLA phenotype of the reagent lymphocytes
- the nature of the HLA reagent lymphocytes (e.g. normal peripheral lymphocytes, separated peripheral B lymphocytes, separated peripheral T lymphocytes, chronic lymphocytic leukemia (CLL) cells, splenic lymphocytes, lymph node lymphocytes, lymphoblastoid cell line)
- the concentration of the lymphocyte suspension should be stated in the instructions for use for HLA reagent lymphocytes issued in individual immediate containers, or on the phenotype listing of batches issued as multi-immediate container products
- HLA reagent lymphocyte sets issued in multi-well trays should include a representation of the tray or reservoir layout indicating the location of the various HLA reagent lymphocytes in the wells of the tray
- for HLA reagent lymphocyte sets issued in multi-well trays or reservoirs the phenotype information may take the form of a listing of the phenotypes of each of the individual donations comprising the set
- the shelf life of the HLA reagent lymphocytes following recovery from long-term storage and subsequent storage in conditions recommended by the manufacturer should be stated in the instructions for use
- when HLA reagent lymphocytes are provided suspended in preservative or medium, the components of the preservative or the name of the medium should be stated in the instructions for use.

**Reagent lymphocytes**

Freshly isolated or previously frozen lymphocytes should have a viability of at least 80% and should contain less than 1% platelets or granulocytes.

Reagent B lymphocytes isolated for the identification of Class II antibodies should contain less than 10% of non-B cells.

The background incidence of spontaneous cell death, as assessed by a negative control serum, should be less than 30%.

Reagent lymphocytes supplied as previously frozen in test trays should contain 1000 to 2000 lymphocytes per well, after recovery following manufacturer’s instructions.

The manufacturer should specify in the instructions for use those antigens known to be present or absent, and those for which no testing has been performed. HLA-A, HLA-B, HLA-C, HLA-DR and HLA-DQ serologically defined specificities should be included in this statement.

**16.6.2.2: HLA antibody characterisation by solid-phase and particle bound methods**

Purified HLA captured onto a microtitre well, nylon membrane or microparticles can be used as sensor molecules for characterising sera containing HLA-specific antibodies.

Antibody binding can be detected by ELISA or fluorescence. The detector reagent should be able to identify IgG and discriminate between IgG, IgA and IgM.

**Human material**
If a product is prepared from human source material then the guidance in section 11.1.4.10 must be followed.

**Instructions for use**

The instructions for use must comply with the requirements of EN ISO 18113:2009 and the information required in section 16.6.1. In addition, the instructions for use should include the following information on each individual preparation or component of a set of HLA screening product:

- the HLA antigens represented in each container
- the concentration of any cells or particles in suspension should be stated in the instructions for use of HLA screening product issued in individual immediate containers or on the antigen information table of batches issued as multi-immediate container products or multi-well trays or reservoirs
- HLA screening products issued in multi-well trays should include a representation of the tray or reservoir layout indicating the location of the HLA antigens in the wells of the tray
- the expiry life of the HLA screening product following reconstitution or preparation and subsequent storage in conditions recommended by the manufacturer should be stated
- when components of an HLA screening product contains preservatives the name of the chemical preservatives and the components which contain them should be stated

**Validation**

Kits for the detection of HLA antibodies should be validated for sensitivity and specificity on a batch basis using a panel of clinically representative HLA antisera. A panel of sera shown to be inert for HNA and HLA antibodies should also be used.

### 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.

**16.8: Application of HLA/HPA testing to patients and donors**

**16.8.1: Donor and patient testing**

The most common cause of immunological refractoriness to random donor platelet transfusion is the presence of HLA-specific antibodies in the patient receiving platelet transfusion. The management of this group of patients may involve the provision of HLA-compatible platelets and/or crossmatch-negative donors.

HLA Class I typed platelets should normally be provided for refractory patients with the aim of minimising exposure to mismatched Class I antigens. In the absence of a zero mismatched donor, a compatible donor
can be selected on the basis of a lack of antigens or alleles corresponding to the antibody specificities identified in the patient. Where a patient's antibodies have not been characterised, a crossmatch can be performed; however, it is best practice to establish the patient's antibody specificities if long-term platelet support is envisaged.

There are several crossmatch techniques for the detection of donor reactive antibodies that may involve the use of donor lymphocytes or donor platelets. The basic principle is the same for most of the techniques in that serum or plasma from the patient is incubated with donor cells and reactivity is detected by flow cytometry or cytotoxicity. Platelets from donors negative in the crossmatch testing may be used for transfusion of the patient whose serum has been crossmatched.

HLA and/or granulocyte-specific antibodies present in donor plasma have been implicated in nearly 80% of TRALI cases (patient leucocyte antibody or inter-donor reactions in pooled products have also been reported as causes of TRALI). The identification of leucocyte-specific antibodies in implicated donors provides support for the diagnosis of TRALI.

### 16.8.2: Apheresis platelet donors

All potential plateletpheresis donors used for the provision of HLA selected platelets should be typed for HLA-A, HLA-B and HLA-C. If serological typing is used the minimum level of typing should be for the HLA Class I specificities listed in Table 16.1. For all donors HLA-Bw4 or HLA-Bw6 should be assigned.

If DNA-based typing is performed on donors a typing strategy should be employed that allows for HLA alleles to be defined at least to the two-digit (first field) level of resolution. Typing should also be capable of determining the presence of the Bw4 and Bw6 epitopes.

Each donor should be HLA typed twice using samples collected on separate occasions, such that only if the second test confirms the first should the donor provide platelets for clinical use.

### 16.8.3: Testing of donors/cord units for related haematopoietic stem cell transplant (EFI Standard I1.000)

DNA-based HLA typing, to at least the two-digit (first field) level of resolution, should be performed on donors. High-resolution typing may also be necessary as detailed below.

Initially, all potential related stem cell donors must be typed for at least HLA-A, HLA-B and/or HLA-DR to assess compatibility. Further testing must then be undertaken to establish a phenotypic match for HLA Class I and II loci, as described in local protocols. HLA types of the matched patient and donor must be confirmed on a second sample. If HLA haplotype inheritance can be established by typing family members, then high-resolution typing is not required to establish a genotypic match. However, if haplotype inheritance is not established, high-resolution typing of HLA Class I and/or Class II should be undertaken as required by the local transplant protocol. Intra-familial donors who are not HLA identical siblings require both Class I and Class II high-resolution typing as required by the local transplant protocol.

As a minimum related cord units must be typed at low resolution for HLA-A, -B and -DRB1. Extended typing must be undertaken if required by the transplant protocol.

Prior to cord unit transplant, confirmatory typing at low resolution must be performed for HLA-A, -B and -DRB1. Typing must be performed on a segment of the tubing integrally attached to the unit, on a satellite vial or on the content of the thawed unit.

### 16.8.4: Testing of donors/cord units for unrelated haematopoietic stem cell transplant (EFI Standard I2.000)
DNA-based HLA typing, to at least the two-digit (first field) level of resolution, should be performed on donors. High-resolution typing may also be necessary as detailed below.

As a minimum all potential unrelated donors should be typed for HLA-A, -B, -C and -DRB1. HLA types of patient and donor should be confirmed, although the original type from the unrelated donor registry is acceptable for this purpose. The need for high-resolution typing of HLA Class I and II will depend upon local transplant protocols.

As a minimum cord units must be typed at low resolution for HLA-A and -B and high resolution for -DRB1. Extended typing must be performed if required by the local transplant protocol. Prior to commencement of patient conditioning, a minimum low-resolution confirmatory type of at least HLA-A, -B and -DRB1 must be performed upon receipt of the shipped unit. Typing must be performed on a segment of the tubing integrally attached to the unit, on a satellite vial or on the content of the thawed unit.
Figure 16.1 Algorithm for laboratory investigation of platelet refractoriness

### 16.8.5: Investigation of refractoriness

The investigation of refractoriness (see Figure 16.1) and the provision of selected platelets in such cases should comply with the British Committee for Standards in Haematology (BCSH) Guidelines for the Use of Platelet Transfusions. Serological investigation of suspected immune refractoriness requires screening for...
HLA Class I-specific antibodies only, but the screening technique must detect HLA-A, HLA-B, and HLA-C-specific antibodies. Any screen-positive patient should be tested further for specificity to include all the Class I antigens listed in Table 16.2.

If a patient has HLA-specific antibodies that cannot be completely characterised, or a specificity corresponding to any of the donor’s HLA Class I antigens cannot be excluded, then a crossmatch between donor and patient may be performed as described above.

16.8.6: Investigation of TRALI

Sera from all implicated donors must be screened for both HLA Class I and Class II-specific antibodies and HNA antibodies (see section 16.6.2).

Any screen-positive serum should be further characterised for HLA Class I and Class II to identify the antibody specificity.

If any of the implicated donors are shown to have HLA-specific antibodies the patient should be typed for HLA Class I and Class II to determine the presence of alleles/antigens corresponding to the antibody specificities found in the donor(s).

If a donor serum has HLA-specific antibodies that cannot be completely characterised, or a specificity corresponding to any of the patient’s HLA antigens cannot be excluded, then a crossmatch between donor and patient should be performed.

See Figure 16.2 which gives an algorithm for laboratory investigation of TRALI.
Figure 16.2 Algorithm for laboratory investigation and reporting of TRALI case

16.8.7: Investigation of febrile transfusion reactions
If an investigation is requested, sera from patients should be screened for both HLA Class I and Class II-specific antibodies. Any screen-positive serum should be further characterised for HLA Class I and Class II specificities to include all those listed in Table 16.2.

16.8.8: Investigation of female donors to reduce the incidence of TRALI

Many transfusion services have introduced screening for HLA or HLA and HNA antibodies to reduce the incidence of TRALI. An initial screen for HLA antibodies may be followed by a screen for HNA antibodies to further reduce the potential incidence of TRALI (see section 16.8.6). Female blood donors should be investigated for HLA antibodies following the guidelines set out in section 16.6. There is no requirement to determine the specificity of any HLA antibodies detected or type the donor for HLA.

16.9: References