

Guidelines for the Blood Transfusion and Tissue Transplantation Services in the UK

16. HLA typing and HLA serology

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16.1. Preamble

DNA-based testing for HLA alleles has now completely replaced serological phenotyping and antibody detection/characterisation involves non-cell-based methods. This HLA section is constructed of three main parts, concerning:

- Reagents (chapter 16.4)
- Testing (chapter 16.5, chapter 16.6 and chapter 16.7)
- Application to donor and patient investigations (chapter 16.8)

For certain patient or donor investigations there is, of course, an overlap with the guidance given in this and the granulocyte/platelet immunology chapters (chapter 17 and chapter 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-specific and human neutrophil antigen (HNA)-specific antibodies and the patient is HLA typed if possible.

The [European Federation for Immunogenetics \(EFI\)](#) has established standards (at the time of writing, version 8.0) for histocompatibility testing and where appropriate the relevant EFI Standards must be followed.

16.3. Terminology and nomenclature

All HLA assignments, irrespective of the method, must comply with the latest report of the WHO Nomenclature Committee for Factors of the HLA System [Marsh, 2010].

HLA typing is now performed by DNA molecular analysis.

HLA typing by DNA-based techniques employs either sequencing or DNA-based probes/primers to 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.

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 [Holdsworth, 2009].

HLA typing results must conform to the recommendations of the WHO Nomenclature committee. Examples of suitable reporting formats as referenced in [EFI Standards v.8.0](#) include the following:

Single alleles	HLA-B*07
Single antigens	HLA-B7
DNA assignment	HLA-A*02,*30; B*07,*44; C*07,*16; DRB1*01,*04; DQB1*05, *03:01
Serological assignment	HLA-A2,30; B7,44; Cw7; DR1,4; DQ5,7

If an HLA typing is performed using DNA methods, it is acceptable to report an HLA serological assignment if required for the purposes of, for example, HLA-matched platelet allocation. The translation of alleles to serological equivalence must be performed according to a documented protocol.

Caution should 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.

References

Holdsworth R et al. (2009). The HLA dictionary 2008: a summary of HLA-A, -B, -C, -DRB1/3/4/5, and -DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR, and -DQ antigens. *Tissue Antigens*, 73, 95–170. <https://doi.org/10.1111/j.1399-0039.2008.01183.x>

Marsh SGE (2010). Nomenclature for Factors of the HLA System. *Tissue Antigens*, 65, 291–455. <https://doi.org/10.1111/tan.15495>

16.4. Reagents

16.4.1. 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.

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.

16.4.1.1. Instructions for use

In addition to chapter 11.1.4.12, the instructions for use must adhere to the relevant EFI Standards 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.

16.4.1.2. 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.

16.4.2. HLA antibody testing reagents

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 3 sera of known HLA specificity from different cross-reacting groups.

HLA-specific antibodies may be detected by 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%.

16.4.2.1. Instructions for use

The instructions for use must comply with the requirements of EN ISO 18113:2009 and the information required in chapter 16.6. 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 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.

16.4.3. External quality assessment schemes

Laboratories should take part in regular external quality assessment (EQA) exercises such as the UK National External Quality Assessment Scheme (NEQAS) for Histocompatibility and Immunogenetics schemes. Effective mechanisms should be in place to correct poor performance in EQA schemes.

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 (available at the [WHO Nomenclature Committee website](https://www.who.int/publications/m/item/who-nomenclature-committee-report)) and Nomenclature for Factors of the HLA System [Marsh, 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 EFL.

The minimum level of resolution by serological typing is given below for each HLA antigen. Typing to the level of broad specificities (shown in brackets) 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.

HLA-A

A1
A2
A3
A11
A23 (A9)
A24 (A9)
A25 (A10)
A26 (A10)
A29 (A19)
A30 (A19)
A31 (A19)
A32 (A19)
A33 (A19)
A34 (A10)
A36
A43
A66 (A10)
A68 (A28)
A69 (A28)
A74 (A19)
A80

HLA-B

B7
B8
B13
B18
B27
B35
B37
B38 (B16)
B39 (B16)
B41
B42
B45
B44
B46
B47
B48
B49 (B21)
B50 (B21)
B51 (B5)
B52 (B5)
B53
B54 (B22)
B55 (B22)
B56 (B22)
B57 (B17)
B58 (B17)
B59
B60 (B40)
B61 (B40)
B62 (B15)
B63 (B15)
B64 (B14)
B65 (B14)
B67
B71 (B70)
B72 (B70)
B73
B75 (B15)
B76 (B15)
B77 (B15)
B78
B81
Bw4
Bw6

HLA-C

Cw1
Cw2
Cw9 (3)
Cw10 (3)
Cw4
Cw5
Cw6
Cw7
Cw8
Cw12*
Cw14*
Cw15*
Cw16*
Cw17*
Cw18*

(*) The products of the Cw12 and Cw14 to Cw18 genes have not been formally designated as recognised antigens and might not be identified serologically.

HLA-DR

DR1
DR15 (2)
DR16 (2)
DR17 (3)
DR18 (3)
DR4
DR11 (5)
DR12 (5)
DR13 (6)
DR14 (6)
DR7
DR8
DR9
DR10
DR103
DR51
DR52
DR53

HLA-DQ

DQ5 (1)
DQ6 (1)
DQ2
DQ7 (3)
DQ8 (3)
DQ9 (3)
DQ4

References

Marsh SGE (2010). Nomenclature for Factors of the HLA System. Tissue Antigens, 65, 291–455. <https://doi.org/10.1111/j.1399-0039.2010.01466.x>

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 2 fluorochromes resulting in a unique signal for each bead and which have 1 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)-labelled anti-human 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 2 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 characterised and HLA specificities accurately determined. This technology is now considered essential for the pretransplant testing of sensitised patients.

The composition of the panel should be sufficient to discriminate the specificities (Class I, Class II, or both as appropriate) given in Tables 16.2.1 to 16.2.5. The full list of antigens comprising a panel should be supplied and typed to the higher level of resolution shown in the tables.

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 4-digit (second field) level (e.g. HLA-A*02:01) and null alleles identified.

Table 16.2.1: Characterisation of HLA-specific antibodies – HLA-A

HLA-A broad specificities	Splits
A1	Not applicable
A2	Not applicable
A3	Not applicable
A9	A23
A9	A24
A10	A25
A10	A34
A10	A66
A11	Not applicable
A19	A29
A19	A30
A19	A31
A19	A32
A19	A33
A19	A74
A28	A68
A28	A69
A36	Not applicable
A43	Not applicable
A80	Not applicable

Table 16.2.2: Characterisation of HLA-specific antibodies – HLA-B

HLA-B broad specificities	Splits
B5	B51
B5	B52
B7	Not applicable
B8	Not applicable
B12	B44
B12	B45
B13	Not applicable
B14	B64
B14	B65
B15	B62
B15	B63

HLA-B broad specificities	Splits
B15	B75
B15	B76
B15	B77
B16	B38
B16	B39
B17	B57
B17	B58
B18	Not applicable
B21	B49
B21	B50
B22	B54
B22	B55
B22	B56
B27	Not applicable
B35	Not applicable
B37	Not applicable
B40	B60
B40	B61
B41	Not applicable
B42	Not applicable
B46	Not applicable
B47	Not applicable
B48	Not applicable
B53	Not applicable
B59	Not applicable
B67	Not applicable
B70	B71
B70	B72
B73	Not applicable
B78	Not applicable
B81	Not applicable
Bw5	Not applicable
Bw6	Not applicable

Table 16.2.3: Characterisation of HLA-specific antibodies – HLA-C

HLA-C broad specificities	Splits
Cw1	Not applicable
Cw2	Not applicable
Cw3	Cw9
Cw3	Cw10
Cw4	Not applicable
Cw5	Not applicable
Cw6	Not applicable
Cw7	Not applicable
Cw8	Not applicable
Cw12	Not applicable
Cw13	Not applicable
Cw15	Not applicable
Cw16	Not applicable
Cw17	Not applicable
Cw18	Not applicable

Table 16.2.4: Characterisation of HLA-specific antibodies – HLA-DR

HLA-DR broad specificities	Splits
DR1	Not applicable
DR103	Not applicable
DR2	DR15
DR2	DR16
DR3	DR17
DR3	DR18
DR4	Not applicable
DR5	DR11
DR5	DR12
DR6	DR14
DR6	DR14
DR7	Not applicable
DR8	Not applicable
DR9	Not applicable
DR10	Not applicable
DR51	Not applicable
DR52	Not applicable
DR53	Not applicable

Table 16.2.5: Characterisation of HLA-specific antibodies – HLA-DQ

HLA-DQ broad specificities	Splits
DQ1	DQ5
DQ1	DQ6
DQ2	Not applicable
DQ3	DQ7
DQ3	DQ8
DQ3	DQ9
DQ4	Not applicable

16.7. Leucocyte crossmatching in blood transfusion

Crossmatching may be used in the diagnosis of transfusion-related acute lung injury (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 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. 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 2-colour or 3-colour FCXM should be used with 1 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) with phycoerythrin (PE) and anti-CD19 (B cells) with 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 relevant EFI Standards.

16.7.1.1. 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 1 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 testing to patient and donor investigations

16.8.1. Investigation of refractoriness

Please refer to the relevant EFI Standards for Transfusion.

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

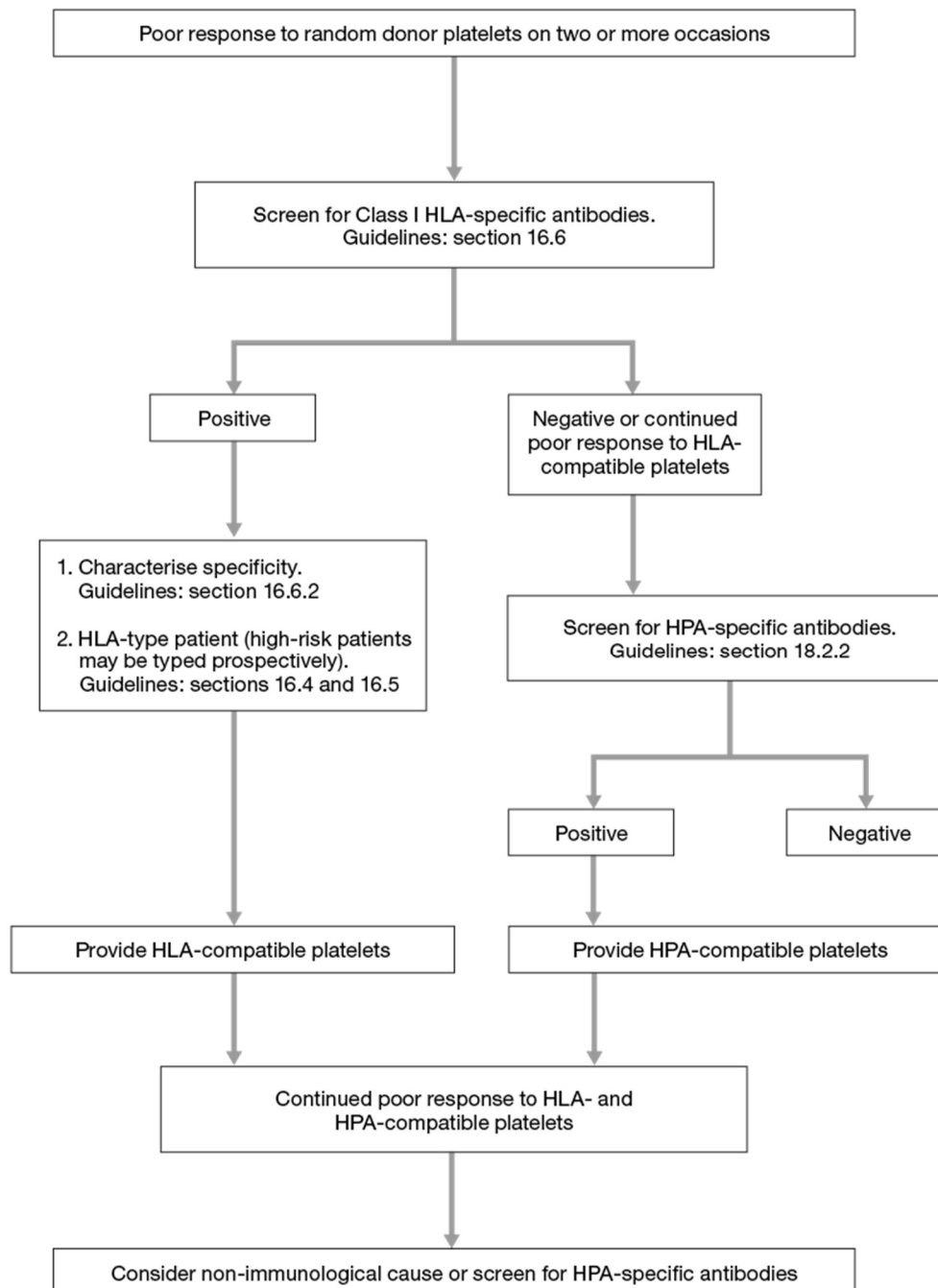
HLA Class I typed platelets should normally be provided for refractory patients with the aim of minimising exposure to mismatched Class I antigens. In the absence of a zero mismatched donor, a compatible donor can be selected on the basis of a lack of antigens or alleles corresponding to the antibody specificities identified in the patient.

The investigation of refractoriness (see Figure 16.1) and the provision of selected platelets in such cases should comply with the British Society for Haematology (BSH) Guidelines for the Use of Platelet Transfusions [BSH, 2017].

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 16.2.1 to Table 16.2.5.

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.

Figure 16.1: Algorithm for laboratory investigation of platelet refractoriness



16.8.2. Investigation of TRALI

Please refer to the relevant EFI Standards for Transfusion.

HLA and/or granulocyte-specific antibodies present in donor plasma have been implicated in nearly 80% of transfusion-related acute lung injury (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.

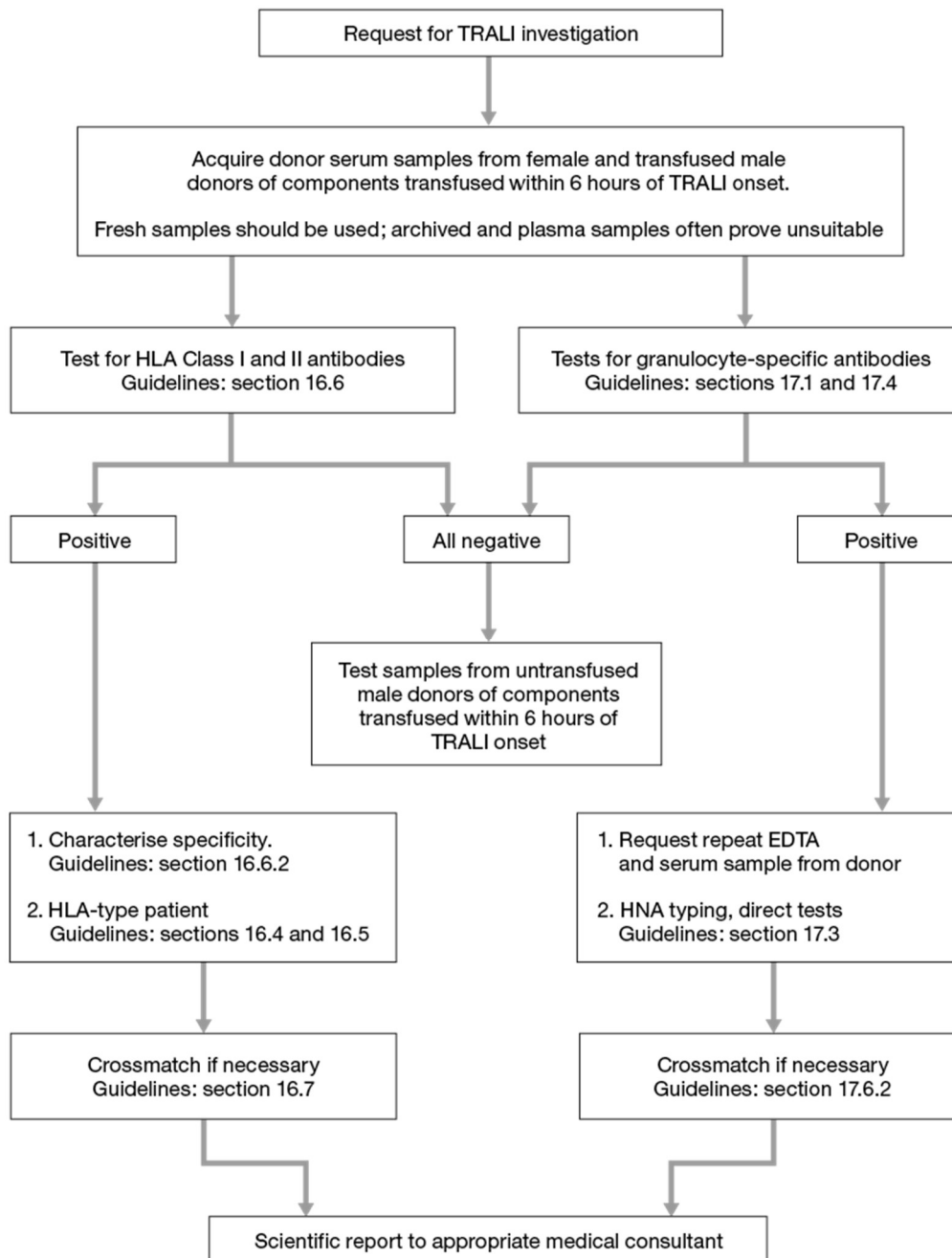
Sera from all implicated donors must be screened for both HLA Class I and Class II specific antibodies and HNA antibodies (see chapter 16.6.2). Any screen positive serum should be further characterised for HLA Class I and Class II to identify the antibody specificity

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.

Figure 16.2 gives an algorithm for laboratory investigation of TRALI.

Figure 16.2: Algorithm for laboratory investigation and reporting of TRALI case



16.8.3. Investigation of febrile transfusion reactions

Please refer to the relevant EFI Standards for Transfusion.

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 Tables 16.2.1 to 16.2.5.

16.8.4. Apheresis platelet donors

Please refer to the relevant EFI Standards for Transfusion.

All potential apheresis platelet donors used for the provision of HLA selected platelets should be typed for HLA-A, HLA-B and HLA-C.

DNA-based typing should allow for HLA alleles to be defined to at least the 2-digit (first field) level of resolution.

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.

16.8.5. Testing of donors/cord units for related haematopoietic stem cell transplant

Please refer to relevant EFI standards for Haematopoietic Stem Cell Transplantation.

DNA-based HLA typing, to at least the 2-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.

16.8.6. Testing of donors/cord units for unrelated haematopoietic stem cell transplant

Please refer to relevant EFI standards for Haematopoietic Stem Cell Transplantation.

DNA-based HLA typing, to at least the 2-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.

16.8.7. Investigation of female donors to reduce the incidence of TRALI

Many transfusion services screen 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 16.8.6 above).

Female blood donors should be investigated for HLA antibodies following the guidelines set out in chapter 16.6. There is no requirement to determine the specificity of any HLA antibodies detected or type the donor for HLA.

References

British Society for Haematology (2017). Guidelines for the Use of Platelet Transfusions. British Journal of Haematology, 176(3):365-394. <https://doi.org/10.1111/bjh.14423>