Guidelines for the Blood Transfusion Services

Chapter 16: HLA typing and HLA serology

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Chapter 16:

HLA typing and HLA serology

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 (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/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 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.

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.[4]

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 e.g. 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.

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 section 11.1.4.12 of these guidelines, 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 three 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 section 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 (EQA) Schemes

Laboratories should take part in regular external quality assessment exercises such as the UK 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[2] and Nomenclature for Factors of the HLA System, 2010[3] . 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)

HLA-A	HLA-B	HLA-C	HLA-DR	HLA-DQ
A1	B7	Cw1	DR1	DQ5 (1)
A2	B8	Cw2	DR15 (2)	DQ6 (1)
A3	B13	Cw9 (3)	DR16 (2)	DQ2
A11	B18	Cw10 (3)	DR17 (3)	DQ7 (3)
A23 (A9)	B27	Cw4	DR18 (3)	DQ8 (3)
A24 (A9)	B35	Cw5	DR4	DQ9 (3)

A25 (A10)	B37	Cw6	DR11 (5)	DQ4
A26 (A10)	B38 (B16)	Cw7	DR12 (5)	
A29 (A19)	B39 (B16)	Cw8	DR13 (6)	
A30 (A19)	B41	Cw12*	DR14 (6)	
A31 (A19)	B42	Cw14*	DR7	
A32 (A19)	B45	Cw15*	DR8	
A33 (A19)	B44	Cw16*	DR9	
A34 (A10)	B46	Cw17*	DR10	
A36	B47	Cw18*	DR103	
A43	B48			
A66 (A10)	B49 (B21)		DR51	
A68 (A28)	B50 (B21)		DR52	
A69 (A28)	B51 (B5)		DR53	
A74 (A19)	B52 (B5)			
A80	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			
* 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

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							

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 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 microlymphocytoxicity 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 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 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 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 Committee for Standards in Haematology (BCSH) Guidelines for the Use of Platelet Transfusions5. 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.

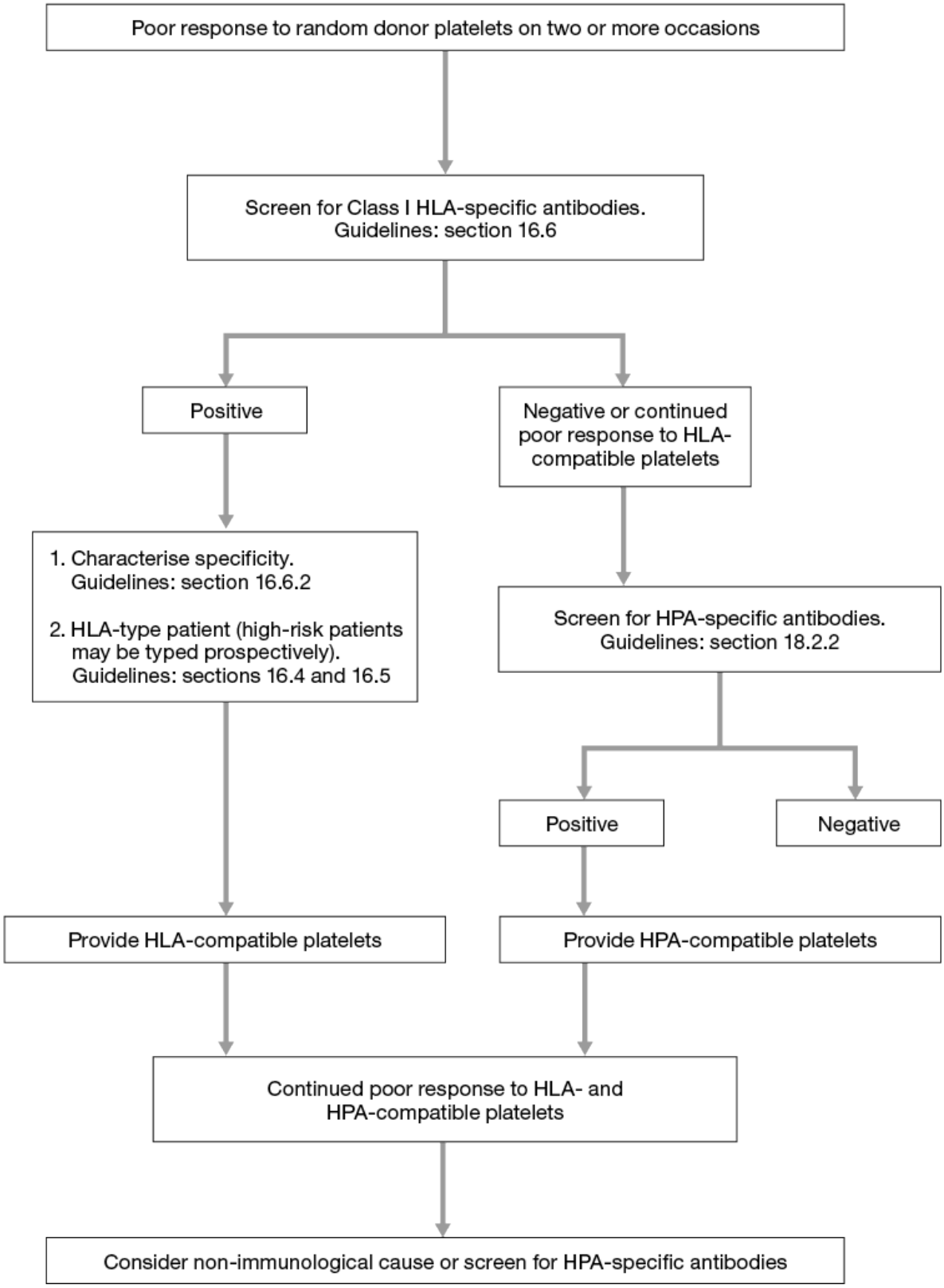

Figure 16.1 Algorithm for laboratory investigation of platelet refractoriness

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 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 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.

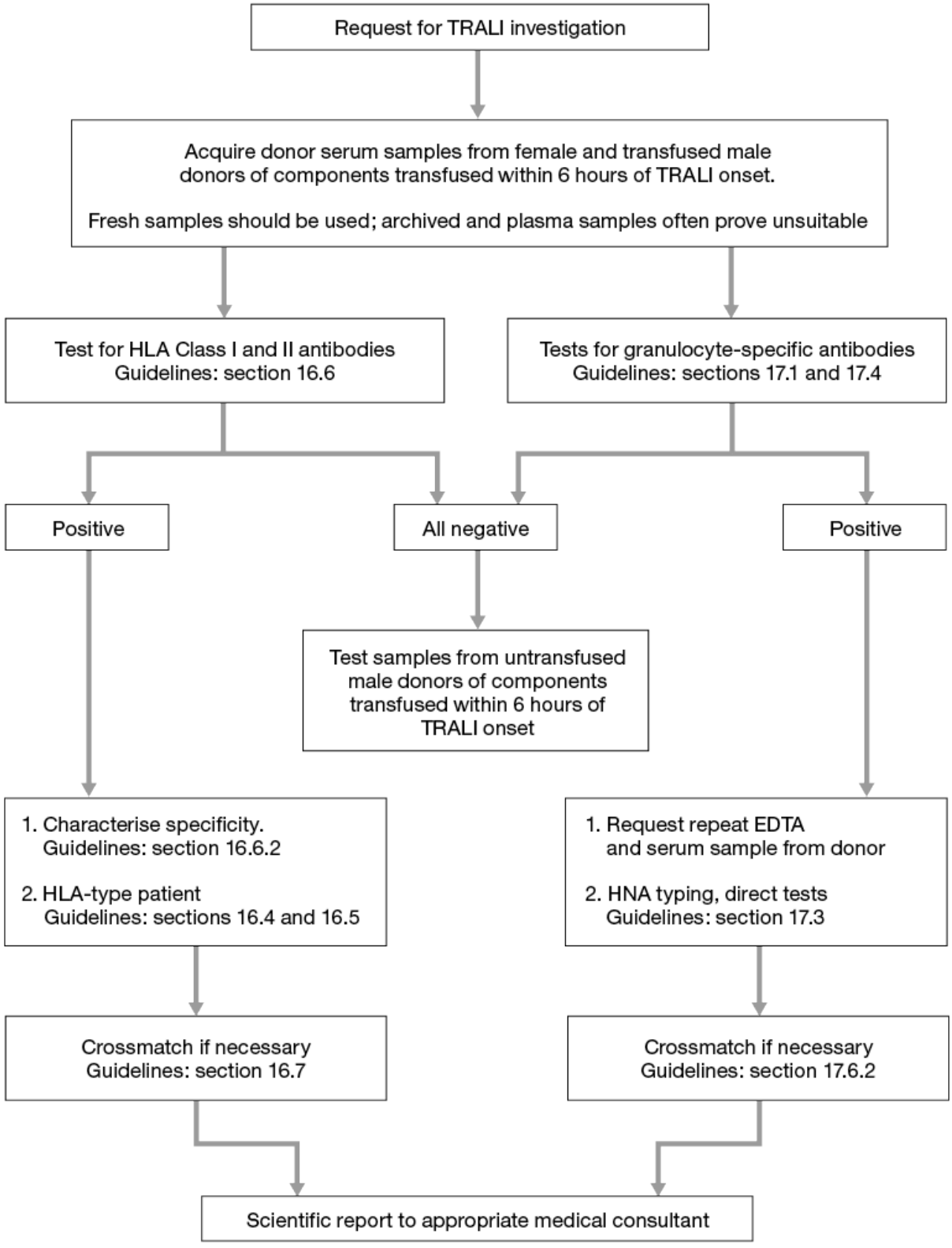

Figure 16.2 Algorithm for laboratory investigation and reporting of TRALI case

16.8.3: 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 Table 16.2.

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 two-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 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.

16.8.6: 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 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.

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 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

1. The European Federation for Immunogenetics (EFI) Standards. Available at www.efiweb.eu
2. WHO Nomenclature Committee for Factors of the HLA System Report. Available at https://hla.alleles.org/nomenclature/index.html
3. Marsh SGE (2010). Nomenclature for Factors of the HLA System. *Tissue Antigens*, 65, 291–455.
4. 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.
5. British Committee for Standards in Haematology (2017). Guidelines for the Use of Platelet Transfusions. *British Journal of Haematology*, 176(3):365-394.