

European Federation for Immunogenetics



STANDARDS FOR HISTOCOMPATIBILITY & IMMUNOGENETICS TESTING

Version 6.2

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SECTION A – GENERAL POLICIES

A1	These Standards have been approved and adopted by the EFI Executive Committee
A2	They are based on Standards originally prepared by the American Society for Histocompatibility and Immunogenetics (ASHI).
A3	These Standards have been established for the purpose of ensuring accurate and dependable histocompatibility testing consistent with the current state of technological procedures and the availability of reagents.
A4	These Standards establish minimal criteria, which all histocompatibility laboratories must meet if their services are to be considered acceptable.
A5	Many laboratories, because of extensive experience, will exceed the minimal requirements of these Standards.
A6	Certain Standards are obligatory. In these instances, the Standards use the word "must".
A7	Some Standards are highly recommended but not absolutely mandatory. In these instances the Standards use words like "should" or "recommended".
A8	Procedures to be used in histocompatibility testing often have multiple acceptable variations. The accuracy and dependability of each procedure must be documented in each laboratory or by published data from other laboratories.
A9	Some procedures have sufficient documentation of effectiveness to warrant their use in clinical service even though they are not available in or obligatory for all laboratories.
A10	The use of the name of the European Federation for Immunogenetics as certification of compliance to these Standards may only be made by laboratories, which have been accredited through the EFI accreditation process

SECTION B – PERSONNEL QUALIFICATIONS

B0	For the purposes of this document, EFI defines the Director as the person who is responsible for the H&I laboratory.
B1	The laboratory must employ one or more individuals who meet the qualifications and fulfil the responsibilities of:
B1.1	A Director, that must:
B1.1.1	Hold a qualification approved by EFI, such as an earned doctoral degree in a biological science, or be a physician, and
B1.1.2	Have minimum qualifying experience equivalent to either of the following:
B1.1.2.1	Four years' relevant experience two of which were devoted to full time training in human H&I testing, or
B1.1.2.2	Four years of working experience at full time in human H&I testing
B1.1.2.3	Additional qualifications required according to national legislation also apply.
B1.1.3	Have documentation of professional competence in the appropriate activities in which the laboratory is engaged. This should be based on sound knowledge of the fundamentals of immunology, genetics and histocompatibility testing
B1.1.4	If a Co-Director is appointed, this person must also fulfil Standards B1.1.1 - B1.1.3
B1.1.5	The Director and/or Co-Director must:
B1.1.5.1	Be available on site to supervise the laboratory for at least 80% of the week
B1.1.5.2	Provide adequate supervision of technical personnel
B1.1.5.3	Utilises his/her special scientific skills in developing new procedures
B1.1.5.4	Be held responsible for the proper performance, interpretation and reporting of all laboratory procedures
B1.1.5.5	Ensure the laboratory's successful participation in Proficiency testing.
B1.1.5.6	Be informed of the relevant national legislation
B1.1.5.7	Comply with the relevant national legislation
B1.1.5.8	Demonstrate active participation in H&I related clinically relevant professional development, such as national or international conferences or workshops
B1.1.6	The Director or Co-Director should:
B1.1.6.1	Have publications in peer-reviewed journals
B1.2	A Technical Supervisor, that must:

B1.2.1	Have minimum qualifying experience equivalent to either of the following:
B1.2.1.1	Hold a bachelor's degree or equivalent and have three years' relevant experience in human histocompatibility and immunogenetics testing under the supervision of a qualified Director or Co-Director
B1.2.1.2	Five years of supervised experience if a bachelor's degree has not been earned
B1.3	A Quality Manager, who must establish and maintain a comprehensive quality management programme covering all aspects for the accredited facility addressed by these standards.
B2	The resources of the laboratory must be sufficient to accommodate the workload.
B3	Testing referred to other laboratories
B3.1	An accredited laboratory may engage another laboratory to perform testing not done by the primary laboratory.
B3.2	The subcontracting laboratory:
B3.2.1	Must be accredited by EFI or by ASHI, if the testing is covered by EFI standards.
B3.2.2	Should have documented expertise and/or accreditation in genetic systems not covered by EFI Standards
B3.2.3	The identity of the subcontracting laboratory and that portion of the testing for which it bears responsibility must be noted in the reports issued

SECTION C – QUALITY ASSURANCE

C1	Facilities
C1.1	The following facilities must be adequate and immediately available to the laboratory:
C1.1.1	Sufficient space so that all procedures can be carried out without crowding to the extent that errors may result, in accordance with national regulations.
C1.1.2	Lighting
C1.1.3	Ventilation
C1.1.4	Refrigerators
C1.1.5	Freezers
C1.1.6	Storage for :
C1.1.6.1	Reagents
C1.1.6.2	Specimens
C1.1.6.3	Records
C1.2	Refrigerators and freezers:
C1.2.1	Optimal ranges for each fridge and freezer must be documented
C1.2.2	Must be monitored to detect unacceptable temperatures
C1.2.3	Should be coupled to recording thermometers
C1.2.4	Should be coupled to alarm systems with an audible alarm where it can be heard 24 hours a day
C1.2.5	Corrective actions for when the temperature is outside the documented acceptable range must be defined and documented
C1.3	In laboratories where liquid nitrogen is utilised for storage of frozen cells, the level of liquid nitrogen in the cell freezers must be monitored at intervals which will ensure an adequate supply at all times
C1.4	To ensure that procedures are carried out within temperature ranges specified in the laboratory's procedure manual, the following must be monitored every working day:
C1.4.1	Ambient temperature
C1.4.2	Temperature of incubators in which test procedures are carried out
C1.5	Laboratories performing procedures which require cell culture must have the following:
C1.5.1	Laminar Flow Hoods or other appropriately aseptic work area
C1.5.2	Incubators, which must be

C1.5.2.1	appropriately humidified and
C1.5.2.2	monitored daily in relation to:
C1.5.2.2.1	Temperature (37°C)
C1.5.2.2.2	CO ₂ concentration (5% ± 1%)
C1.6	Laboratories using radioactive materials must have a designated section of the laboratory for
C1.6.1	The storage of materials
C1.6.2	Conducting procedures
C1.6.3	Radioactive materials must be disposed of at locations designated by local institutions.
C1.7	Laboratories performing amplification of nucleic acids must:
C1.7.1	Use physical and/or biochemical barriers to prevent DNA contamination
C1.7.2	Perform pre-amplification procedures in an area which excludes amplified DNA that has the potential to serve as a template for amplification in any of the genetic systems tested in the laboratory
C1.8	The laboratory must establish and employ policies and procedures for the proper maintenance of equipment, instruments and test systems by:
C1.8.1	Defining its preventive maintenance programme for each instrument and piece of equipment at least once a year
C1.8.2	Performing and documenting function checks on equipment with at least the frequency specified by the manufacturer.
C1.8.3	The laboratory must use calibrated dispensing instruments (e.g. pipettes, etc) to perform assays
C1.8.3.1	Calibration of dispensing instruments must be performed at least once a year
C1.8.3.2	Calibration must be documented
C1.9	The laboratory must document compliance with all applicable national and local laws which relate to:
C1.9.1	Employee health and safety
C1.9.2	Fire safety
C1.9.3	Storage, handling and disposal of:
C1.9.3.1	Chemical material
C1.9.3.2	Biological material
C2	Computer assisted analyses
C2.1	The Laboratory Director and/or the Supervisor must
C2.1.1	Review

C2.1.2	Verify
C2.1.3	Sign computer assisted analyses before issue
C2.2	The computer software programme used for analyses must be:
C2.2.1	Identified
C2.2.2	Validated/Verified before use
C3	Specimen submission and requisition
C3.1	The laboratory must have available and follow written policies and procedures regarding specimen collection
C3.2	The laboratory must perform tests only at the written or electronic request of an authorised person
C3.3	The laboratory must assure that the requisition includes:
C3.3.1	The patient's or donor's name or other method of specimen identification to assure accurate reporting of results
C3.3.2	The name and address of the authorised person or of the service who ordered the test
C3.3.3	Date of specimen collection
C3.3.4	Time of specimen collection, when pertinent to testing
C3.3.5	Source of specimen (e.g. bone marrow, spleen cells) if pertinent
C3.4	Blood or tissue samples must be individually labelled with:
C3.4.1	The name, and/or other unique identification marker of the individual
C3.4.2	Date of collection
C3.5	When multiple blood containers are collected, each container must be individually labelled
C3.6	The laboratory must:
C3.6.1	Maintain a system to ensure reliable specimen identification
C3.6.2	Document each step in the processing and testing of patient specimens to assure that accurate test results are recorded.
C3.6.3	Have criteria for specimen rejection
C3.6.4	Have mechanism to assure that specimens are not tested when they do not meet the laboratory's criteria for acceptability
C3.7	If the laboratory provides a phlebotomy service:
C3.7.1	Blood samples must be obtained using a location, which does not compromise aseptic techniques
C3.7.2	The donor's skin must be prepared by a technique, which ensures minimal possibility of:

C3.7.2.1	Infection of the donor
C3.7.2.2	Contamination of the sample
C3.7.3	All needles and syringes must be disposable.
C3.8	All biological samples must be handled and transported in accordance with the understanding that they could transmit infectious agents
C3.9	The laboratory must provide all service users with information about the requirement for
C3.9.1	Sample labelling
C3.9.2	Anticoagulant / preservation media
C3.9.3	Sample packaging
C3.9.4	Regulations relating to postal transport
C3.9.5	The laboratory should warn users that failure to meet these requirements may result in sample rejection.
C4	Reagents
C4.1	All reagents must be properly labelled and stored according to manufacturers' instructions or locally-specified conditions to maintain reactivity and specificity.
C4.2	Reagents, solutions, culture media, controls, calibrators and other materials must be labelled to indicate:
C4.2.1	Identity and when significant, titre, strength or concentration
C4.2.2	Recommended storage requirements
C4.2.3	Preparation and/or expiration date and other pertinent information
C4.3	For storage of larger numbers of identical samples, it might be acceptable to use short-cut labelling of individual samples if the short-cut notation is explained on the outside of the storage container
C5	Laboratory Procedure Manual.
C5.1	All procedures in use in the laboratory must be detailed in a procedure manual which is immediately available where the procedures are carried out. The use of product inserts provided by manufacturers is not acceptable in place of the procedure manual.
C5.2	For each procedure:
C5.2.1	A review by the Director/Co-Director or a delegated individual with appropriate qualifications must be performed at least biennially.
C5.2.2	Documented evidence of this review must be available
C5.2.3	Any changes in procedures must be approved and documented by the Director/Co-Director/ delegated individual at the time they are initiated.
C6.	External Proficiency Testing(EPT)
C6.1	The laboratory must participate in EPT programme(s) to cover

C6.1.1	All the accredited laboratory applications (HLA typing, antibody screening and identification, crossmatching, etc.)
C6.1.2	All techniques used individually or in combination as routinely employed to produce a final result
C6.1.3	If no established scheme exists for a specific category (e.g. HNA antibody detection and identification) the laboratory must participate in an exchange of samples based workshop.
C6.2	Procedure of EPT
C6.2.1	The procedure for testing EPT samples including the allocation to techniques must be documented prior to the annual commencement of the EPT cycle.
C6.2.2	The laboratory must have a predetermined policy if they select individual shipments or samples for EPT
C6.2.3	The minimum number of samples as defined in C6.4 applies to all technique(s) used to produce a final result
C6.2.4	If the same sample is tested for more than one laboratory application, e.g. both low and high resolution HLA typing, the results must be analysed independently
C6.2.5	If the same sample is tested by more than one technique at low or high resolution DNA typing, the laboratory should make only one report to the Provider, but keep results obtained by different techniques available for inspection
C6.3	EPT samples must be
C6.3.1	tested by the same techniques as routinely employed for clinical samples, either individually or in combination
C6.3.2	interpreted in a manner comparable to routine clinical samples
C6.3.3	incorporated into the laboratory's routine workload
C6.4	Minimum number of samples for EPT per year
C6.4.1	HLA typing:
C6.4.1.1	Serological typing: 10 samples
C6.4.1.2	Low resolution DNA-based typing: 10 samples
C6.4.1.3	High resolution DNA-based typing: 10 samples
C6.4.2	HPA/HNA typing: 10 samples
C6.4.3	HLA antibody detection: 10 samples
C6.4.4	HLA antibody identification: 10 samples
C6.4.5	HPA antibody detection and identification: 5 samples
C6.4.6	Crossmatching: 20 tests of different donor/recipient combinations which must include a minimum of 10 different sera
C6.4.7	Haematopoietic chimaerism and engraftment monitoring: 10 tests of different donor/recipient mixtures in the range 0% - 100% excluding the reference donor and recipient samples
C6.5	Reporting of EPT results
C6.5.1	Participants must report:

C6.5.1.1	the antigen specificities and alleles identified
C6.5.1.2	the method(s) used.
C6.5.2	The following convention for reporting groups of HLA alleles is recommended:
C6.5.2.1	Groups of alleles should be reported as allele x/allele y, where “/” means “or”, e.g. <i>DRB1*01:01/02/04</i> means <i>DRB1*01:01</i> or <i>DRB1*01:02</i> or <i>DRB1*01:04</i> . This shortened presentation of alleles within a string must not be used if alleles differ within the first field, e.g. <i>DPB1*03:01/104:01/124:01</i> must not be given as <i>DPB1*03:01/104/124</i> .
C6.5.2.2	Groups of alleles that include sequential allele numbers may be reported as allele x-allele y, where “-” means “to”, e.g. <i>DRB1*15:01-04</i> means that the allele could be any between <i>DRB1*15:01</i> to <i>DRB1*15:04</i> inclusive.
C6.5.2.3	Alleles should be reported in full for <i>DRB3</i> , <i>DRB4</i> , and <i>DRB5</i> , e.g. <i>DRB4*01:02-01:03</i> and not as <i>DRB4*01:02-03</i> .
C6.5.3	For the detection of HLA class I and/or class II antibodies, participants must report
C6.5.3.1	the presence or absence of HLA class I and/or class II antibodies
C6.5.3.2	the antibody specificities identified
C6.5.3.3	the methods used
C6.5.4	For crossmatching, participants must report
C6.5.4.1	the test results
C6.5.4.2	the method(s) used
C6.5.5	For haematopoietic chimaerism and engraftment monitoring, participants must report
C6.5.5.1	the test results as a percentage of donor chimaerism
C6.5.5.2	the method(s) used
C6.5.5.3	details of the kit(s) and the manufacturer(s) used
C6.6	Laboratory performance
C6.6.1	If a laboratory's performance in EPT programme(s) is unsatisfactory in any category for which EFI accreditation is sought, the laboratory must:
C6.6.1.1	Participate in an additional EPT programme in that category
C6.6.1.2	Document the Director's review and any corrective action taken
C6.6.2	Laboratories must not engage in inter-laboratory communication pertaining to EPT results until after the reporting deadline has passed
C6.6.3	Laboratories must not send their own EPT samples or results for analysis to another laboratory until after the reporting deadline has passed
C6.6.4	Participating laboratories must ensure that all the following EPT related documents are maintained and are made available to EFI inspectors for assessment:
C6.6.4.1	Submitted worksheets
C6.6.4.2	EPT summary/scheme reports

C6.6.4.3	Annual performance
C6.6.4.4	Participation certificates
C6.6.4.5	Outcomes of investigations of any unsatisfactory results
C6.6.4.6	Corrective or preventive actions
C7	Competency Evaluation and Continuous Education
C7.1	The Director/Co-Director or designee must:
C7.1.1	Evaluate the competence of each technologist to accurately perform tests. This must be done at least yearly for each technique the technologist performs and must be based on a defined process
C7.1.2	Maintain records of these evaluations for each individual.
C7.2	The Laboratory Director and the technical staff must participate in continuing education relating to each category for which EFI accreditation is sought
C8	Systems for Continuous Test Evaluation and Monitoring
C8.1	The laboratory must establish and employ policies and procedures, and document actions taken when:
C8.1.1	Test systems do not meet the laboratory's established criteria
C8.1.2	Quality control results are outside of acceptable limits
C8.1.3	Errors are detected in the reported patient results. In this instance, the laboratory must:
C8.1.3.1	Promptly notify the authorised person ordering or individual utilising the test results of reporting errors
C8.1.3.2	Issue corrected reports
C8.1.3.3	Maintain copies of the original report as well as the corrected report for at least two years
C8.2	The laboratory must have mechanisms in place for continuous monitoring of all test systems and equipment used , including:
C8.2.1	Validation/verification, before introduction into routine use, of all new tests, by systematic comparative evaluation of results obtained in parallel with the new and the standard system
C8.2.2	Regular evaluation of results obtained in external and internal QC testing
C8.2.3	Regular monitoring of test validity in routine testing, by recording observations diverging from the expected results (e.g. cross-reactivity of probes or primer mixes, day-to-day variations).
C8.2.4	Comparing test results and documenting inconsistencies, if the same test is performed using different techniques.
C8.2.5	Identifying and evaluating inconsistencies between test results and clinical data or diagnostic parameters provided

C8.2.6	Written evidence of the ongoing monitoring process must be available in the laboratory for each method performed
C9	Client Service Evaluation
C9.1	All complaints and problems reported to the laboratory must be:
C9.1.1	documented
C9.1.2	investigated
C9.1.3	followed by corrective action when necessary
C9.2	The laboratory must, upon request, make available to clients a list of tests employed by the laboratory
C10	Quality Assurance Evaluation
C10.1	The laboratory must:
C10.1.1	Hold quality assurance reviews
C10.1.2	Document, assess problems identified in these reviews and discuss them with the staff
C10.1.3	Take corrective actions necessary to prevent recurrences
C10.1.4	Have an ongoing mechanism to evaluate corrective action taken. Ineffective policies and procedures must be revised based on the outcome of the evaluation
C10.2	The laboratory must maintain documentation of all quality assurance activities including problems identified and corrective actions taken, for a minimum of two years or longer, depending on local, or national regulations.
C10.3	The laboratory must maintain permanent files of all internal and external quality control tests according to any regulation to which the laboratory is obliged to abide, but for a minimum of four years
C11	Records and Test Reports
C11.1	The laboratory must maintain the following records:
C11.1.1	Records of subjects tested for two years or longer, depending on local regulations. These records must include:
C11.1.1.1	Log books
C11.1.1.2	Worksheets, that must clearly identify:
C11.1.1.2.1	The sample tested
C11.1.1.2.2	The reagents used
C11.1.1.2.3	The methods used
C11.1.1.2.4	The test performed
C11.1.1.2.5	The date of the test
C11.1.1.2.6	The person performing the test
C11.1.1.3	A summary of results obtained

C11.2	Records may be only saved in computer files, provided that back-up files are maintained to ensure against loss of data.
C11.3	For molecular typing, a record must be kept which is appropriate to the technique used, such as a photographic record of a gel, a membrane, an autoradiograph, an electronic file, or the read out from a sequencer.
C11.3.1	The record must be kept according to any regulation to which the laboratory is obliged to abide, but for a minimum of two years.
C11.4	Reports or records, as appropriate, must include a brief description of the specimen (blood, lymph node, spleen, bone marrow, etc.) used for testing
C11.5	The report must contain:
C11.5.1	The name of the individual tested or unique identifier of each individual tested and relationship to the patient if applicable
C11.5.2	The date(s) of collection of sample when pertinent
C11.5.3	The date of the report
C11.5.4	The test results
C11.5.5	The techniques used
C11.5.6	Appropriate interpretations and the signature of the Laboratory Director/Co-Director, or a designated individual.
C11.5.7	Information regarding the condition and disposition of specimen that did not meet the laboratory's criteria for acceptability.
C11.6	The laboratory must have adequate systems in place to report results in a timely, accurate and reliable manner.
C11.7	Laboratories must have a procedure in place for resolving any discrepancies that may occur between laboratories.

SECTION D – HLA ALLELES AND ANTIGENS

D1	Terminology
D1.1	Terminology of HLA alleles and antigens must conform to the latest report of the WHO Committee on Nomenclature.
D1.2	Potential new alleles or antigens not yet approved by the WHO Committee must have a local designation which cannot be confused with WHO terminology.
D1.3	NMDP codes must only be used for recording donors or cord blood unit typings into databases or for communication of the donor, cord blood unit or recipient typing with the registries.
D1.4	High resolution typing is defined as the identification of HLA alleles that encode the same protein sequence within the antigen binding site.
D1.4.1	HLA alleles must be identified at the level of resolution which defines the first and second fields according to WHO nomenclature by at least resolving all ambiguities:
D1.4.1.1	resulting from polymorphisms located within exons 2 and 3 for HLA class I loci, and exon 2 for HLA class II loci.
D1.4.1.2	that encompass a null allele, wherever the polymorphism is located, unless it can be demonstrated that an expressed antigen is present on the cells.
D2	Phenotypes and Genotypes
D2.1	Phenotypes and genotypes must be expressed as recommended by the WHO Committee, as in the following examples:
D2.1.1	Single alleles: HLA-B*07. Single antigens: HLA-B7 (or B7 if HLA is obvious from context).
D2.1.2	Phenotype
D2.1.2.1	Serological assignment: HLA-A2,30; B7,44; Cw7; DR1,4; DQ5,7.
D2.1.2.2	DNA assignment: HLA-A*02,*30; B*07,*44; C*07,*16; DRB1*01,*04; DQB1*05,*03:01.
D2.1.2.3	If an HLA typing is performed using DNA methods, it is acceptable to report an HLA serological assignment if required for the purposes of organ allocation.
D2.1.2.4	The translation of alleles to serological equivalence must be performed according to a documented protocol.
D2.1.3	Genotype
D2.1.3.1	Serological assignment: HLA-A2, B44, Cw-, DR1, DQ5 / A30, B7, Cw7, DR4, DQ7.
D2.1.3.2	DNA assignment: HLA-A*02, B*44, C*16, DRB1*01, DQB1*05 / A*30, B*07, C*07, DRB1*04, DQB1*03:01.
D2.2	The locus designation must always be included.

D2.3	Reporting Homozygosity and Heterozygosity
D2.3.1	If no more than one single antigen or allele is found at a locus by serological typing or DNA typing, the phenotype may include it twice only if homozygosity is proven by family studies or if 2-digit DNA typing unequivocally demonstrates the presence of heterozygosity for two different alleles from the same specificity.
D2.3.2	A “blank antigen or allele” can only be assigned if proven by family studies.
D2.3.3	If homozygosity has not been proven, the HLA type may be reported using a hyphen. For example:
D2.3.3.1	HLA-A1,3; B7,44; Cw7,- to indicate a phenotypic blank, or
D2.3.3.2	HLA-A*01,*03; B*07,*44; C*07,- for DNA based typing.
D2.3.4	If typing unequivocally demonstrates the presence of heterozygosity for two different alleles from the same specificity (e.g. DRB1*13:01/13:59, DRB1*13:03/13:33), the report may include it twice (e.g. DRB1*13,*13) even in the absence of family studies.
D2.4	Reporting High Resolution Typing
D2.4.1	When reporting high resolution typing, where ambiguous allele combinations cannot be resolved, all the alternatives must be documented
D2.4.2	If all ambiguities are not included on the report, a comment must be added stating that:
D2.4.2.1	Other ambiguous HLA (define loci) results have not been excluded and
D2.4.2.2	this information is available upon request.
D3	Haplotype Assignment
D3.1	Determination of haplotypes must be done by typing immediate family members including parents, siblings and/or children of the patient.
D3.2	Genotypic identity can only be proven if both parents are available or if the segregation of the four haplotypes is clearly defined.
D3.3	When appropriate, ambiguities in haplotype assignment must be resolved by:
D3.3.1	typing for HLA-C, and/or DQ and/or DP
D3.3.2	high resolution typing
D3.4	If recombination occurs, this must be reported with the HLA haplotype assignment.
D3.5	For unrelated individuals, when probable haplotypes based on population frequencies are used:
D3.5.1	Reports must clearly indicate that they were so derived
D3.5.2	The relevant references or sources must be available.

SECTION E – SEROLOGICAL HLA CLASS I AND CLASS II TYPING

E1	Recording Test Results
E1.1	For HLA testing by Complement Dependent Cytotoxicity, each serum-cell combination must be recorded in a manner which indicates the percentage of cells killed. Numerical scores used should be:
E1.1.1	Scores used by the International Workshop (0,1,2,4,6,8), or
E1.1.2	Other numerical codes.
E2	Typing
E2.1	For each of the following loci, the laboratory must be able to type for HLA specificities which are officially recognized by the WHO and for those deemed relevant by EFI:
E2.1.1	HLA A and B when applying for accreditation in the category of class I by serology
E2.1.2	HLA DR when applying for accreditation in the category of class II by serology
E2.2	Techniques used must be those, which have been established to define HLA Class I and II specificities optimally.
E3	Control Reagents
E3.1	Each typing tray must include :
E3.1.1	at least one positive control antibody, which reacts with cells expressing class I and class II antigens
E3.1.2	at least one negative control serum that should lack leukocyte reactive antibodies
E3.2	Procedures that deal with control serum failures in typing or crossmatch trays must be described in the laboratory manual.
E3.3	If the positive control fails to react as expected, there must be a procedure in place as whether to accept or reject the test.
E3.4	The minimum viability of the cells and the reactivity of control sera required for the validation of a serological typing must be described in the laboratory manual.
E4	Antigen Assignments
E4.1	Each HLA-A and B antigen must be defined by:
E4.1.1	At least two sera when available, if both are operationally monospecific, or
E4.1.2	if multispecific, at least three partially non-overlapping sera
E4.2	Each HLA Class II antigen should be defined by:
E4.2.1	at least three sera, if all are operationally monospecific
E4.2.2	at least five partially non-overlapping sera if multispecific

E4.3	Criteria for antigen assignment must be described in the laboratory manual.
E4.4	Ambiguity in antigen definition by serological typing must be referred for confirmation by DNA based methods.
E5	Typing Reagents
E5.1	Cells panel of known HLA type:
E5.1.1	must be used to prove the specificity of new antibodies
E5.1.2	should include at least one example of each HLA antigen the laboratory is required to define.
E5.2	Each monoclonal antibody used for alloantigen assignment must be used with an established technique at a dilution which demonstrates specificity comparable to antigen assignment by alloantisera on a well-defined cell panel.
E5.3	For reagent grade typing serum:
E5.3.1	confirmation of specificity must be performed
E5.3.2	supporting statistical analysis must be recorded
E5.4	Specificity of individual sera received from other laboratories or commercial sources must be confirmed to ensure that they reveal the same specificities in the receiving laboratory.
E5.5	Typing trays lots and shipments
E5.5.1	Each lot of typing trays must be evaluated by testing, either:
E5.5.1.1	at least five different cells of known phenotype representing major specificities
E5.5.1.2	in parallel with previously evaluated trays with at least five cells of known phenotype.
E5.5.2	Each new shipment of previously evaluated typing trays must be verified with at least one cell of known phenotype.
E6	Complement
E6.1	Complement must be kept at the recommended temperature.
E6.2	Complement lot and shipment testing
E6.2.1	Each lot and shipment of complement must be evaluated by either:
E6.2.1.1	testing with at least 3 previously evaluated trays for every application for which it is intended, or
E6.2.1.2	testing a combination of at least 3 sera and 2 cells selected to include negative, weak positive and strong positive reactions.
E6.2.2	The test must employ multiple dilutions of complement to ensure that it is maximally active at least one dilution beyond that intended for use.
E6.2.3	Complement must be tested separately for use with each type of target cell.

E6.2.4	Evaluation of each new lot and shipment of Complement must be tested to determine that:
E6.2.4.1	it mediates cytotoxicity in the presence of specific HLA antibody
E6.2.4.2	it is not cytotoxic in the absence of HLA specific antibody

SECTION F – ANTIBODY SCREENING AND CROSSMATCHING

F1	Sera
F1.1	Serum samples stored must be retained in the frozen state.
F1.2	Sera must be tested at a concentration determined to be optimal for detection of antibody to HLA antigens. The dilution(s) must be documented.
F1.3	Negative control:
F1.3.1	Each assay must include a negative control
F1.3.2	The negative control must be a serum from non-alloimmunised human donor(s).
F1.4	Positive control:
F1.4.1	Each assay must include a positive control
F1.4.2	The positive control must be either a validated monoclonal antibody, or sera from highly alloimmunised individuals and documented to react with HLA antigens
F1.4.3	The antibodies used must be of the appropriate isotype for each assay
F2	Techniques
F2.1	For the detection of antibody to HLA antigens, the laboratory must either use:
F2.1.1	A complement-dependent cytotoxic technique, or
F2.1.2	Another technique performed by the laboratory with documented validation testing, demonstrating that this technique identifies alloantibody to HLA antigens at a level of sensitivity equivalent or superior to that of its cytotoxic technique.
F2.1.3	To detect antibodies to HLA class II antigens, a technique must be used that distinguishes them from antibodies to HLA class I antigens.
F2.2	Other techniques:
F2.2.1	Laboratories performing assays using flow cytometry and/or Luminex must also conform to the standards in Section M.
F2.2.2	Laboratories using micro-plate ELISA techniques for antibody screening must additionally conform to standards in section N (ELISA).
F3	Antibody Screening by Complement-Dependent Cytotoxicity
F3.1	The following controls must be included on each tray:
F3.1.1	Positive control
F3.1.2	Negative control
F3.1.3	If sera are screened after treatment with dithiothreitol, IgG and IgM positive controls must be used
F3.2	Laboratories using a CDC technique must also conform to standard E6 (Complement).

F4	Panels
F4.1	The panel of HLA antigens must include sufficient panel cell donors to ensure that they are appropriate for the population served and the use of the test results.
F4.2	For assays intended to provide information on antibody presence or antibody identification, documentation of the HLA class I and/or class II specificities of the panel must be provided.
F5	Crossmatching
F5.1	Crossmatching for the detection of HLA specific antibodies:
F5.1.1	must use techniques at least as sensitive as the basic lymphocytotoxicity test.
F5.1.2	should use at least one technique documented to have increased sensitivity in comparison with the basic microlymphocytotoxicity test, such as prolonged incubation, antiglobulin test, ELISA, B-cell crossmatch or flow cytometry
F5.2	The screening result used must be at least as sensitive as the routine crossmatch technique.
F5.3	Each serum must be tested:
F5.3.1	Undiluted
F5.3.2	In duplicate
F5.4	Crossmatches must be performed:
F5.4.1	with unseparated lymphocytes or with T lymphocytes
F5.4.2	With B lymphocytes if required by the relevant transplantation programmes.
F5.5	The following controls must be included on each tray:
F5.5.1	Positive control
F5.5.2	Negative control
F5.5.3	If sera are tested after treatment with dithiothreitol, IgG and IgM positive controls must be used
F5.6	Laboratories using a CDC technique must also conform to standard E6 (Complement)
F5.7	Standards in sections M (Flow Cytometry) and N (ELISA) must be followed when applicable.

SECTION G – RENAL and/or PANCREAS TRANSPLANTATION

G1	If deceased donor transplants are done:
G1.1	The following personnel must be available 24 hours a day, seven days a week:
G1.1.1	personnel for the required histocompatibility testing,
G1.1.2	personnel for interpretation of results
G1.1.3	personnel for advice for the clinical transplant team
G1.2	Laboratories not able to perform tests 24h/day, 7d/week must arrange with an EFI or ASHI accredited laboratory to perform tests.
G2	Antibody Screening
G2.1	Laboratories must :
G2.1.1	have a documented policy in place to evaluate the sensitisation of each patient at the time of their initial evaluation
G2.2	have a programme to periodically screen serum samples from each patient for antibodies to HLA antigens by:
G2.2.1	determining and recording the specificity of detected HLA antibodies
G2.2.2	performing testing to distinguish HLA specific antibodies from non HLA antibodies and autoantibodies
G2.3	Have a policy establishing the frequency of screening serum samples and must have data to support this policy. Samples must be collected and tested , either:
G2.3.1	every three months, or
G2.3.2	as stipulated by the national and/or international organ exchange organisations.
G3	Sensitising Events
G3.1	Laboratories should maintain a record of potentially sensitising events for each patient.
G3.2	Serum samples should be collected and stored after each of these events for possible subsequent screening for antibodies to HLA and/or use in crossmatch tests.
G4	Crossmatching
G4.1	Crossmatching must be performed according to national legislation applying to the laboratory and/or regulations from the national / international exchange organisation.
G4.1.1	Crossmatching must be performed prospectively, or may be omitted if Virtual Crossmatching is performed
G4.1.2	Prospective crossmatching must be performed for all living donor transplants
G4.1.3	If the prospective crossmatch is omitted, a retrospective crossmatch must be performed.

G4.2	Virtual Crossmatching
G4.2.1	A transplant protocol for Virtual Crossmatching must be agreed with the clinical transplant teams and documented.
G4.2.2	There must be evidence that the eligibility of each patient has been evaluated when a virtual crossmatch has been performed.
G4.2.3	The transplant protocol must include evidence that the clinical teams are aware of the possibility of errors in donor offer typing.
G4.2.4	The Virtual Crossmatch result must be reported by the laboratory to the transplant clinician before the transplant proceeds.
G4.2.5	Evidence that the Virtual Crossmatch was reported must be documented.
G4.2.6	Patients are only eligible for Virtual Crossmatching if
G4.2.6.1	There have been no potential sensitising events since the last serum sample screened.
G4.2.6.2	Sera must have been collected as defined in standard G2.3
G4.2.6.3	At least two different samples must have been tested.
G4.2.6.4	At least one serum screening result obtained within the previous 3 months must be included
G4.2.6.5	Sera must be tested for antibody specificity identification by a technique of at least equivalent sensitivity to that used for crossmatching
G4.3	Virtual Crossmatching for Unsensitised Patients
G4.3.1	A prospective crossmatch may be omitted for patients:
G4.3.1.1	Who test consistently negative for the presence of HLA-specific antibodies, as relevant for the transplant protocol.
G4.3.1.2	for whom there must be documented evidence that the laboratory maintains a record of potentially sensitising events
G4.4	Virtual Crossmatching for Sensitised Patients
G4.4.1	The prospective crossmatch may be omitted in carefully selected HLA immunised recipients in whom acceptable and/or unacceptable mismatches have been clearly defined and documented.
G4.4.2	If a prospective crossmatch is omitted from an alloimmunised recipient, the method of antibody identification must rely on single antigen technology.
G4.4.3	The Virtual Crossmatch must include data from single antigen testing performed on a sample collected within the last 3 months.
G4.4.4	Patients are ineligible for Virtual Crossmatching if they have antibodies against specificities for which the donor has not been typed.

G4.5	Retrospective Crossmatching
G4.5.1	If retrospective cross-matches are performed according to standard G4.1.3
G4.5.1.1	They must be shown to be in concordance with the predicted negative result and this must be documented
G4.5.1.2	The physician in charge must be notified immediately of an unpredicted positive result.
G4.5.1.3	There must be a re-evaluation of this policy at least annually.
G4.5.1.4	Any additional regulations either national, or of the exchange organisation must also be applied.
G4.6	Sera
G4.6.1	The laboratory must have a policy regarding the selection of relevant sera to be used in the final crossmatch procedure
G4.7	Final crossmatches performed prior to transplantation
G4.7.1	must use a recipient serum sample collected within the previous 48 hours before transplant if the recipient has had a recent sensitising event.
G4.7.2	must use the most recent available serum collected as defined in standard G2.3
G4.7.3	should use sera obtained 14 days after a potentially sensitising event.
G5	HLA Typing
G5.1	Prospective typing of donor and recipient:
G5.1.1	Must include typing for HLA-A, B and DR antigens.
G5.1.2	Must include additional loci if required by national regulations.
G5.2	Every effort must be made to perform verification typing for recipients prior to transplantation.
G5.3	Verification typing must be performed on living donors prior to transplantation.

SECTION H – OTHER ORGAN TRANSPLANTATION

H1	In cases where patients are at high risk for allograft rejection (e.g. patients with histories of allograft rejection, patients with preformed HLA antibodies), donors and recipients must be typed for HLA-A, B and DR antigens.
H2	Cardiothoracic patients must be screened for the presence of HLA alloantibodies, and
H2.1	Unacceptable specificities must be defined, or
H2.2	A prospective crossmatch must be performed
H2.3	Standards G2 (Antibody Screening) also applies
H3	Crossmatching
H3.1	Crossmatching must be performed according to standards in F5 (Crossmatching).
H3.1.1	Sera from patients at high risk for allograft rejection should be prospectively crossmatched.
H3.1.2	Crossmatch results should be available prior to transplantation of a pre-sensitised patient.
H3.2	Final crossmatches performed prior to transplantation should either:
H3.2.1	utilise a recipient serum sample collected within the previous 48 hours before transplant if the recipient has had a recent sensitising event. Or,
H3.2.2	utilise a serum collected within three months
H3.3	Sera obtained 14 days after a potential sensitising event should be used in the final cross-match.
H3.4	Whenever possible, non-renal organs for recipients at high risk for allograft rejection should come from cross-match negative donors as defined by the laboratory and the transplant programme.

SECTION I – HAEMATOPOIETIC STEM CELL TRANSPLANTATION

I1	HLA typing
I1.1	If required by the transplant protocol, the laboratory must be able to type the donor and the recipient for HLA Class I and HLA Class II by DNA methods, to a level of resolution as defined in standard D1.4.
I1.2	Laboratories performing transplantation using related donors who are not able to perform high resolution Class I and/or Class II typing by DNA methods must arrange for an EFI or ASHI accredited laboratory to perform these tests.
I1.3	Laboratories performing transplantation using unrelated donors who are not able to perform high resolution Class I typing by DNA methods must arrange for an EFI or ASHI accredited laboratory to perform these tests.
I2	Histocompatibility testing for related transplants
I2.1	HLA-A, B or DR typing must be carried out on available members of the immediate family
I2.2	Must include adequate testing
I2.2.1	to definitively establish HLA genotype identity (D3.2 applies), or
I2.2.2	to type at high resolution for the relevant loci defined in the transplant protocol, if only phenotype identity has been established, or
I2.2.3	to include high resolution typing for recipient and potential intra-familial donors who are not HLA identical siblings
I2.3	HLA-A, B and DR typing as a minimum requirement must be repeated on both the recipient and the potential donor prior to transplantation using a new typing sample from each, so that each individual's typing is confirmed.
I3	HLA typing for Donors (related cord blood unit)
I3.1	The cord blood unit must be typed using DNA methods for HLA-A, B and DRB1 at a minimum of low resolution (eg. <i>A*02, B*44, DRB1*11</i>)
I3.2	Extended typing must be included if required by the transplant protocol (standards I2.1, I2.2 and I2.3 also apply)
I3.3	Prior to transplantation, a verification typing:
I3.3.1	Must be performed for HLA-A, B and DRB1 at a minimum of low resolution
I3.3.2	Must be performed on a segment of the tubing integrally attached to the unit, if available, or otherwise, on a satellite vial.
I3.4	If verification typing was not performed on a segment of the tubing integrally attached, the laboratory must recommend that an additional typing is performed on the content of the thawed unit.
I4	Histocompatibility Testing for Unrelated Transplants
I4.1	Volunteer Bone Marrow Donor Registries
I4.1.1	Typing of the donors must be performed

I4.1.1.1	By serology or
I4.1.1.2	By DNA methods at a minimum of low resolution (eg A2 or A*02, DR11 or DRB1*11)
I4.2	Typing of Units for Cord Blood Banks
I4.2.1	Typing must be performed using DNA methods for HLA-A, B and DRB1, at a minimum of low resolution (e.g. A*02, B*44, DRB1*11).
I4.2.2	Typing of additional loci or high resolution typing must be included if required by the policy of the registry, or if requested.
I4.2.3	The identity of the Cord Blood Unit must be verified by HLA typing on a separate sample to demonstrate concordance of results.
I4.2.4	Additional typing may be performed using any stored DNA sample, provided that the identity of the unit has previously been verified.
I4.2.5	The verification of identity and the source of the sample tested must be reported back to the registry.
I4.3	Histocompatibility Testing for Transplants from Unrelated Donors.
I4.3.1	HLA typing for recipient and unrelated donors must:
I4.3.1.1	Be performed by DNA based methods
I4.3.1.2	include as a minimum requirement:
I4.3.1.2.1	HLA-A/B/C and DRB1 typing at high resolution
I4.3.1.3	Include additional loci if required by the transplant protocol.
I4.3.1.4	Include other resolution levels if required by the transplant protocol
I4.3.1.5	Be performed by the laboratory affiliated with the transplant centre
I4.3.2	Prior to transplantation using an unrelated donor, HLA typing of the recipient and donor must be repeated for verification:
I4.3.2.1	by the laboratory affiliated with the transplant centre
I4.3.2.2	using a different typing sample
I4.3.2.3	for HLA-A, -B, and -DR, as a minimal requirement
I4.3.3	For unrelated donors HLA-A,-B,-DR concordant results are required on two separate samples. Registry typing is acceptable as one of the two required results.
I4.4	Unrelated Cord Blood Unit Typing for Donor Selection
I4.4.1	Verification typing must be performed
I4.4.1.2	Including as a minimum requirement
I4.4.1.2.1	HLA-A and -B at low resolution, and
I4.4.1.2.2	HLA-DRB1 at high resolution

I4.4.1.2.3	Extended typing if required by the transplant protocol
I4.5	Unrelated Cord Blood Unit Typing Prior to Transplantation
I4.5.1	Prior to the conditioning regimen of the recipient, a verification typing must be performed
I4.5.1.1	at a minimum level of low resolution for HLA-A, -B, and -DRB1
I4.5.1.2	upon reception of the shipped unit
I4.5.1.3	on a segment of the tubing integrally attached to the unit, if available; otherwise a satellite vial shipped with the unit may be used.
I4.5.2	If no segment is available, this step can be performed after transplantation and must be initiated as soon as possible after thawing the unit.
I5	Crossmatching
I5.1	Crossmatching must be performed
I5.1.1	prior to related and unrelated transplantation if required by the local transplant protocol
I5.1.2	according to standards F5 (Crossmatching)
I6	Haemopoietic Chimaerism and Engraftment (HCE) Monitoring
I6.1	Standards L1, L5.1, L5.3, L6, L7.1 and L7.2 also apply.
I6.2	The polymorphic gene system(s) used for HCE monitoring must be identified and documented with regards to allelic variability
I6.3	The sensitivity of the HCE assay must be validated using DNA mixtures from two individuals at defined ratios/concentrations, before implementation into clinical use.
I6.4	For locally developed PCR primers/probes the following must be documented
I6.4.1	Sequence
I6.4.2	Specificity
I6.5	Donor and patient specific allele profiles must be
I6.5.1	determined using appropriate reference material
I6.5.2	Documented
I6.6	Optimal ranges of DNA quantity and purity must be:
I6.6.1	Defined
I6.6.2	Documented
I6.6.3	If a sample falls outside these optimal ranges, a statement must be included in the report.
I6.7	Criteria for assignment of HCE results, on a qualitative or quantitative basis, must be defined.

I6.8	When multiple PCR primers are used in the same tube (multiplex PCR), results must take into account possible amplification bias
I6.9	When HCE testing is performed on cellular subsets isolated by cell sorting, the purity of the sorted population:
I6.9.1	must be documented and
I6.9.2	taken into account in the analysis of the results
I6.9.3	If this is not possible it must be clearly stated in the report.
I6.10	For quantitative HCE monitoring by quantitative PCR (Q-PCR), the following must be defined
I6.10.1	Chemistry used
I6.10.2	internal control gene
I6.10.3	thresholds for positive and negative results of each reaction.
I6.11	All steps of locally developed Q-PCR assays must be validated.
I6.12	In addition to the requirements from standard C11.5.7, the report must contain
I6.12.1	a description of the specimen used for testing (bone marrow, peripheral blood, cellular subsets isolated by cell sorting etc)
I6.12.2	the date of transplant
I6.12.3	other information if deemed relevant for HCE interpretation (i.e. limited informative markers or clinical condition of the patient)

SECTION J – HLA / HPA / HNA AND TRANSFUSION

J1	Documented protocols for testing each of the following must be provided:
J1.1	HLA
J1.2	Human Platelet Antigens (HPA)
J1.3	Human Neutrophil Antigens (HNA)
J2	HLA and Transfusion
J2.1	Platelet refractoriness
J2.1.1	Platelet refractory patients who require HLA matched platelets
J2.1.1.1	must be typed for HLA-A and HLA-B
J2.1.1.2	If alloimmune refractoriness is suspected the patient must be tested for HLA class I antibodies.
J2.1.2	To provide compatible platelets, either:
J2.1.2.1	The specificity of detected HLA antibodies must be defined and recorded, or
J2.1.2.2	crossmatching must be performed
J2.1.3	For crossmatching using lymphocytes standards F5 (Crossmatching) must be followed.
J2.1.4	All selected plateletpheresis donors used for the provision of HLA matched platelets must be typed for HLA-A and HLA-B.
J2.2	Transfusion Related Acute Lung Injury (TRALI).
J2.2.1	For the laboratory investigation of TRALI, the sera from implicated donors must be tested for both HLA class I and class II antibodies.
J2.2.2	The specificity of detected HLA antibodies must be defined and recorded.
J2.2.3	If HLA specific antibodies are identified, HLA typing must be performed at least for all relevant loci on
J2.2.3.1	The patient
J2.3.3.2	The donor
J2.3	Transfusion Associated Graft versus Host Disease (TAGVHD)
J2.3.1	The patient and the donor must be typed for HLA-A, HLA-B, and HLA-DR.
J2.4	Febrile Non Haemolytic Transfusion Reactions (FNHTR)
J2.4.1	The patient's serum must be tested for the presence of HLA antibodies
J3	HPA and Transfusion
J3.1	Current HPA Nomenclature (Platelet Nomenclature Committee; <i>Vox Sanguinis</i> 2003 85, 240-245) must be used for recording and reporting HPA alloantigen and HPA alloantibody specificities.
J3.2	HPA Typing

J3.2.1	HPA typing must be performed using a validated HPA typing technique. If typing is performed using DNA based methods, standards in section L (Nucleic Acid Analysis) apply.
J3.2.2	Clinically significant HPA specificities must be defined and documented.
J3.3	Investigation of HPA antibodies
J3.3.1	For bead array techniques, relevant standards from section M (Flow Cytometry) also apply
J3.3.2	For ELISA based assays, standards in section N (ELISA) also apply.
J3.3.3	Laboratories must make all reasonable efforts to include HPA antigens in their antibody screening protocol which will aid the identification of clinically significant HPA alloantibodies.
J3.3.4	The antibody screening technique must
J3.3.4.1	be validated before use
J3.3.4.2	Include positive and negative controls in each assay
J3.3.4.3	In glycoprotein specific assays, a positive control for each glycoprotein used should be included.
J3.3.5	The specificity of detected HPA alloantibodies must be defined and recorded.
J3.4	Neonatal Alloimmune Thrombocytopenia (NAIT)
J3.4.1	The maternal serum must be investigated for the presence of HPA antibodies
J3.4.2	HPA typing of the mother, father and neonate should be performed
J3.5	Post Transfusion Purpura (PTP)
J3.5.1	The patient must be HPA typed
J3.5.2	The patient's serum must be investigated for HPA antibodies
J4	HNA and Transfusion
J4.1	Current HNA Nomenclature (ISBT Working Party; <i>Vox Sanguinis</i> 2008 94, 277-285) must be used for recording and reporting HNA alloantigen and HNA alloantibody specificities.
J4.2	HNA Typing
J4.2.1	HNA typing must be performed using a validated HNA typing technique. If typing is performed using DNA based methods, standards in section L (Nucleic Acid Analysis) apply.
J4.2.2	The clinically significant HNA specificities must be defined and documented.
J4.3	Investigation of HNA Antibodies
J4.3.1	For bead array and flow cytometry techniques, relevant standards from section M (Flow Cytometry) also apply
J4.3.2	For ELISA based assays, standards in section N (ELISA) also apply.
J4.3.3	Laboratories must make all reasonable efforts to include HNA antigens in their antibody screening protocol which will aid the identification of clinically significant HNA alloantibodies

J4.3.4	The antibody screening technique must
J4.3.4.1	be validated before use
J4.3.4.2	Include positive and negative controls in each assay
J4.3.4.3	In glycoprotein specific assays, laboratories must make all reasonable efforts to include a positive control for each glycoprotein used.
J4.3.5	The specificity of detected HNA alloantibodies must be defined and recorded.
J4.4	Neonatal Alloimmune Neutropenia (NAIN)
J4.4.1	The maternal serum sample must be investigated for the presence of HNA antibodies.
J4.4.2	HNA typing of the mother, father and neonate should be performed.

SECTION K – DISEASE ASSOCIATION

K1	The following standards apply:
K1.1	If complete HLA typing is performed by serology standards in section E (Serological HLA Class I and Class II typing) must be followed.
K1.2	Typing may also be limited to all products of a single or limited number of HLA antigens, alleles or loci.
K1.3	If HLA typing is performed by DNA techniques standards in section L (Nucleic Acid Analysis) must be followed
K1.4	The clinically relevant HLA loci and alleles must be documented for each disease association service provided.
K2	Typing for a single antigen by CDC
K2.1	Typing Reagents
K2.1.1	Sera to define each antigen must meet requirements of section E (Serological HLA Class I and Class II typing) as appropriate.
K2.2	Cell Controls must:
K2.2.1	Be tested on each batch
K2.2.2	Include at least two cells known to express the specified antigen
K2.2.3	Include at least two cells for each cross reacting antigen, which might be confused with the specific antigen
K2.2.4	Include at least two cells lacking the specific and cross reacting antigens
K2.3	Serum Controls must
K2.3.1	Be tested at the time of typing
K2.3.2	include a positive and negative control
K2.3.3	Serum controls should also include two sera for each antigen which cross reacts with the specified antigen (if available)
K3	Typing for a single allele-group by molecular techniques:
K3.1	a positive control DNA known to encode the allele-group of interest must be included in each test.
K3.2	a negative control DNA known not to encode an allele belonging to the allele-group of interest must be included in each test.

SECTION L – NUCLEIC ACID ANALYSIS

L1	General laboratory design
L1.1	Laboratories performing amplification of nucleic acids must use:
L1.1.1	A dedicated work area with restricted traffic flow
L1.1.2	Physical barriers to prevent DNA contamination, including the use of dedicated
L1.1.2.1	Equipment
L1.1.2.2	laboratory coats
L1.1.2.3	Disposable supplies
L1.2	Pre-amplification procedures must be performed in an area which excludes amplified DNA that has the potential to serve as a template for amplification in any of the genetic systems tested in the laboratory.
L1.3	All activities occurring from and including thermal cycling must take place in the post-amplification area.
L1.4	For methods that use two consecutive steps of logarithmic amplification, the addition of the template for subsequent amplifications:
L1.4.1	must occur in an area isolated by physical or chemical barriers from both the pre- and post-amplification work areas
L1.4.2	must use dedicated equipment and consumables.
L2	Equipment
L2.1	Accuracy of thermal cycling instruments:
L2.1.1	must be verified by maintenance according to the manufacturer, or
L2.1.2	must be verified by annual thermal verification of the block using a calibrated device designed specifically for this purpose.
L2.2	Incubators and water baths must be monitored for accurate temperature every time an assay is performed.
L3	Reagents
L3.1	All reagents (solutions containing one or multiple components) must either be:
L3.1.1	dispensed in aliquots for single use, or
L3.1.2	dispensed in aliquots for multiple use if documented to be free of contamination at each use
L3.2	When reagents are combined to create a master mix, one critical component (e.g. DNA polymerase) should be left out of the mixture.
L3.3	The appropriate performance of individual products must be documented before results using these reagents are reported for:
L3.3.1	each shipment, and
L3.3.2	each lot

L3.4	For commercial kits, the following information must be documented:
L3.4.1	Source
L3.4.2	lot number
L3.4.3	expiry date
L3.4.4	storage conditions
L3.5	Reagents from different lots of commercial kit must not be mixed, unless either:
L3.5.1	specified by the manufacturer, or
L3.5.2	validated and documented with appropriate quality control in the laboratory
L4	Primers
L4.1	The specificity of primer combinations and the annealing positions must be defined.
L4.2	Laboratories must:
L4.2.1	have a policy for quality control of each lot or shipment of primers
L4.2.2	Confirm the specificity and quantity of the amplified product using reference material.
L4.2.3	test each lot and shipment of commercial kits against at least one DNA sample of known type.
L4.2.4	Use primers under empirically determined conditions that achieve the defined specificity for templates used in routine testing.
L4.2.5	Test each lot of local primers for amplification specificity and quantity using reference material whenever available.
L4.2.6	Test each lot of local primers with reference DNA for appropriate sensitivity and specificity.
L5	Nucleic acid extraction:
L5.1	The method used for nucleic acid extraction:
L5.1.1	must be published and documented
L5.1.2	must be validated in the laboratory
L5.2	Purity and concentration of Nucleic Acids:
L5.2.1	must be sufficient to ensure reliable test results.
L5.2.2	should be determined for each sample, or
L5.2.3	if not determined for each sample, the laboratory must have tested and validated this policy.
L5.3	If the DNA is not used immediately after purification, suitable methods of storage must be available that will protect the integrity of the material.
L6	Electrophoresis
L6.1	optimal electrophoretic conditions must be documented

L6.2	The laboratory must establish criteria for accepting each slab or capillary gel migration, and each lane of a gel or capillary injection.
L6.3	When the size of an amplicon is a critical factor in the analysis of data, size markers that produce discrete electrophoretic bands spanning and flanking the entire range of expected fragment sizes must be included in each gel.
L7	Analysis
L7.1	Signal intensity
L7.1.1	Acceptable limits of signal intensity must be specified for positive and negative results
L7.1.2	If these are not achieved, acceptance of the results must be justified and documented.
L7.2	The method of allele assignment must be designated
L7.3	The allele database must be:
L7.3.1	Documented
L7.3.2	Updated at least once a year with the most current version of the IMGT/HLA database
L7.4	If a manual allele call or interpretation of positive/negative reactions is performed for SSOP or SSP, two independent interpretations of primary data must be performed, except under justified special emergency situations.
L7.5	Databases of HLA sequences used to interpret the primary data must be:
L7.5.1	documented
L7.5.2	accurate
L7.5.3	updated at least once a year with the most current version of the IMGT/HLA database
L7.5.4	must be archived or a record retained according any regulation the laboratory is obliged to abide, but for a minimum of four years.
L8	Contamination control ("wipe-test")
L8.1	Contamination must be monitored for amplification products produced in the laboratory.
L8.2	Routine wipe-tests must:
L8.2.1	include pre-amplification work areas
L8.2.2	include pre-amplification equipment
L8.2.3	be performed at least every two months
L8.2.4	be performed using a method that is at least as sensitive as routine test methods.
L8.2.5	include positive controls to assure proper performance of monitoring
L8.2.6	include other areas of the laboratory as relevant
L8.3	If amplified product is detected, there must be:
L8.3.1	written description of how to eliminate the contamination
L8.3.2	measures taken to prevent future contamination
L8.3.3	evidence of elimination of the contamination

L9	Typing using sequence-specific primers (SSP)
L9.1	Each amplification reaction must include controls to detect technical failures (e.g. an internal control such as additional primers or templates that produce a product that can be distinguished from the typing product).
L9.2	When a typing exhibits lanes with no specific amplicon or internal control amplification, the laboratory must have a policy in place on how to accept or reject the whole typing.
L9.3	The laboratory must use the following data in the interpretation phase of the typing:
L9.3.1	information derived from the validation process
L9.3.2	information derived from previous typings with the same lot of primers
L9.4	The following must be defined and documented:
L9.4.1	Non-specific and weak amplifications
L9.4.2	Any tendency to form primer-dimer
L10	Sequence-Based typing (SBT)
L10.1	Sequencing Templates:
L10.1.1	must have sufficient purity, specificity, quantity and quality to provide interpretable sequencing data.
L10.1.2	Should be purified after amplification to eliminate the presence of dNTPs, Taq polymerase and amplification primers.
L10.1.3	must not contain any inhibitors or contaminants affecting the sequencing reaction.
L10.1.4	Validation of the methods for template preparation must ensure that the accuracy of the final typing is not altered (e.g. mutations during cloning, preferential amplification).
L10.1.5	If cloning is used as template preparation, the sequence of at least three different clones for each allele must be determined for accurate results
L10.2	Sequencing Reaction:
L10.2.1	The specificity of the template in combination with the sequencing primer (HLA locus and alleles) must be defined.
L10.2.2	Quantity and quality of templates, sequencing primers and sequencing reagents must be sufficient to provide interpretable primary sequencing data.
L10.2.3	The conditions for the sequencing reaction must be documented and appropriate for obtaining reliable primary sequencing data.
L10.3	Nucleotide Assignment:
L10.3.1	The following criteria for acceptance of primary data must be established (peak intensity, baseline fluctuation, Peak shape, correct assignment for non-polymorphic positions)
L10.3.2	The signal to noise ratio must be sufficient to ensure reliable nucleotide assignments
L10.3.3	A scientific and technically sound method must be established for interpretation, acceptance and/or rejection of sequences from regions which are difficult to resolve (e.g. compression).

L10.3.4	Established sequence-specific characteristics should be documented and utilized in routine interpretation of data.
L10.4	Allele assignment
L10.4.1	Methods must ensure that sequences contributed by amplification primers are not considered in the assignment of alleles.
L10.4.2	Criteria for allele assignment must be established.
L10.4.3	Established sequence-specific artefacts must be documented and the information used in the routine interpretation of data.
L10.4.4	Uni- and bi-directional sequencing
L10.4.4.1	If allele assignments are difficult to obtain by sequencing only one strand, routine sequencing of both strands should be performed
L10.4.4.2	If a sequence suggests a novel allele or a rare combination of alleles, the sequences of both strands must be determined
L11	Sequence-Specific Oligonucleotide Probe (SSOP) hybridization assays
L11.1	Oligonucleotide probes
L11.1.1	The specificity of each probe and target sequence must be defined.
L11.1.2	Probes must be stored under conditions that maintain specificity and sensitivity
L11.2	Quality Control
L11.2.1	Laboratories must have a policy in place for quality control of each lot and shipment of probes
L11.2.2	For home-made kits each lot must be tested with reference DNA so that each probe is tested for specificity and signal intensity at least once.
L11.2.3	For commercial kits each lot and shipment must be tested in parallel against at least one DNA sample of known type.
L11.2.4	The specificity and signal intensity for each probe must be defined and monitored.
L11.2.5	Probes must be utilised under empirically determined conditions that achieve the defined specificity.
L11.2.6	For commercial kits, any deviation from the manufacturer's specifications must be validated and documented.
L11.3	Hybridization
L11.3.1	The amplification should be monitored by gel electrophoresis before the hybridization is performed
L11.3.2	Each assay must include:
L11.3.2.1	a probe internal to a conserved region of the amplified fragment
L11.3.2.2	appropriate controls to validate the hybridization and the detection steps
L11.3.2.3	a negative (no DNA) control :

L11.3.2.3.1	that must be included in the hybridization and revelation steps of the assay in forward SSOP assays
L11.3.2.3.2	that must either be included in the hybridization and detection step of the assay or monitored by gel electrophoresis in reverse SSOP assays
L11.4	Equipment
L11.4.1	Standard L2.2 must be followed for the incubators, water baths and for heated reagents.
L11.4.2	For automated hybridization devices:
L11.4.2.1	the calibration of the pumps and of the heating elements must be performed according to the manufacturer's specification at least once a year
L11.4.3	For tests using an ELISA washer:
L11.4.3.1	calibration must be performed at least annually according to the manufacturer's specifications
L11.4.3.2	monthly functional checks of dispensing/aspirating must be performed.
L11.5	Where a scanner is used for acquisition of the raw data, a second visual reading must be performed to confirm data.
L11.5.1	For automated systems for the acquisition of the primary data
L11.5.1.1	all critical elements influencing the function of the instrument must be monitored at each use
L11.5.1.2	The instrument must be calibrated according to manufacturer's instructions or at least once a year
L11.5.1.3	The laboratory must define and document functional checks.
L11.5.2	For flow cytometer-like devices, there must be evidence of:
L11.5.2.1	regular cleaning
L11.5.2.2	satisfactory calibration functions performed prior to use
L11.6	Interpretation
L11.6.1	Acceptable limits of signal intensity must be specified for positive and negative results.
L11.6.2	If a test is accepted with probe signals outside the set limits, this must be documented and justified.
L11.6.3	The laboratory must use the data derived from the validation process and from previous typings with the same lot of primers and probes in the interpretation phase of the typing.
L11.6.4	Nonspecific and weak hybridization signals must be defined and documented.

L12	Other Methods
L12.1	If alternative methods (e.g. SSCP, heteroduplex, DGGE) are used for HLA typing, there must be established procedures in place which
L12.1.1	must be validated
L12.1.2	must include sufficient controls to ensure accurate assignment of types for every sample
L12.1.3	must comply with all relevant standards from section L (Nucleic Acid Analysis)

SECTION M – FLOW CYTOMETRY

M1	Application
M1.1	Standards M2 (General instrument standardisation and maintenance) applies to flow cytometry and flow analysis using equipment designed for beads only (fluoro-analyser)
M1.2	Standards M2 (General instrument standardisation and maintenance) to M5 (Cell-based HLA typing by flow cytometry) apply to flow cytometry
M2	General Instrument standardisation and maintenance
M2.1	For instruments that perform an automated integrated multi-parameter standardisation (eg Luminex):
M2.1.1	This function may be used instead of the individual optical alignment and fluorescence standardisation described in standards M2.2 and M2.3.
M2.1.2	The reagents specified by the manufacturer to perform this test must be used.
M2.1.3	The result of the standardisation must be recorded.
M2.1.4	The instrument must only be used if the test has passed.
M2.1.5	The frequency of standardisation
M2.1.5.1	must conform to manufacturer's instructions and must be performed at any time that the temperature delta check is not correct, or
M2.1.5.2	must be performed as specified in standards M2.2 or M2.2.4 if there are no manufacturer's instructions
M2.2	Optical Standardisation
M2.2.1	The optical standard must be run:
M2.2.1.1	every day of instrument use unless otherwise specified by the manufacturer
M2.2.1.2	Any time maintenance or adjustment of the instrument during operation is likely to have altered optical alignment.
M2.2.2	The optical standard must consist of latex beads or other uniform particles
M2.2.3	A threshold value for acceptable optical standardisation must be established for all relevant signals
M2.2.4	The results of optical focusing / alignment must be recorded and fall within the defined acceptable range.
M2.3	Fluorescence standardisation
M2.3.1	The fluorescence standard:
M2.3.1.1	Must be run every day of instrument use unless otherwise specified by the manufacturer

M2.3.1.2	Must be run any time maintenance or adjustment of the instrument during operation is likely to have altered settings.
M2.3.1.3	Must be used for each fluorochrome employed in analytical procedures
M2.3.1.4	The results of fluorescence standardisation must fall within the defined acceptable range.
M2.3.1.5	The results of fluorescence standardisation must be recorded
M2.3.2	Compensation
M2.3.2.1	If performing analyses that require the simultaneous use of two or more fluorochromes, an appropriate procedure to compensate for overlap in their emission spectra must be used.
M2.3.2.2	Compensation settings must be determined every day of use, and
M2.3.2.3	at any time maintenance or adjustment of the instrument during operation is likely to have altered them
M2.3.2.4	Compensation must be carried out for all fluorochromes used.
M2.3.2.5	Compensation values
M2.3.2.5.1	Acceptable compensation values must be defined
M2.3.2.5.2	The values used must be recorded
M2.4	Equipment Cleaning and Maintenance
M2.4.1	Cleaning
M2.4.1.1	There must be a procedure for regular cleaning of the instrument
M2.4.1.2	The frequency and protocol for cleaning must conform to manufacturer's instructions, if available
M2.4.1.3	Cleaning must be documented
M2.4.2	Maintenance
M2.4.2.1	The instrument must be maintained according to manufacturer's instructions, but at least once a year.
M3	General Reagents
M3.1	Specificity of labelling reagents for identification of cell subsets:
M3.1.1	The specificity of labelling reagents must be verified using a published method and/or the manufacturer's documentation and/or by local documented quality control testing.
M3.1.2	If locally defined, the specificity of labelling reagents must be verified using appropriate control cells, prepared and tested by the same method employed in the laboratory's test sample analysis
M3.2	Secondary labelling reagents:
M3.2.1	must be titrated to determine the dilution with optimal activity (signal to noise ratio).

M3.2.2	If a multicolour technique is employed, the reagent must not cross-react with the other immunoglobulin reagents used to label the cells.
M3.3	Reagents which have been reconstituted from lyophilised powder must be centrifuged according to the manufacturer's instructions or locally documented procedures to remove micro aggregates prior to use.
M3.4	Each lot and shipment of labelling reagents must be tested for proper performance.
M3.5	Thresholds for adequate intensity must be defined and documented.
M3.6	The quantity of reagents used for each test sample must be determined by the manufacturers or from published data and verified locally by appropriate titration procedures.
M4	Antibody screening and crossmatching
M4.1	Cell based testing
M4.1.1	Labelling of target cells
M4.1.2	An individual fluorochrome must be used for the identification of each population subset (multicolour technique).
M4.1.3	If a single colour technique is used, the purity of the isolated cell population
M4.1.3.1	must be sufficient to define the population for analysis
M4.1.3.2	must be documented
M4.1.4	The target sub-populations analysed
M4.1.4.1	Must be defined
M4.1.4.2	Must include a sufficient number of acquired events per sub-population, relevant to the test performed
M4.1.4.3	Must be identified by appropriate labelling antibodies
M4.1.5	The binding of human immunoglobulin on target cells must be assessed with a fluorochrome labelled F(ab') anti-human IgG specific for the Fc region of the heavy chain
M4.2	Cell and bead based antibody screening
M4.2.1	For the detection of anti-HLA antibodies or assignment of antibody specificity, the composition of the panel must conform to the standards in section F4 (Panels)
M4.3	Controls
M4.3.1	Negative control
M4.3.1.1	A negative control must be used, which must be
M4.3.1.2	A serum from non-alloimmunised human donor(s), and
M4.3.1.3	screened and found to be negative by flow cytometry.

M4.3.2	Positive control
M4.3.2.1	A positive control must be used, which must be a human serum,
M4.3.2.2	specific for HLA antigens
M4.3.2.3	of the appropriate isotype
M4.3.3	Control sera must be tested at the same time and under the same conditions as the sera under test.
M4.4	Procedures and policies
M4.4.1	There must be policies and procedures to address at least:
M4.4.2	Antibody screening and crossmatching technical instructions, including:
M4.4.2.1	Reagent standardisation and optimisation
M4.4.2.2	Reagent validation
M4.4.2.3	Incubation times
M4.4.2.4	Incubation temperatures
M4.4.3	Interpretation instructions must include details of:
M4.4.3.1	The threshold for significant levels of antibody binding (positivity)
M4.4.3.2	The mechanism for reporting positive results (mean, mode or median channel shifts, relative mean fluorescence, or number of molecules of fluorescent marker)
M4.4.4	Acceptable reactivity required for negative, positive and secondary control reagents, in order for the test to be valid must be
M4.4.4.1	Defined
M4.4.4.2	documented
M5	Cell-based HLA typing by flow cytometry (eg HLA B27)
M5.1	Labelling reagents for the identification of HLA specificities
M5.1.1	The specificity of each lot and shipment must be determined by testing:
M5.1.2	at least five cells known to express the target antigen
M5.1.3	at least two cells for each cross-reacting antigen
M5.1.4	at least two cells which lack the specific and cross-reacting antigens
M5.1.5	Acceptable criteria for validation must be defined and results must be recorded.
M5.1.6	Each lot and shipment of labelling reagents must be shown to have comparable reactivity to the previously validated lot and shipment
M5.2	Controls

M5.2.1	Controls for HLA typing by flow cytometry must be run for each test cell preparation
M5.2.2	Negative Controls
M5.2.2.1	For direct labelling, a negative control must be conjugated with the same fluorochrome as the test.
M5.2.2.2	For indirect labelling, a negative control should be used in conjunction with the same secondary antibody conjugated with the same fluorochrome as used for the specific antibody under test.
M5.2.3	Positive Controls
M5.2.3.1	Must include a pan-reacting anti-HLA monoclonal antibody, which
M5.2.3.1.1	Must be tested against each cell
M5.2.3.1.2	Must be tested under the same conditions as the antibodies under test
M5.2.3.2	A control cell known to express the HLA specificity under test must be included in each run.
M5.3	Policies and Procedures
M5.3.1	There must be policies and procedures to address at least:
M5.3.2	HLA typing technical instructions including:
M5.3.2.1	Reagent standardisation and optimisation
M5.3.2.2	Reagent validation
M5.3.2.3	Incubation times
M5.3.2.4	Incubation temperatures
M5.3.3	Interpretation instructions must define:
M5.3.3.1	The required reaction criteria in the negative and positive control samples for the test results to be valid.
M5.3.3.2	Criteria for positivity of the HLA antigen under test
M5.3.3.3	A documented procedure must be followed for monoclonal antibodies which react with antigens other than those expected.

SECTION N – ENZYME-LINKED IMMUNO SORBENT ASSAY (ELISA)

N1	Equipment
N1.1	ELISA Reader
N1.1.1	The light source must produce the intensity and wavelength of light required for the test system
N1.1.2	Periodic calibration must be performed according to the manufacturer's instructions
N1.1.3	The result of the calibration must be documented
N1.2	Microplate Washer
N1.2.1	The performance of the microplate washer must be checked at least monthly.
N1.2.2	The result of the performance test must be
N1.2.2.1	Acceptable
N1.2.2.2	Documented
N2	ELISA Reagents
N2.1	The dilution of reagents, controls and test specimens must be
N2.1.1	Defined
N2.1.2	Documented
N2.2	Sera must be tested at a concentration determined to be optimal for the detection of antibody to HLA antigens with the test system used.
N2.3	Commercial kits must be used according to the manufacturer's instructions, or
N2.4	The laboratory must perform and document testing to support a deviation in the technique or analysis.
N2.5	Each lot of reagents must be validated and shown to have comparable reactivity to a previously validated lot
N3	Quality Management and Controls
N3.1	Sample identity and proper plate orientation must be maintained throughout the procedure.
N3.2	The lot numbers and optical density values for the reference reagents, controls and test samples must be recorded for each assay.
N3.3	The test results must meet defined criteria for reference reagents and controls in order for the test to be valid
N3.4	Negative Control
N3.4.1	A negative control must be included in each assay, and
N3.4.2	Must include a serum from a non-alloimmunised human donor(s)
N3.5	Positive Control

N3.5.1	A positive control must be included in each assay and
N3.5.2	must be a human serum specific for HLA antigens and of the appropriate isotype.
N3.6	Reagent Controls
N3.6.1	A control lacking only HLA antigen must also be included in each assay.
N4	Panels
N4.1	For the detection of antibodies or the assignment of antibody specificity, the composition of the cell panel must conform to the standards in section F4.

ABBREVIATIONS

CDC	Complement Dependent Cytotoxicity
CWD	Common and Well-Documented
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribo Nucleic Acid
dNTP	DeoxyriboNucleotide TriPhosphates
EFI	European Federation for Immunogenetics
EPT	External Proficiency Testing
ELISA	Enzyme Linked Immunosorbent Assay
FNHTR	Febrile Non Haemolytic Transfusion Reaction
HCE	Haematopoietic Chimaerism and Engraftment (monitoring)
HLA	Human Leukocyte Antigen
HNA	Human Neutrophil Antigen
HPA	Human Platelet Antigen
HSCT	Haematopoietic Stem Cell Transplantation
IgG	Immunoglobulin Gamma
IgM	Immunoglobulin Mu
IMGT	ImMunoGeneTics (project)
MHC	Major Histocompatibility Complex
NAIN	Neonatal Allo Immune Neutropenia
NAIT	Neonatal Allo Immune Thrombocytopenia
NMDP	National Marrow Donor Program
PCR	Polymerase Chain Reaction
PTP	Post Transfusion Purpura
QC	Quality Control
Q-PCR	Quantitative PCR
SBT	Sequence Based Typing
SSCP	Single Strand Conformation Polymorphism
SSOP	Sequence Specific Oligonucleotide Probe
SSP	Sequence Specific Primer
TAGVHD	Transfusion Associated Graft Versus Host Disease
<i>Taq</i>	<i>Thermus Aquaticus</i>
TRALI	Transfusion Related Acute Lung Injury
WHO	World Health Organisation

DEFINITIONS

Allele	alternate forms or varieties of a gene
Alloimmunised	is a response to foreign antigens (alloantigens) from genetically dissimilar members of same species
Ambiguity	possibility of interpretation in more than one way
Antigens	a foreign substance in the body that stimulates an immune response especially the production of antibodies
Chromosome	thread-like, gene carrying bodies in the nucleus of a cell and are composed of DNA , which itself is composed of 4 nucleotides: A (adenine), T (thymine), C (cytosine) and G (guanine)
Cloning	to make multiple identical copies of a DNA sequence
Common	HLA alleles which have been observed in multiple populations, although not necessarily in every population or in every region of the world. Their frequencies are known, and they have been observed at frequencies of <0.001 in reference populations of at least 1500 individuals. (<i>Tissue Antigens</i> 2013 81 :194-203)
Conditioning regimen	chemotherapy or irradiation given immediately prior to a transplant the purpose of which is to help eradicate the patient's disease prior to the transplant of HSC and to suppress the immune system
Discrepancy (typing)	a conflict or variation
DNA	a large genetic molecule that stores the genetic code for the synthesis of proteins. DNA is made out of two strands that are held together by hydrogen bonds, each strand being made up of a sugar, a phosphate group and one of four bases (adenine, guanine, cytosine or thymine)
Exon	is any nucleotide sequence encoded by a gene that remains present within the final RNA product of that gene after introns have been removed by RNA splicing
Extended typing	HLA typing performed to add additional information to an existing HLA assignment
Genes	units of inheritance usually occurring at specific locations, or loci, on a chromosome OR the fundamental physical and functional unit of heredity, which carries information from one generation to the next.
Genotype	the genetic makeup of an individual. Genotype can refer to an organism's entire genetic makeup or the alleles at a particular locus
Haplotype	a set of closely linked alleles (genes or DNA polymorphisms) inherited as a unit

Heteroduplex	a DNA double helix formed by annealing single strands from different sources
Heterozygous	a genotype consisting of two different alleles of a gene
Homozygous	having the same allele at the same locus on both members of a pair of homologous chromosomes
High resolution typing	is defined as the identification of HLA alleles that encode the same protein sequence within the antigen recognition site OR As a set of alleles that specify and encode the same protein sequence for the antigen binding domain of an HLA molecule and that excludes alleles that are not expressed as cell- surface proteins
Histocompatibility	compatibility between the tissues of two different individuals so that one accepts a graft from the other without having an immune reaction
HLA system	the body's genetically inherited system for recognising and rejecting foreign tissues, such as transplanted organs
Immunogenetics	is a branch of medical science that explores the relationship between the immune system and genetics
Isotype (antibody)	defines the role of antibodies in the body. There are five different antibody isotypes seen in humans: IgG, IgA, IgM, IgE and IgD.
Locus	the position of a particular gene on a chromosome OR a specific location on a chromosome.
Low resolution typing	The DNA-based typing result is at the level of the digits comprising the first field in the DNA-based nomenclature
Luminex	bead array solid phase based assay
Markers (genetic)	alleles of genes, or DNA polymorphisms, used as experimental probes to keep track of an individual, a tissue, a cell, a nucleus, a chromosome or a gene
Molecular techniques	the study of the molecular constitution of genes and chromosomes
Mutation	an alteration of genetic material such that a new variation is produced OR a disruption in the normal sequence of a DNA strand resulting in a different trait produced. A point mutation is caused by a change in a single base pair
Null allele (HLA)	allele in which no expressed antigen is present on the cells
Phenotype	the detectable expression of a genotype or the observable or detectable characteristics of an individual organism or the visible characteristics of an individual.

Polymerase chain reaction (PCR)	a method of DNA analysis that amplifies a specific DNA region allowing rapid DNA analysis.
Polymorphisms	different forms of a single gene
Primer	an oligonucleotide that binds to a specific target sequence of a gene or template by complementarities under defined conditions and is used to initiate DNA amplification
Probe	an oligonucleotide that binds to and identifies the presence of target sequences of a gene by complementarities under defined conditions. Probes may be free in liquid phase or bound to solid substrates
Primer-dimer	is a potential by-product in PCR. As its name implies a primer-dimer consists of primer molecules that have attached (hybridised) to each other
Prospectively	looking towards the future
Recombination	the interchange of genetic sections between pairing chromosomes during meiosis that produces variations in genetic characteristics by rearranging genes. Also referred to as a “crossover”
Refractoriness	not responding to treatment/therapy eg. Transfusion of randomly selected platelets
Retrospectively	looking backwards to the past
Sensitisation	to render an individual sensitive to foreign antigens. May follow a sensitising event such as transplantation, transfusion, pregnancy etc.
Verification typing	HLA typing performed on an independent sample with the purpose of verifying concordance of that typing assignment with the initial HLA typing assignment
Virtual crossmatching	Determining the presence of pre-transplant donor specific HLA – Antibodies (DSA) by comparing recipient’s HLA antibody specificities with the donor’s HLA antigens , and replacing the pre-transplant laboratory crossmatching test with an interpretive assessment of compatibility.
Well-Documented	Alleles which have been observed in at least five times in unrelated individuals through the use of a Sequence Based Typing method or at least three times if the allele has been observed in a specific haplotype in unrelated individuals (<i>Tissue Antigens</i> 2013 81 :194-203).