# European Federation for Immunogenetics

# STANDARDS FOR HISTOCOMPATIBILITY & IMMUNOGENETICS TESTING

**Version 6.1**

Accepted by the Standards and Quality Assurance Committee on 10th May 2013
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Effective from 1st October 2013

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## SECTION A – GENERAL POLICIES

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| **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

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| **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 an earned doctoral degree in a biological science, or be a physician, or have an equivalent qualification |
| **B1.1.2** | Have minimum qualifying experience equivalent to either of the following: |
| **B1.1.2.1** | Four years experience in immunology or cell biology, two of which were devoted to full time training in human histocompatibility testing, or |
| **B1.1.2.2** | Five years of working experience at full time in human histocompatibility 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, Standards B1.1.1 - B1.1.3 apply. |
| **B1.1.5** | **The Director or Co-Director must:** |
| **B1.1.5.1** | Be available on site at least 20h/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 |
| **B1.1.6** | **The Director or Co-Director should:** |
| **B1.1.6.1** | Demonstrate active participation in national or international workshops |
| **B1.1.6.2** | 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 |

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| **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

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| **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** | Must be maintained at temperatures optimal for storage of each type of sample or reagent |
| **C1.2.2** | Must be monitored every working day |
| **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 |
| **C1.3** | 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: |

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| **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 |

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| **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 |

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| **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 annually |
| **C5.2.2** | Documented evidence of this review must be available |
| **C5.2.3** | Any changes in procedures must be initialled and dated by the Director/Co-Director/ delegated individual at the time they are initiated. |
| **C6** | **Quality assurance** |
| **C6.1** | **External Proficiency Testing( EPT)** |
| **C6.1.1** | The laboratory must participate in EPT programme(s) to cover |
| **C6.1.1.1** | All the accredited laboratory applications (HLA typing, antibody screening and identification, crossmatching, etc.). |

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| **C6.1.1.2** | All techniques used individually or in combination as routinely employed to produce a final result |
| **C6.2** | The procedure for testing EPT samples including the allocation to techniques must be documented prior to the annual commencement of the EPT cycle. |
| **C6.3** | For proficiency testing, the laboratory must be in compliance with published regulations formulated by the EFI EPT Committee and approved by the EFI Board. |
| **C6.4** | If a laboratory's performance in EPT programme(s) is unsatisfactory in any category for which EFI accreditation is sought, the laboratory must: |
| **C6.4.1** | Participate in an additional EPT programme in that category |
| **C6.4.2** | Document the Director's review and any corrective action taken |
| **C6.5** | EPT samples must be tested and interpreted in a manner comparable to that for routine testing of clinical samples |
| **C6.6** | Participating laboratories must ensure that all the following EPT related documents are maintained and are made available to EFI inspectors for assessment: |
| **C6.6.1** | Submitted worksheets |
| **C6.6.2** | EPT summary/scheme reports |
| **C6.6.3** | Annual performance |
| **C6.6.4** | Participation certificates |
| **C6.6.5** | Outcomes of investigations of any unsatisfactory results |
| **C6.6.6** | 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: |

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| **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. |

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| **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. |

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| **C11.7** | Laboratories must have a procedure in place for resolving any HLA typing discrepancies that may occur between laboratories. |

## SECTION D – HLA ALLELES AND ANTIGENS

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| **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; Cw5; DR1, 4; DQ5, 7. |
| **D2.1.2.2** | DNA assignment: HLA-A*02, *30; B*07, *44; C*05, *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, Cw5, DR1, DQ5 / A30, B7, Cw-, DR4, DQ7. |
| **D2.1.3.2** | DNA assignment: HLA-A*02, B*44, C*05, DRB1*01, DQB1*05 / A*30, B*07, C*16, DRB1*04, DQB1*03:01. |
| **D2.2** | The locus designation must always be included. |

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| **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** | Reports of HLA haplotype assignments must include an explanation of recombination when this occurs. |
| **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

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| **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** |
| **E.2.1** | 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 |

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| **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. |

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| **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

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| **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 (classical non-dedicated cytometers) must also conform to the standards in sections M1 and M2. |
| **F2.2.2** | Laboratories using micro-plate ELISA techniques for antibody screening must additionally conform to standards in Section N. |
| **F2.2.3** | Laboratories performing assays using fluorescent microbead arrays in conjunction with a dedicated cytometer-like instrument must additionally conform to relevant parts of section M4. |
| **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 |

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| **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 and N must be followed when applicable. |

## SECTION G – RENAL and/or PANCREAS TRANSPLANTATION

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| **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 (G4.2) |
| **G4.1.2** | Prospective crossmatching must be performed for all living donor transplants |

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| **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 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. (G4.2.1) |
| **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. |

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| **G4.5** | **Retrospective Crossmatching** |
| **G4.5.1** | If retrospective cross-matches are performed according to 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 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

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| **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** | G2 also applies |
| **H3** | **Crossmatching** |
| **H3.1** | Crossmatching must be performed according to standards in section F5. |
| **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 and tissues for recipients at high risk for allograft rejection should come from cross-match negative donors as defined by the laboratory and the transplant program. |

## SECTION I – HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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| **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 under 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 all available members of the immediate family |
| **I2.2** | Must include adequate testing to definitively establish HLA identity by descent (D3.2 applies), or |
| **I2.3** | Must include high resolution Class I and/or Class II typing by DNA methods to determine the degree of HLA matching as documented in the transplant protocol. |
| **I2.4** | Must include high resolution Class I and Class II typing for recipient and potential intra-familial donors who are not HLA identical siblings as documented in the transplant protocol. |
| **I2.5** | 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. A*02, B*44, DRB1*11*) |
| **I3.2** | Extended typing must be included if required by the transplant protocol (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 |

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| **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 typing at a minimum level of low resolution which allows assignment of all serologically defined antigens |
| **I4.3.1.2.2** | high resolution DRB1 typing |
| **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 |

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| **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 |
| **I6** | **Haemopoietic Chimaerism and Engraftment (HCE) Monitoring** |
| **I6.1** | Standards in 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. |

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| **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

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| **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 in section F5 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; *Vox Sanguinis 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 apply. |

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| **J3.2.2** | Clinically significant HPA specificities must be defined and documented. |
| **J3.2.3** | For patients with HPA alloimmunisation, HPA verification typing must be performed on donors whose products may be used. |
| **J3.3** | **Investigation of HPA antibodies** |
| **J3.3.1** | For bead array techniques, relevant standards from Section M also apply |
| **J3.3.2** | For ELISA based assays, standards in Section N 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; *Vox Sanguinis 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 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 also apply |
| **J4.3.2** | For ELISA based assays, standards in Section N 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 |

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| **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

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| **K1** | **The following standards apply:** |
| **K1.1** | If complete HLA typing is performed by serology standards in section E 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 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 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

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| **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 |

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| **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. |

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| **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)** |

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| **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. |

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| **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 |
| **L10.4.5** | If a determined sequence is ambiguous, all possible allelic combinations must be documented. |
| **L10.4.6** | If ambiguities are not included on the report, a comment that additional data are available in the laboratory must be added. |
| **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 : |

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| **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** | Non specific and weak hybridization signals must be defined and documented. |
| **L12** | **Other Methods** |

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| **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 |

## SECTION M – FLOW CYTOMETRY

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| **M1** | **Application** |
| **M1.1** | Section M2 applies to flow cytometry and flow analysis using equipment designed for beads only (fluoro-analyser) |
| **M1.2** | Sections M2 to M5 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 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 M2.2 to 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. |

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| **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. |

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| **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 cross-matching** |
| **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 |
| **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, |

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| **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 |

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| **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)

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| **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 |

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| **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. |