

European Federation for Immunogenetics



**STANDARDS FOR
HISTOCOMPATIBILITY
TESTING**

Version 5.5

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HISTOCOMPATIBILITY TESTING

A - GENERAL POLICIES

- A1.000** These Standards have been approved and adopted by the EFI Executive Comr They are based on Standards originally prepared by the American Socie Histocompatibility and Immunogenetics (ASHI).
- A2.000** These Standards have been established for the purpose of ensuring accurat dependable histocompatibility testing consistent with the current sta technological procedures and the availability of reagents.
- A3.000** These Standards establish minimal criteria, which all histocompatibility labor must meet if their services are to be considered acceptable. Many labora because of extensive experience, will exceed the minimal requirements of Standards.
- A4.000** Certain Standards are obligatory. In these instances, the Standards use the "must". Some Standards are highly recommended but not absolutely mandato these instances the Standards use words like "should" or "recommended".
- A5.000** Procedures to be used in histocompatibility testing often have multiple ace variations. The accuracy and dependability of each procedure must be documer each laboratory or by published data from other laboratories.
- A6.000** Some procedures have sufficient documentation of effectiveness to warrant th in clinical service even though they are not available in or obligatory f laboratories.
- A7.000** The use of the name of the European Federation for Immunogenetics as certi of compliance to these Standards may only be made by laboratories, which hav accredited through the EFI accreditation process.

B - PERSONNEL QUALIFICATIONS

- B1.000** The laboratory must employ one or more individuals who meet the qualificatio fulfill the responsibilities of the Director/Co-Director, and Technical Supervisor
- B2.000** A *Director/Co-Director* must hold an earned doctoral degree in a biological sc or be a physician, or have an equivalent qualification. In addition, the Direct Director1) must have had four years experience in immunology or cell biolog; of which were devoted to full time training in human histocompatibility testing five years of working experience at full time in human histocompatibility testin Director/Co-Director must have documentation of professional competence appropriate activities in which the laboratory is engaged. This should be base sound knowledge of the fundamentals of immunology, genetics and histocompa testing and reflected by external measures such as participation in nation international workshops or publications in peer-reviewed journals. The Direc Co-Director is available on site at least 20h/week, provides adequate supervis technical personnel, utilises his/her special scientific skills in developing

procedures and is held responsible for the proper performance, interpretation and reporting of all laboratory procedures and the laboratory's successful participation in proficiency testing. The Director/Co-Director must be informed of the results of the national legislation.

B3.000 A *Technical Supervisor* must hold a bachelor's degree or equivalent and have three years' experience in human histocompatibility testing under the supervision of a qualified Director/Co-Director or five years of supervised experience if a bachelor's degree has not been earned.

B4.000 The number of staff must be large enough to carry out the volume and variety of work required.

C - QUALITY ASSURANCE

C1.000 *Facilities.*

C1.100 In accordance with national regulations, laboratory space must be sufficient so that all procedures can be carried out without crowding to the extent that errors may be introduced. The following facilities must be adequate and immediately available in the laboratory: refrigerators, freezer storage of reagents and specimens, storage of records.

C1.200 Lighting and ventilation must be adequate.

C1.300 Refrigerators and freezers must be maintained at temperatures optimal for storage of each type of sample or reagent. They must be monitored every working day. Recording thermometers are recommended for mechanical refrigerators or freezers. These should be coupled to alarm systems with an audible alarm where it can be heard 24 hours a day. 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. Ambient temperature and/or the temperature of incubators in which test procedures are carried out must be monitored every working day to ensure that these procedures are carried out within the temperature ranges specified in the laboratory's procedure manual.

C1.400 Laboratories performing procedures which require cell culture must have a laminar flow hood or other appropriately aseptic work area. Incubators must be monitored every working day in relation to temperature (37°C) and CO₂ concentration (5%±1%) and should be appropriately humidified.

C1.500 Laboratories using radioactive materials must store these and conduct procedures using such materials in a designated section of the laboratory. Radioactive materials must be disposed of at locations designated by local institutions.

C1.600 Laboratories performing amplification of nucleic acids must use physical and biochemical barriers to prevent DNA contamination. 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.

C1.700 The laboratory must establish and employ policies and procedures for the prevention and maintenance of equipment, instruments and test systems by 1) defining its preventive maintenance programme for each instrument and piece of equipment, and

- performing and documenting function checks on equipment with at least a frequency specified by the manufacturer.
- C1.800 The laboratory must document compliance with all applicable national and local regulations which relate to laboratory employee health and safety; fire safety; and the safe handling and disposal of chemical, biological and radioactive materials.
- C1.900 *Computer assisted analyses.*
- C1.910 Computer assisted analyses must be reviewed, verified and signed by the Superintendent and/or Laboratory Director before issue.
- C1.920 The computer software programme used for analyses must be identified, validated/verified before use.
- C2.000** *Specimen submission and requisition.*
- C2.100 The laboratory must have available and follow written policies and procedures regarding specimen collection.
- C2.110 The laboratory must perform tests only at the written or electronic request of an authorised person. The laboratory must assure that the requisition includes: 1) patient's or donor's name or other method of specimen identification to ensure accurate reporting of results; 2) the name and address of the authorised person or the service who ordered the test; 3) date of specimen collection; 4) time of specimen collection, when pertinent to testing; 5) source of specimen (e.g. bone marrow, sputum, cells) if pertinent.
- C2.120 Blood or tissue samples must be individually labelled with the name, and/or unique identification marker of the individual and the date of collection. If multiple blood containers are collected, each container must be individually labelled.
- C2.130 The laboratory must maintain a system to ensure reliable specimen identification. The laboratory must document each step in the processing and testing of patient specimens to ensure that accurate test results are recorded.
- C2.140 The laboratory must have criteria for specimen rejection and a mechanism to ensure that specimens are not tested when they do not meet the laboratory's criteria for acceptability.
- C2.200 Blood samples must be obtained using a technique, which does not compromise the integrity of the sample. The donor's skin must be prepared by a technique, which ensures the possibility of infection of the donor or contamination of the sample. All needles and syringes must be disposable.
- C2.210 All blood and tissue samples should be handled and transported in accordance with the understanding that they could transmit infectious agents.
- C2.220 The laboratory must provide all service users with information on the requirements for sample labelling, anticoagulant/preservation media and sample packaging including regulations relating to postal transport. Users should be warned that failure to meet the requirements may result in sample rejection.
- C2.300 *Reagents.*
- C2.310 All reagents must be properly labelled and stored according to manufacturer's instructions or locally-specified conditions to maintain reactivity and specificity.
- C2.320 Reagents, solutions, culture media, controls, calibrators and other materials must be labelled to indicate 1) identity and when significant, titre, strength or concentration; 2) recommended storage requirements; 3) preparation and/or expiration date; and 4) other pertinent information. For storage of larger numbers of identical samples, the container must be clearly labelled.

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.

C3.000

Laboratory Procedure Manual.

C3.100

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 pictorial inserts provided by manufacturers is not acceptable in place of the procedure manual. Each procedure must be reviewed at least annually by the Director/Co-Director. Written evidence of this review must be in the manual. Any changes in procedure must be initialled and dated by the Director/Co-Director at the time they are initiated.

C4.000

Quality Assurance.

C4.100

Proficiency Testing and Competency Evaluation.

C4.110

The laboratory must participate in EPT programme(s) to cover all the accreditation laboratory applications (HLA typing, antibody screening and identification, crossmatching, etc.). EPT results must be obtained for all techniques individually in combination as routinely used to produce a final result. The procedure for testing EPT samples including the allocation to techniques must be documented prior to the annual commencement of the EPT cycle.

C4.120

For proficiency testing, the laboratory must be in compliance with published regulations formulated by the EFI EPT Committee and approved by the EFI Board.

C4.130

If a laboratory's performance in EPT programme(s) is unsatisfactory in any category for which EFI accreditation is sought, the laboratory must participate in an additional EPT programme in that category and document the Director's review and corrective action taken.

C4.140

EPT samples must be tested and interpreted in a manner identical to that for routine testing of clinical samples.

C4.150

The laboratory must, at least once a year, give each individual performing a test a characterised specimen as an unknown to verify his or her ability to reproduce results. This standard must be applied to all tests regularly performed by the individual. The laboratory must maintain records of these results for each individual.

C4.160

The Director/Technical Supervisor or designee must evaluate the competence of each technologist annually.

C4.170

The laboratory Director and the technical staff must participate in continuing education relating to each category for which EFI accreditation is sought.

C4.200

Systems for continuous test evaluation and monitoring.

C4.210

The laboratory must establish and employ policies and procedures, and document actions taken when 1) test systems do not meet the laboratory's established criteria including quality control results that are outside of acceptable limits; and when errors are detected in the reported patient results. In the latter instance, the laboratory must promptly a) notify the authorised person ordering or individual utilising the results of reporting errors; b) issue corrected reports, and c) maintain copies of the original report as well as the corrected report for at least two years.

C4.220

The laboratory must have mechanisms in place for continuous monitoring of test systems used. These mechanisms must include: a) validation/verification, before introduction in routine use, of all new tests, by systematic comparative evaluation of results obtained in parallel with the new and the standard system, b) re-evaluation of results obtained in external and internal QC testing, c) re-monitoring of test validity in routine testing, by recording observations during

from the expected results (e.g. cross-reactivity of probes or primer mixes, day-variations). Written evidence of the ongoing monitoring processes must be available in the laboratory for each method performed.

- C4.230 If a laboratory performs the same test using different techniques, test results must be compared and inconsistencies documented.
- C4.240 The laboratory must have a mechanism to identify and evaluate inconsistencies between test results and clinical data or diagnostic parameters provided.
- C4.300 *Client service evaluation.*
- C4.310 The laboratory must document problems that result from breakdown communication between the laboratory and the authorised individual who orders or receives results.
- C4.320 All complaints and problems reported to the laboratory must be documented. Complaints must be investigated and corrective action taken when necessary.
- C4.330 The laboratory must, upon request, make available to clients a list of tests employed by the laboratory.
- C4.400 *Quality assurance evaluation.*
- C4.410 The laboratory must document and assess problems identified during quality assurance reviews, discuss them with the staff, and take corrective actions necessary to prevent recurrences.
- C4.420 The laboratory must have an ongoing mechanism to evaluate corrective actions. Ineffective policies and procedures must be revised based on the outcome of the evaluation.
- C4.430 The laboratory must maintain documentation of all quality assurance activities including problems identified and corrective actions taken, for a minimum of 5 years or longer, depending on local, or national regulations.
- C4.440 The laboratory must maintain permanent files of all internal and external quality control tests.
- C5.000** *Records and test reports.*
- C5.100 The laboratory must maintain records of subjects tested for two years or longer depending on local regulations.
- C5.110 These records must include log books, worksheets, and at least a summary of results obtained.
- C5.120 Work sheets must clearly identify the sample tested, the reagents and methods used, the test performed, the date of the test and the person performing the test.
- C5.130 For HLA typing by complement-dependant cytotoxicity each serum-cell combination must be recorded in a manner, which indicates the approximate percent of cells killed. The numerical scores used in the International workshop procedure (0,1,2,3,4) should be used. Other numerical codes can also be used.
- C5.140 Reports or records, as appropriate, should include a brief description of the specimen (blood, lymph node, spleen, bone marrow, etc.) used for testing.
- C5.150 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.
- C5.160 Records may be only saved in computer files, provided that back-up files are maintained to ensure against loss of data.
- C5.200 For marrow transplantation, donor records should be maintained so that donors can be rapidly retrieved according to HLA type.

- C5.300 The laboratory must have adequate systems in place to report results in a timely, accurate and reliable manner.
- C5.400 The report must contain:
- a. The name of the individual tested or unique identifier of each individual test and the relationship to the patient if applicable.
 - b. The date(s) of collection of sample when pertinent.
 - c. The date of the report.
 - d. The test results.
 - e. The techniques used.
 - f. Appropriate interpretations and the signature of the Laboratory Director, or, in his/her absence, by a designee who meets the requirements of the Technical Supervisor.
- C5.410 The laboratory must indicate on the test report information regarding the condition and disposition of specimen that did not meet the laboratory's criteria for acceptability.
- C5.500 Laboratories must have a procedure in place for resolving any tissue discrepancies that may occur between laboratories.
- C6.000** *Testing referred to other laboratories.*
- C6.100 An accredited laboratory may engage another laboratory to perform testing not covered by the primary laboratory. In that event, the subcontracting laboratory must be accredited by EFI or by ASHI, if the testing is covered by EFI Standards. If testing systems not covered by EFI Standards are subcontracted, the subcontracting laboratory should have documented expertise and/or accreditation in those systems. The identity of the subcontracting laboratory and that portion of the testing for which it bears responsibility must be noted in the reports.

D - HLA ALLELES AND ANTIGENS

- D1.000** *Terminology* of HLA alleles and antigens must conform to the latest report of the WHO Committee on Nomenclature.
- D1.100 Potential new alleles or antigens not yet approved by the WHO Committee must have a local designation which cannot be confused with WHO terminology.
- D1.200 Phenotypes and genotypes must be expressed as recommended by the WHO Committee, as in the following examples:
- D1.210 Single alleles: HLA-B*07. Single antigens: HLA-B7 (or B7 if HLA is obvious in context).
- D1.220 HLA type. Serological assignment: HLA-A2, 30; B7, 44; Cw5; DR1, 4; DQ5. DNA assignment: HLA-A*02, *30; B*07, *44; Cw*05, *16; DRB1*01, DQB1*05, *0301.
- D1.230 Genotype. Serological assignment: HLA-A2, B44, Cw5, DR1, DQ5 / A30, B7, DR4, DQ7. DNA assignment: HLA-A*02, B*44, Cw*05, DRB1*01, DQB1*03, A*30, B*07, Cw*16, DRB1*04, DQB1*0301.
- D1.240 The locus designation must always be included.
- D1.250 Broad antigens and the epitopes Bw4 and Bw6 may be reported in brackets.
- D1.300 If only a 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 DNA typing.

studies. Conversely, a "blank antigen or allele" can only be assigned if prov family studies.

- D1.310 If 2-digit DNA typing unequivocally demonstrates the presence of heterozygous two different alleles from the same specificity (e.g. DRB1*1301/DRB1*1303/1333), the report may include it twice (e.g. DRB1*13,*13) even in the absence of family studies.
- D1.320 High resolution typing is defined as a) identifying HLA alleles at the resolution of 4 digits or more, at least resolving all ambiguities resulting from polymorphisms located within exons 2 and 3 for HLA class I loci, and exon 2 for HLA class II loci and b) all ambiguities that encompass a null allele, wherever the polymorphism is located, unless it can be demonstrated that an expressed antigen is present on the cells.
- D2.000** *Haplotype assignment.*
- D2.100 Determination of haplotypes can only be done by typing immediate family members including parents, siblings and/or children of the patient.
- D2.110 Typing for HLA -A, B and DR locus alleles or antigens is mandatory.
- D2.120 Genotypic identity can only be proven if both parents are available or if the segregation of the four haplotypes is clearly defined.
- D2.130 Ambiguities in haplotype assignment must be resolved by typing for HLA -C, -E, -DQ and/or -DP. When appropriate, high resolution typing must be used to resolve ambiguities.
- D2.140 Reports of HLA haplotype assignments must include an explanation of recombination when this occurs.
- D2.200 *Unrelated individuals.*
- D2.210 Reports of probable haplotypes based on population frequencies must clearly indicate that they were so derived and the relevant references or sources must be available.

E - SEROLOGICAL HLA CLASS I AND CLASS II TYPING

- E1.000** *HLA-A, -B locus antigens.*
- E1.100 The laboratory must be able to type for the HLA-A and -B specificities, which are officially recognised by the WHO and for those deemed relevant by EFI.
- E1.200 Typing for HLA -C locus antigens is not mandatory.
- E2.000** *HLA-DR, -DQ antigens.*
- E2.100 The laboratory must be able to type for the HLA-DR specificities officially recognised by the WHO and for those deemed relevant by EFI.
- E2.200 Typing for HLA -DQ antigens is not mandatory.
- E3.000** *HLA Class I and II typing techniques.*
- E3.100 Techniques used must be those, which have been established to define HLA Class I and II specificities optimally.
- E3.200 *Control reagents.*
- E3.210 Each typing tray must contain at least one positive control antibody, previously shown to react with cells expressing class I and class II antigens.
- E3.220 Typing results may be invalid if the positive control fails to react as expected.
- E3.230 Each typing tray must include at least one negative control serum. The negative control should be one previously shown to lack leukocyte reactive antibodies.

- E3.240 Cell viability in the negative control well at the end of incubation must be sufficient to permit accurate interpretation of results. For most techniques, viability should exceed 80%.
- E3.250 Procedures that deal with control serum failures in typing or crossmatch trays are described in the laboratory manual.
- E3.300 *Target cells.*
- E3.310 Separation of B lymphocytes is not required if a technique is used that distinguishes between T and B lymphocytes or in assays in which antibodies of well-defined specificity are used which only define HLA Class II molecules.
- E3.400** *Antigen assignments.*
- E3.410 Each HLA -A, B antigen must be defined by at least two sera when available, if they are operationally monospecific. If multispecific sera are used, at least three partially non-overlapping sera must be used when available to define each HLA -A, B antigen.
- E3.420 Each monoclonal antibody used for alloantigen assignment must be used at a dilution and with a technique in which it demonstrates specificity comparable to that of an assignment by alloantisera on a well-defined cell panel.
- E3.430 Each HLA Class II antigen should be defined by at least three sera, if they are operationally monospecific. If multispecific sera are used, at least five partially non-overlapping sera must be used to define each HLA Class II antigen.
- E3.440 Criteria for antigen assignment must be described in the laboratory manual.
- E3.450 Ambiguity in antigen definition by serological typing must be referred to for confirmation by DNA based methods.
- E3.500 *Control of antibody specificity.*
- E3.510 Cell panels of known HLA type must be used to prove the specificity of typing antibodies. The panel cells should include at least one example of each HLA type and the laboratory should be able to define them.
- E3.600 *Typing sera.*
- E3.610 It is recommended that the specificity of typing sera obtained locally be confirmed by at least one other HLA laboratory.
- E3.620 A reagent grade typing serum is validated only after confirmation of specificity. Specificity determinations must include supporting statistical analysis.
- E3.630 Specificity of individual sera received from other laboratories or commercial sources must be confirmed to ensure that they reveal the same specificities in the recipient laboratory.
- E3.640 Each lot of new typing trays must be evaluated by testing either with at least two different cells of known phenotype representing major specificities or in parallel with previously evaluated trays.
- E3.700 *Complement.*
- E3.710 Each batch of complement must be tested to determine that it mediates cytotoxicity in the presence of specific antibody but is not cytotoxic in the absence of specific antibody. The complement must be kept at an appropriate temperature.
- E3.720 The test should employ multiple dilutions of complement to ensure that it is maximally active at least one dilution beyond that intended for use.
- E3.730 The test should be carried out with at least two antibodies, which should react with at least two different test cells and at least one cell, which should not react. A strong or a weak antibody should be selected for the test, or serial dilutions of a single antibody may be used.

E3.740 Complement should be tested separately for use with each type of target cell, s different dilution or preparation may be required for optimal performance.

F - ANTIBODY SCREENING AND CROSSMATCHING

F1.000 *Techniques.*

F1.100 A complement-dependent cytotoxic technique must be used for the detecti antibody to HLA antigens unless the laboratory has performed and docum testing to validate that another technique identifies alloantibody to HLA antigen level of sensitivity equivalent or superior to that of its cytotoxic technique.

F1.110 To detect antibodies to HLA class II antigens, a technique must be usec distinguishes them from antibodies to HLA class I antigens.

F1.120 Reports of results of antibody screening must include identification of the tecl used.

F1.200 *Sera.*

F1.210 Sera must be tested at a concentration determined to be optimal for detecti antibody to HLA antigens. The dilution(s) must be documented.

F1.220 Negative control sera must include a serum from non-alloimmunised human do. Each assay must include negative control(s).

F1.230 Positive control sera should be from highly alloimmunised individual documented to react with HLA antigens. The antibodies must be of the appr isotype for each assay. Each assay must include positive control(s).

F1.300 *Panel cells.*

F1.310 Target cells may be mononuclear cells from peripheral blood, lymph nodes, spl cell lines.

F1.320 To detect HLA class II specific antibodies, B lymphocytes, chronic lymph leukaemia cells or B cell lymphoblastoid lines may be used.

F1.330 The panel of HLA antigens must include sufficient panel cell donors to ensu they are appropriate for the population served and the use of the data.

F1.340 For assays intended to provide information on antibody presence or an identification, documentation of the HLA class I and/or class II phenotypes donors of the panel cells must be provided.

F1.350 To identify the specificity of an antibody with certainty, the laboratory should t serum with additional cells expressing and lacking the candidate antigen and reacting antigens.

F2.000 *Antibody screening by complement-dependent cytotoxicity.*

F2.100 An HLA specific positive control for the activity of the complement and a ne control for the viability of the test cells must be included on each tray.

F2.200 If sera are screened after treatment with dithiothreitol, then IgG and IgM specific positive controls must be included.

F2.300 Laboratories using a CDC technique must also conform to standard F Complement.

F3.000 *Antibody screening using classical non-dedicated cytometers.*

F3.100 Laboratories performing assays using flow cytometry must also conform t standards in sections M1 and M2.

F4.000 *Antibody screening by micro-plate ELISA.*

- F4.100 Laboratories using ELISA techniques for antibody screening must additionally conform to standards in Section N.
- F5.000** *Antibody screening using fluorescent microbead arrays in conjunction with a dedicated cytometer-like instrument.*
- F5.100 Laboratories performing assays using fluorescent microbead arrays in conjunction with a dedicated cytometer-like instrument must additionally conform to requirements of parts of section M.
- F6.000** *Crossmatching.*
- F6.100 Crossmatching for the detection of HLA specific antibodies must use techniques at least as sensitive as the basic lymphocytotoxicity test. At least one technique must be documented to have increased sensitivity in comparison with the basic microlymphocytotoxicity test, such as prolonged incubation, antiglobulin enhanced ELISA, B-cell crossmatch or flow cytometry should be used in addition to this.
- F6.110 The screening result must be predictive of the routine crossmatch result.
- F6.120 For lymphocytotoxic crossmatching each serum must be tested in duplicate and undiluted.
- F6.130 Crossmatches must be performed with unseparated lymphocytes or with separated lymphocytes from the potential donor. B-cell crossmatches must be performed in accordance with requirements required by the relevant transplantation programmes.
- F6.140 For lymphocytotoxic crossmatching, an HLA specific positive and negative control must be included for each tray.
- F6.150 If crossmatches are performed after treatment of the patient sera with dithiothreosol then IgG and IgM positive and negative HLA specific controls must be included.
- F6.160 Laboratories using a CDC technique must also conform to standard I for Complement.
- F6.200 Standards in sections M and N must be followed when applicable.

G - RENAL and/or PANCREAS TRANSPLANTATION

- G1.000** If cadaver donor transplants are done, personnel for the required histocompatibility testing, interpretation of results and provision of advice for the clinical transplant team must be available 24 hours a day, seven days a week. Laboratories not able to perform tests 24h/day, 7d/week must arrange with an EFI or ASHI accredited laboratory to perform tests.
- G2.000** *Antibody Screening.*
- G2.100 Laboratories must have a documented policy in place to evaluate the sensitisation of each patient at the time of their initial evaluation.
- G2.110 Laboratories must have a programme to periodically screen serum samples from each patient for antibodies to HLA antigens. Samples must be collected and tested monthly or as stipulated by the national and/or international organ exchange organisations. The laboratory must have a policy establishing the frequency of screening serum samples and must have data to support this policy.
- G2.120 Laboratories should maintain a record of potentially sensitising events for each patient. 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 testing.

- G2.200 Testing must be performed to distinguish HLA specific antibodies from non antibodies and autoantibodies. The specificity of detected HLA antibodies must be defined and recorded.
- G3.000** *Crossmatching.*
- G3.100 Crossmatching must be performed prospectively.
- G3.110 Prospective crossmatch may be omitted in recipients that have shown consistently negative for the presence of HLA-specific antibodies, as relevant to the transplant protocol. Sera must have been collected as defined in G2.110, covering at least one year of immunisation history and must have been tested by at least two different techniques. Screening data must include at least one result obtained within the previous 3 months, using a technique of equivalent sensitivity to that used for crossmatching.
- G3.120 If prospective crossmatch is not systematically performed, there must be evidence that the laboratory maintains a record of potentially sensitising events for each patient. Otherwise such a record should be maintained.
- G3.130 If a prospective crossmatch is omitted, a retrospective crossmatch must be performed. It must be shown to be in concordance with the predicted negative result, and must be documented. If this is not the case, the physician in charge must be immediately notified. A re-evaluation of this policy must be performed at least annually.
- G3.140 A prospective crossmatch cannot be omitted if this is contrary to the national legislation applying to the laboratory, and/or regulations from the national/international exchange organisation. If national regulations, or those of the exchange organisation mandate other criteria than those mentioned in G3.110, these must also be applied.
- G3.150 Crossmatching must be performed according to section F6.000.
- G3.200 *Sera samples.*
- G3.210 Sera obtained 14 days after a potentially sensitising event should be included in the final crossmatch.
- G3.220 Final crossmatches performed prior to transplantation should utilise a recipient serum sample collected within the previous 48 hours before transplant if the recipient has HLA antibodies or has had a recent sensitising event. Otherwise, the most recent available serum collected as defined in G2.110 must be used.
- G3.230 The laboratory must have a policy regarding the selection of the relevant serum sample to be used in the final crossmatch procedure.
- G3.240 Serum samples used for crossmatching should be retained in the frozen state for future use.
- G4.000** *HLA typing.*
- G4.100 Prospective typing of donor and recipient for HLA-A, B and DR antigens is mandatory.

H - OTHER ORGAN TRANSPLANTATION

- H1.000** In cases where patients are at high risk for allograft rejection (e.g. patients with a history of allograft rejection, patients with preformed HLA antibodies), donor and recipient should be typed for HLA-A,B and DR antigens.

- H2.000** Patients should be screened for the presence of HLA alloantibodies.
- H3.000** *Cross-matching.*
- H3.100 Crossmatching must be performed according to section F6.000.
- H3.200 Sera from patients at high risk for allograft rejection should be prospectively matched. Cross-match results should be available prior to transplantation presensitised patient.
- H3.300 Final crossmatches performed prior to transplantation should utilise a recipient sample collected within the previous 48 hours before transplant if the recipient has HLA antibodies or has had a recent sensitising event. Otherwise, a serum collected within three months should be used.
- H3.400 Sera obtained 14 days after a potential sensitising event should be used in the cross-match.
- H3.500 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.

I - HAEMATOPOIETIC STEM CELL TRANSPLANTATION

- I1.000** *Histocompatibility testing for related transplants.*
- I1.100 HLA-A, B or DR typing of all available members of the immediate family is mandatory.
- I1.110 HLA typing for HLA phenotypically identical siblings must include adequate typing to definitively establish HLA identity by descent (D2.120 applies), or use high resolution Class I and/or Class II typing (4-digit allele assignment) by DNA methods to determine the degree of HLA matching as appropriate for the transplant protocol.
- I1.120 HLA typing for recipient and potential intra-familial donors who are not phenotypically identical siblings must include high resolution Class I and Class II typing (4-digit allele assignment) by DNA methods as required by the transplant protocol.
- I1.130 Prior to transplantation using a related donor, HLA typing of both donor and recipient must be repeated using a new typing sample from each such that each individual's typing is confirmed for HLA -A, -B, and -DR, as a minimal requirement.
- I1.140 If required by the transplant protocol, laboratories not able to perform high resolution Class I and/or Class II typing by DNA methods must arrange for an EFIL or an accredited laboratory to perform these tests at the required level of resolution. If the ambiguities cannot be resolved, all the alternatives must be reported.
- I2.000** *Histocompatibility testing for unrelated transplants.*
- I2.100 *Volunteer bone marrow donors registries.*
- I2.110 The donor must give his/her informed consent according to the national legislation before blood is taken for typing and before the donor is placed on a list of donors available to be called.
- I2.120 Typing of the donors must be performed by serology or DNA methods at a level of resolution with at least 2 digits (e.g. A2 or A*02, DR11 or DRB1*11).
- I2.200 *Histocompatibility testing for transplants from unrelated donors.*
- I2.210 HLA typing for recipient and unrelated donors must include as a minimum requirement: low resolution HLA -A/B/Cw typing (2-digit allele assignment) and high resolution DRB1 typing (4-digit allele assignment) by DNA methods. Class I typing is not required.

must, as a minimum, be at a resolution which allows assignment of all serology defined antigens. Additional loci must be included if required by the transplant protocol.

- I2.220 If required by the transplant protocol, the laboratory must be able to type the donor and the recipient for HLA Class I by DNA methods, to a level of resolution as defined under D1.320. Where the ambiguities cannot be resolved, all the alternatives must be reported.
- I2.230 Laboratories not able to perform high resolution Class I typing by DNA method must arrange for an EFI or ASHI accredited laboratory to perform these tests as required.
- I2.240 Prior to transplantation using an unrelated donor, HLA typing of the recipient must be repeated using a different typing sample such that typing is confirmed for HLA -A, -B, and -DR, as a minimal requirement.
- I2.250 For unrelated donors, requirements of I2.210, I2.220, and I2.230 must be met. Typing on a sample obtained by the laboratory affiliated with the transplant centre and Registry data cannot be used for this purpose.
- I2.260 For unrelated donors HLA -A,-B,-DR concordant results are required on two separate samples. Registry data are acceptable for one of these samples.
- I2.270 Typing of donor and recipient at the highest level of resolution required by the transplant protocol must be performed in the laboratory affiliated with the transplant centre or as defined in I2.230.
- I3.000** *Crossmatching.*
- I3.110 If required by the local transplant protocol, crossmatching must be performed pre-transplant for related and unrelated transplantation.
- I3.120 Crossmatching must be performed according to standard F6.000.

J - PLATELET AND GRANULOCYTE TRANSFUSION

- J1.000** *HLA typing.*
- J1.100 The patient should be typed for HLA -A and HLA-B.
- J1.200 The donors should be selected according to national regulation/legislation.
- J2.000** *Cross-matching.*
- J2.100 Lymphocyte cross-matches are optional.
- J2.200 Cross-matching by techniques which utilise donor platelets or granulocytes as target cells are preferred.

K - DISEASE ASSOCIATION

- K1.000** Complete HLA typing is an appropriate option.
- K1.100 Typing may also be limited to all products of a single or limited number of HLA antigens.
- K2.000** *Typing for a single antigen by CDC (e.g. HLA-B27).*
- K2.100 Cell controls must be tested on each batch.
- K2.110 The control cells must include at least two cells known to express the specific antigen.
- K2.120 The control cells must also include two cells for each crossreacting antigen, which might be confused with the specific antigen.

- K2.130 The control cells must also include at least two cells lacking the specific crossreacting antigens.
- K2.200 Serum controls must be tested at the time of typing.
- K2.210 Serum controls must include a positive and negative control.
- K2.220 Serum controls should also include two sera for each antigen which crossreact the specified antigen (if available).
- K2.300 Sera to define each antigen must meet requirements of Section E as appropriate.

L – NUCLEIC ACID ANALYSIS

The nucleic acid analysis standards apply to histocompatibility testing.

L1.0000 General laboratory design, equipment and reagents.

L1.1000 Laboratory design.

- L1.1100 All pre-amplification procedures must be performed in a dedicated work area. Physical separation and restricted traffic flow are recommended (see C1.600).
- L1.1200 Pre-amplification physical containment must include use of dedicated equipment, coats, and disposable supplies.
- L1.1300 All activities occurring from and including thermal cycling must take place in a post-amplification area.
- L1.1400 Methods that use two consecutive steps of logarithmic amplification are especially susceptible to contamination. Addition of the template for subsequent amplification must occur in an area isolated by physical or chemical barriers from both the pre- and post-amplification work areas and must use dedicated equipment and consumables.

L1.2000 Contamination control (≠ wipe-test²).

- L1.2100 Contamination must be monitored for all amplification products that are produced in the laboratory.
- L1.2200 Routine wipe-tests of pre-amplification work areas must be performed at least every two months. Testing of other areas is recommended.
- L1.2300 Monitoring must be performed using a method that is at least as sensitive as routine test methods. Positive controls must be included to assure proper performance of monitoring.
- L1.2400 If amplified product is detected there must be a written description of how to eliminate the contamination and measures must be taken to prevent future contamination. There must be evidence of elimination of the contamination.

L1.3000 Equipment and reagents.

- L1.3100 Accuracy of thermal cycling instruments must be verified a) by maintenance according to the manufacturer, and b) by locally performed functional checks at least every six months.
- L1.3200 Incubators and water baths must be monitored for accurate temperature every time the assay is performed.
- L1.3300 All reagents (solutions containing one or multiple components) must be dispensed in aliquots for single use or reagents can be dispensed in aliquots for multiple use, documented to be free of contamination at each use. When reagents are combined

create a master mix, it is recommended that one critical component (e.g. polymerase) be left out of the mixture.

L1.3400 The appropriate performance of individual products must be documented for shipment and each lot before results using these reagents are reported.

L1.3500 For commercial kits, the source, lot number, expiry date, and storage condition be documented.

L1.3600 Reagents from different lots of commercial kits must not be mixed unless specified by the manufacturer or validated and documented with appropriate quality control at the laboratory.

L1.4000 Primers.

L1.4100 The specificity of primer combinations and the annealing positions must be defined.

L1.4200 Laboratories must have a policy for quality control of each shipment of primer. Specificity and quantity of amplified product must be confirmed with reference material. For commercial kits, each new shipment must be tested against at least one DNA sample of known type. The signal intensity must be defined, monitored and maintained within an acceptable range.

L1.4300 Primers must be utilized under empirically determined conditions that achieve the defined specificity for templates used in routine testing. Each new set of local primers must be tested for amplification specificity and quantity using reference material whenever available.

L1.4400 Local primer pairs must be monitored for appropriate sensitivity and specificity at regular intervals. The frequency between intervals should not exceed six months.

L2.0000 Nucleic acid extraction, electrophoresis and analysis.

L2.1000 Nucleic acid extraction.

L2.1100 Nucleic acids must be extracted and purified by a standard method that is documented and has been validated in the laboratory.

L2.1200 If the DNA is not used immediately after purification, suitable methods of storage must be available that will protect the integrity of the material.

L2.1300 Nucleic acids must be of sufficient purity and concentration to ensure reliable results. DNA purity and concentration should be determined for each sample; if no measurement, the laboratory must have tested and validated this policy.

L2.2000 Electrophoresis.

L2.2100 Optimal electrophoretic conditions must be determined, acceptable ranges must be established and their use documented.

L2.2200 The laboratory must establish criteria for accepting each slab or capillary migration, and each lane of a gel or capillary injection.

L2.2300 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.

L2.3000 Analysis.

L2.3100 Acceptable limits of signal intensity must be specified for positive and negative results. If these are not achieved, acceptance of the results must be justified and documented.

- L2.3200 Two independent interpretations of primary data must be performed for SSO SSP when the read out of positive/negative reactions or the allele call are performed manually. A single interpretation may be performed by a single individual in justified special emergency situations.
- L2.3300 The method of allele assignment must be designated and the allele database must be documented.
- L2.3400 If the typing result is ambiguous, the report must indicate all possible combinations.

L3.0000 Typing methods.

L3.1000 Typing using sequence-specific primers (SSP).

- L3.1100 Each amplification reaction must include controls to detect technical failures (e.g. internal control such as additional primers or templates that produce a product that can be distinguished from the typing product).
- L3.1200 When a typing exhibits lanes with no specific amplicon nor internal control amplification, the laboratory must have a policy in place on how to accept or reject the whole typing.
- L3.1300 The laboratory must use data derived from the validation process and from previous typings with the same lot of primers in the interpretation phase of the typing to identify specific and weak amplifications as well as a tendency to primer-dimer formation. These must be defined and documented.

L3.2000 Sequence-based typing (SBT).

L3.2100 Sequencing templates.

- L3.2110 Sequencing templates must have sufficient purity, specificity, quantity and quality to provide interpretable sequencing data.
- L3.2120 If cloning is used as template preparation, the sequence of at least 3 different clones for each allele must be determined for accurate results.
- L3.2130 Methods for template preparation must ensure that the accuracy of the final typing is not altered (e.g. mutations during cloning, preferential amplification).
- L3.2140 Templates must not contain any inhibitors or contaminants affecting the sequencing reaction. Purification of templates after amplification should be performed to eliminate the presence of dNTP, Taq polymerase and amplification primers.

L3.2200 Sequencing reaction.

- L3.2210 The specificity of the template in combination with the sequencing primer (locus and alleles) must be defined.
- L3.2220 Quantity and quality of templates, sequencing primers and sequencing reagents must be sufficient to provide interpretable primary sequencing data.
- L3.2230 The conditions for the sequencing reaction must be documented and appropriate for obtaining reliable primary sequencing data.

L3.2400 Nucleotide assignment.

- L3.2410 Criteria for acceptance of primary data must be established (peak intensity, baseline fluctuations, peak shapes, correct assignment for nonpolymorphic positions).
- L3.2420 The signal to noise ratio must be sufficient to ensure reliable nucleotide assignment.
- L3.2430 A scientifically and technically sound method must be established for interpretation, acceptance and/or rejection of sequences from regions which are difficult to type.

(e.g. compression). Established sequence-specific characteristics should be documented and utilized in routine interpretation of data.

L3.2500 Allele assignment.

L3.2510 The method used for allele assignment must be designated.

L3.2520 Methods must ensure that sequences contributed by amplification primers are considered in the assignment of alleles.

L3.2530 Criteria for allele assignment must be established. Established sequence-specific artifacts must be documented and utilized in the routine interpretation of data.

L3.2540 If allele assignments are difficult to obtain by sequencing only one strand, resequencing of both strands is recommended. If a sequence suggests a novel allele or rare combination of alleles, the sequences of both strands must be determined.

L3.2550 Databases of HLA sequences used for allele assignment must be accurately updated at least each year.

L3.2560 The laboratory must document the sequence database utilized to interpret the data. Records of the databases used must be maintained.

L3.2570 If a determined sequence is ambiguous, the report must indicate all possible combinations.

L3.3000 Sequence-specific oligonucleotide probes hybridization assays (SSOP).

L3.3100 Oligonucleotide probes.

L3.3110 The specificity of each probe and target sequence must be defined.

L3.3120 Probes must be stored under conditions that maintain specificity and sensitivity.

L3.3130 Laboratories must have a policy in place for quality control of each lot and shipment of probes. The specificity of hybridization must be confirmed with reference material. For home made kits, each new lot should be tested with reference DNA so that the probe is tested for specificity and signal intensity at least once. For commercial kits, each new shipment must be tested in parallel against at least one DNA sample of a known type. The specificity and signal intensity for each probe must be defined and monitored.

L3.3140 Probes must be utilized under empirically determined conditions that achieve defined specificity. For commercial kits, any deviation from the manufacturer's specifications must be validated and documented.

L3.3200 Hybridization.

L3.3210 The amplification should be monitored by gel electrophoresis before hybridization is performed.

L3.3220 Each assay must include a probe internal to a conserved region of the amplicon fragment.

L3.3230 Each assay must include appropriate controls to validate the hybridization and revelation (detection) step of the assay.

L3.3240 Each amplification assay must include a negative (no DNA) control. In forward SSOP this negative control must be included in the hybridization and revelation step of the assay. For reverse SSOP the negative control must be included in the hybridization and revelation step of the assay whenever false positive hybridization of the amplicons is observed.

L3.3250 It is recommended that a DNA of known type is run with each hybridization as:

- L3.3260 Standards in L1.3200 must be followed for the incubators and water baths and heated reagents.
- L3.3270 For automated hybridization devices, the calibration of the pumps and of the elements must be performed according to the manufacturer's specifications, at least once a year. For tests using an ELISA washer, calibration must be performed annually according to the manufacturer's specifications, and monthly function checks of dispensing/aspirating must be performed.
- L3.3271 Where a scanner is used for acquisition of the raw data, a second visual review should be performed to confirm data.
- L3.3272 When the acquisition of the primary data is automated, all critical elements influencing the function of the instrument must be monitored at each use. The instrument must be calibrated according to manufacturer's instructions or at least once a year. The laboratory must define and document function checks. For cytometer-like devices, there must be evidence that regular cleaning and calibration functions have been performed prior to use and that these are satisfactory.
- L3.3280 There must be in place systems to ensure reliable identification of the samples throughout all stages of the testing.
- L3.3281 Acceptable limits of signal intensity must be specified for positive and negative results. If a test is accepted with probe signals out of the set limits, this must be documented and justified.
- L3.3282 The laboratory must use the data derived from the validation process and previous typings with the same lot of primers and probes in the interpretation of the typing. Non specific and weak hybridization signals must be defined and documented.
- L3.4000 Other methods.**
- L3.4100 If alternative methods (e.g. SSCP, heteroduplex, DGGE) are used for HLA typing, established procedures must be validated and must include sufficient controls to ensure accurate assignment of types for every sample. All relevant standards from the above section must be applied.
- L3.4200 Automated systems and computer programmes must be validated prior to use and tested routinely for accuracy and reproducibility of manipulations.

M - FLOW CYTOMETRY

- M1.0000** The Flow Cytometry standards apply to MHC testing including B27 typing. Sections M1, M2, and M3 apply only when using a classical cytometer not dedicated to the use of a fluorescent bead array. Some of the M1 standards, as outlined in section M1.1000, apply when using a dedicated cytometer-like instrument in conjunction with arrays of fluorescent microbeads.
- M1.1000 *Instrument Standardisation/Calibration.*
- M1.1100 An optical standard consisting of latex beads or other uniform particles must be used to ensure proper focusing and alignment of all lenses in the path for both the excitation light source and signal (light scatter, fluorescence, etc.) detectors.

- M1.1110 The optical standard must be run at the start of each daily or shift operation ; time maintenance or adjustment of the instrument during operation is likely to altered optical alignment.
- M1.1200 The results of optical focusing/alignment must be recorded in quality control log
- M1.1300 A threshold value for acceptable optical standardisation must be established ; relevant signals for each instrument and the focusing procedure repeated until values are achieved or surpassed.
- M1.1310 In the event a particular threshold value cannot be attained, a written protocol instituting corrective action must be available. This protocol should include appropriate corrective actions including clear guidelines describing when a service call is warranted.
- M1.1400 The flow cytometer should be cleaned regularly in accordance with manufacturer's instructions.
- M1.1500 The flow cytometer must be serviced regularly exactly as recommended by manufacturer. The dates of the service visits, the faults detected and the comments the service engineer must be recorded.
- M1.2000 A fluorescent standard for each fluorochrome to be used must be run to ensure adequate amplification of the fluorescent signal(s) on a day-to-day basis.
- M1.2100 This standard may be incorporated in the beads or other particles used for cell standardisation or may be a separate bead or fixed cell preparation.
- M1.2200 The fluorescent standard must be run at the start of each daily or shift operation ; any time after maintenance or adjustment of the instrument during operation ; has altered the gain or high voltage settings.
- M1.2300 The results of fluorescent standardisation must be recorded in a daily quality control log.
- M1.2400 In the event that acceptable fluorescence separation cannot be attained, a written protocol for instituting corrective action must be available. This protocol should include appropriate corrective action including clear guidelines describing when a service call is warranted.
- M1.3000 If performing analyses using two or more fluorochromes simultaneously, appropriate compensation procedure must be used to eliminate "spill over" in other fluorescence detectors.
- M1.3100 Compensation must be carried out using peripheral blood lymphocytes stained with suitable fluorochromes or with other dual stained particles, which have been shown to be suitable by the laboratory.
- M1.4000 For laser based instruments, the current input (amps) and laser light power (milliwatts), at the normal operating wavelength measured after the laser is powered and normal operating power set, should be recorded as part of a daily quality control record.
- M2.0000** *Flow cytometric crossmatch technique/Antibody screening.*
- M2.1000 A multi-colour technique is recommended. However, if a single colour technique is used, the purity of the isolated cell population must be documented and shown to be sufficient to define the population for analysis.
- M2.1100 The binding of human immunoglobulin must be assessed with a fluorescently labelled F(ab') anti-human IgG specific for the Fc region of the heavy chain.
- M2.1200 In order to assess binding of human immunoglobulin to cell population(s), the population(s) should be identified by two or three colour staining with different

- labelled monoclonal antibodies to the appropriate CD marker(s) (e.g. phycoerythrin conjugated CD3 monoclonal antibody to identify T cells).
- M2.1300 Two or three colour staining of other immunoglobulin classes may also be justified.
- M2.1400 *Antibody screening using cell targets.*
- M2.1410 If cells pooled from multiple individuals are used for a present/not present detection of antibody, the cells used must cover the major antigen specificities. The laboratory must document the criteria for defining major specificities and the number of individuals included.
- M2.1420 For assignment of antibody specificity, the composition of the cell panel must conform to the standards in section F1.300 *Panel Cells*.
- M2.1500 *Antibody screening using microparticle targets.*
- M2.1510 For the detection of antibodies or assignment of antibody specificity, the composition of the cell panel must conform to the standards in section F1.300 *Panel Cells*.
- M2.2000 *Controls.*
- M2.2100 Control sera must be tested at the same time and under the same conditions as the sera under test. Tests must be done in duplicate as a minimum requirement. Assays must include positive and negative controls.
- M2.2200 The normal human serum (negative) control should be from non-immunised otherwise healthy individuals and may be a pool of several donors. It must be screened by flow cytometry to ensure lack of reactivity against human leukocyte antigens.
- M2.2300 The positive control should be a human serum specific for HLA antigens and appropriate isotype. Additionally, other positive controls for other alloantigens deemed to be important for detection in the crossmatch may be used.
- M2.2400 The secondary antibody reagent should be titrated to determine the dilution of optimal activity (signal to noise ratio). If a multicolour technique is employed, the reagent must not demonstrate crossreactivity with the other immunoglobulin reagents used to mark the cells.
- M2.2500 There must be methods to control for non-specific binding of the secondary antibody to target material.
- M2.3000 *Reagents.*
- M2.3100 The specificity of monoclonal antibodies must be verified by published manufacturer's documentation and/or local documented quality control testing.
- M2.3200 Reagents must be stored according to manufacturer's instructions or according to conditions verified to maintain stability by documented local tests.
- M2.3300 Monoclonal antibodies which have been reconstituted from lyophilised powder for storage at 4°C should be centrifuged according to the manufacturer's instructions or locally documented procedures to remove micro aggregates prior to use in the preparation of working stains.
- M2.4000 *Interpretation.*
- M2.4100 Each laboratory must establish its own crossmatch protocol, standardising and optimising all reagents used, incubation times and temperatures.
- M2.4110 Each laboratory must document its own threshold for significant levels of antibody binding. Any change in technique, protocol or instrumentation requires that the characterisation of the positive threshold be repeated.
- M2.4200 Whether reporting mean, mode or median channel shifts, relative mean fluorescence or number of molecules of fluorescent marker, each laboratory must establish its

threshold for positive crossmatches. Any significant change in protocol instrumentation requires that characterisation of the positive threshold be repeated.

- M3.0000** *HLA typing by flow cytometry (e.g. HLA B27).*
- M3.1000 Terminology used must be defined and/or conform to nomenclature recommended/approved by the most recent WHO nomenclature committee meeting.
- M3.2000 *Cell preparation.*
- M3.2100 The method used for cell preparation should be documented to yield appropriate preparations of viable cells.
- M3.2200 The viability of cell preparations should be recorded and should exceed the laboratory's established minimum standards for each procedure used.
- M3.2300 *Labelling of specimens.*
- M3.2310 A negative reagent control(s) must be run for each test cell preparation. This control should consist of monoclonal antibody(ies) of the same species and subclass as the test antibody(ies) and should be prepared/purified in the same way as the monoclonal(s) used for phenotyping.
- M3.2311 For indirect labelling, an irrelevant primary antibody, if available, and in all cases a secondary antibody(ies) conjugated with the same fluorochrome(s) must be used in all relevant test combinations.
- M3.2312 For direct labelling, an irrelevant antibody conjugated with the same fluorochrome and at the same fluorochrome: protein ratio must be used in all relevant test combinations.
- M3.2320 Whether analysed directly or fixed prior to analysis, labelled cells must be analysed within a time period demonstrated by the laboratory to avoid significant changes in test results. Control samples must be analysed within the same period after staining as the test samples.
- M3.3000 *Reagents.*
- M3.3100 The specificity of monoclonal antibodies must be verified through tests using appropriate control cells prepared and tested by the same method employed for the laboratory's test sample analysis.
- M3.3200 Cell controls must be tested for each batch of monoclonal antibodies received.
- M3.3210 The control cells must include at least five cells known to express the specific antigen.
- M3.3220 The control cells must also include two cells for each cross-reacting antigen, which might be confused with the specific antigen.
- M3.3230 The control cells must also include at least two cells lacking the specific and reacting antigens.
- M3.3300 The quantities of reagents used for each test sample must be determined by the manufacturers or from published data and whenever possible should be verified locally by appropriate titration procedures.
- M3.3400 Reagents must be stored according to manufacturer's instructions or according to conditions verified to maintain stability by documented local tests.
- M3.3500 Monoclonal antibodies, which have been reconstituted from lyophilised powder for storage at 4°C should be centrifuged according to the manufacturer's instructions or locally documented procedures to remove microaggregates prior to the preparation of working stains.
- M3.3600 A single monoclonal antibody may be used to define an antigen provided its specificity has been sufficiently verified by local testing.

- M3.3700 Minimum reactivity for assignment of a positive reaction must be established laboratory.
- M3.3710 Each batch of tests must include a cell sample known to express the antigen and as a positive control.
- M3.3800 If the monoclonal antibody(ies) is (are) known or found to react with antigens than the one specified, a written protocol must explain how its presence or absence is finally determined.
- M4.000** *Bead array techniques.*
- M4.100 This section applies to antibody screening and HLA typing when using a dedicated cytometer-like instrument in conjunction with an array of fluorescent microbeads.
- M4.200 The optical and fluorescence calibrating standard must be run as specified by the manufacturer or at least weekly. The fluorescence standard must be run additionally any time the temperature delta check is not correct.
- M4.300 The lot number of each kit and the reference parameters for controls must be recorded for each assay. The reference values must fall within acceptable limits for the assay to be valid.
- M4.400 For antibody screening, identification and HLA typing the following standards in section M also apply : M1.1100, M1.1200, M1.1310, M1.1400, M1.1500.
- M4.500 For antibody screening and identification the following standards in section N also apply : N1.200, N2.100, N2.200, N2.210, N2.220, N2.400, N2.500, N2.700.
- M4.600 For HLA typing using bead array techniques the standards in L3.3200 also apply.

N - ENZYME-LINKED IMMUNO SORBENT ASSAY (ELISA)

- N1.000** *Instrument Standardisation/Calibration of ELISA reader and washer.*
- N1.100 The light source must produce the intensity and wavelength of light required for the test system.
- N1.110 Periodic calibration must be performed according to the instrument manufacturer's instructions and must be documented.
- N1.200 Assays must be performed with calibrated dispensing instruments (pipettes). Calibration must be performed routinely and must be documented.
- N1.300 Microplate washer performance must be checked monthly and acceptance performance documented.
- N2.000** *ELISA technique.*
- N2.100 If commercial kits are used, the manufacturer's instructions must be followed and the laboratory has performed and documented testing to support a deviation technique or analysis.
- N2.200 Each assay must contain a positive control, a negative control and reagent controls. The dilution of reagents and test specimens must be documented.
- N2.210 Negative control sera must include a serum from non-alloimmunised human donor.
- N2.220 The positive control should be a human serum specific for HLA antigens and appropriate isotype.
- N2.230 A control reaction lacking only HLA antigen must be included in the test system.
- N2.300 Sera must be tested at a concentration determined to be optimal for detection of antibody to HLA antigens.

- N2.400 For the detection of antibodies or assignment of antibody specificity, the composition of the cell panel must conform to the standards in section F1.300 *Panel Cells*.
 - N2.500 Sample identity and proper plate orientation must be maintained throughout the procedure.
 - N2.600 The lot numbers and optical density values of the reference reagents and the controls must be recorded for each assay. These values must fall within acceptable limits for the assay to be valid.
 - N2.700 New lots of reagents must be validated by side-by-side testing with a lot known to give acceptable performance or by testing with test specimens of known reactivity.
-