European Federation for Immunogenetics

STANDARDS FOR HISTOCOMPATIBILITY & IMMUNOGENETICS TESTING

Version 5.7

Accepted by the Standards and Quality Assurance Committee on 31st May 2012
Accepted by the EFI Executive Committee on 1st June 2012
Effective from 1st October 2012

A - GENERAL POLICIES

A1.000 These Standards have been approved and adopted by the EFI Executive Committee. They are based on Standards originally prepared by the American Society for Histocompatibility and Immunogenetics (ASHI).

A2.000 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.

A3.000 These Standards establish minimal criteria, which all histocompatibility laboratories must meet if their services are to be considered acceptable. Many laboratories, because of extensive experience, will exceed the minimal requirements of these Standards.

A4.000 Certain Standards are obligatory. In these instances, the Standards use the word "must". Some Standards are highly recommended but not absolutely mandatory. In these instances the Standards use words like "should" or "recommended".

A5.000 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.

A6.000 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.

A7.000 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.

B - PERSONNEL QUALIFICATIONS

B1.000 The laboratory must employ one or more individuals who meet the qualifications and fulfil the responsibilities of the Director/Co-Director, Technical Supervisor and Quality Manager.

B2.000 A **Director/Co-Director** must hold an earned doctoral degree in a biological science, or be a physician, or have an equivalent qualification. In addition, the Director/Co-Director 1) must have had four years experience in immunology or cell biology, two of which were devoted to full time training in human histocompatibility testing, or 2) five years of working experience at full time in human histocompatibility testing. Additional qualifications required according to national legislation also apply. The Director/Co-Director must have documentation of professional competence in the appropriate activities in which the laboratory is engaged. This should be based on a sound knowledge of the fundamentals of immunology, genetics and histocompatibility testing and reflected by external measures such as participation in national or international workshops or publications in peer-reviewed journals. The Director or Co-Director is available on site at least 20h/week, provides adequate supervision of technical personnel, utilises his/her special scientific skills in

developing new 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, and be compliant with the relevant national legislation.

B3.000 A **Technical Supervisor** must hold a bachelor's degree or equivalent and have had 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 A Quality Manager must establish and maintain a comprehensive quality management programme covering all aspects for the accredited facility addressed by these Standards.

B5.000 The resources of the laboratory must be sufficient to accommodate the workload.

B6.000 Testing referred to other laboratories.

B6.100 An accredited laboratory may engage another laboratory to perform testing not done 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 genetic 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.

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 result. The following facilities must be adequate and immediately available to 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 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^{o}C) and CO_2 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 The laboratory must establish and employ policies and procedures for the proper maintenance of equipment, instruments and test systems by 1) defining its preventive maintenance programme for each instrument and piece of equipment at least once a year, and by 2) performing and documenting function checks on equipment with at least the frequency specified by the manufacturer.

C1.700 Assays must be performed with calibrated dispensing instruments (e.g. pipettes, etc.). Calibration must be performed at least once a year and must be documented.

C1.800 The laboratory must document compliance with all applicable national and local laws which relate to laboratory employee health and safety; fire safety; and the storage, 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 Supervisor and/or Laboratory Director before issue.

C1.920 The computer software programme used for analyses must be identified and 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) the patient’s or donor’s name or other method of specimen identification to assure accurate reporting of results; 2) the name and address of the authorised person or of 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, spleen cells) if pertinent.

C2.120 Blood or tissue samples must be individually labelled with the name, and/or other unique identification marker of the individual and the date of collection. When multiple blood containers are collected, each container must be individually labelled.

C2.130 The laboratory must maintain a system to ensure reliable specimen identification, and must document each step in the processing and testing of patient specimens to assure that accurate test results are recorded.

C2.140 The laboratory must have criteria for specimen rejection and a mechanism to assure 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 location, which does not compromise aseptic techniques. The donor's skin must be prepared by a technique, which ensures minimal possibility of infection of the donor or contamination of the sample. All needles and syringes must be disposable.

C2.210 All biological samples must 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 manufacturers' 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 other pertinent information. 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.

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 product 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 or a delegated individual with appropriate qualifications, and documented evidence of this review must be available. Any changes in procedures must be initialled and dated by the Director/Co-Director/delegated individualk at the time they are initiated.

C4.000 Quality Assurance.

C4.100 **External Proficiency Testing(EPT) and Competency Evaluation.**

C4.110 The laboratory must participate in EPT programme(s) to cover all the accredited laboratory applications (HLA typing, antibody screening and identification, crossmatching, etc.). EPT results must be obtained for all techniques individually or 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 any corrective action taken.

C4.140 EPT samples must be tested and interpreted in a manner comparable to that for routine testing of clinical samples.

C4.145 Participating laboratories must ensure that all EPT related documents including submitted worksheets, EPT summary/scheme reports, annual performance and participation certificates, outcomes of investigations of any unsatisfactory results, corrective or preventive actions are maintained and are made available to EFI inspectors for assessment.

C4.150 The Director/Co-director must 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. The laboratory must maintain records of these evaluations for each individual.

C4.160 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 2) 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 test 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 all 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) regular evaluation of results obtained in external and internal QC testing, c) 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). 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 All complaints and problems reported to the laboratory must be documented. Complaints must be investigated and corrective action taken when necessary.

C4.320 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 action taken. 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 two 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 according to any regulation to which the laboratory is obliged to abide, but for a minimum of four years.

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 that were used, the test performed, the date of the test and the person performing the test.

C5.130 Reports or records, as appropriate, must include a brief description of the specimen (blood, lymph node, spleen, bone marrow, etc.) used for testing.

C5.140 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. The record must be kept according to any regulation to which the laboratory is obliged to abide, but for a minimum of two years.

C5.150 Records may be only saved in computer files, provided that back-up files are maintained to ensure against loss of data.

C5.200 The laboratory must have adequate systems in place to report results in a timely, accurate and reliable manner.

C5.300 The report must contain:

a. The name of the individual tested or unique identifier of each individual tested and 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/Co-Director, or a designated individual with appropriate qualifications.

C5.400 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 HLA typing discrepancies that may occur between laboratories.

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.

D1.110 Use of NMDP codes is only allowed 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.

D1.200 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 from context).

D1.220 HLA type. Serological assignment: HLA-A2,30; B7,44; Cw5; DR1,4; DQ5,7. DNA assignment: *HLA-A*02,*30; B*07,*44; C*05,*16; DRB1*01,*04; DQB1*05,*03:01.*

D1.230 Genotype. Serological assignment: HLA-A2, B44, Cw5, DR1, DQ5 / A30, B7, Cw-, DR4, DQ7. DNA assignment: *HLA-A*02, B*44, C*05, DRB1*01, DQB1*05 / A*30, B*07, C*16, DRB1*04, DQB1*03:01.*

D1.240 The locus designation must always be included.

D1.300 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 typing unequivocally demonstrates the presence of heterozygosity for two different alleles from the same specificity. If homozygosity has not been proven, the HLA type may be reported using a hyphen to indicate

phenotypic blank eg HLA-A1,3; B7,44; Cw7,-. Similarly for DNA typing, if homozygosity has not been proven, a hyphen may be used. eg. HLA-A*01,*03; B*07,*44; C*07,-

D1.310 If typing unequivocally demonstrates the presence of heterozygosity for two different alleles from the same specificity (e.g. *DRB1*13:01/59*, *DRB1*13:03/33*), 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 the identification of HLA alleles that encode the same protein sequence within the antigen binding site. Alleles must be defined by the first and second fields according to WHO Nomenclature, by at least resolving a) 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 must be done by typing immediate family members including parents, siblings and/or children of the patient.

D2.110 Genotypic identity can only be proven if both parents are available or if the segregation of the four haplotypes is clearly defined.

D2.120 Ambiguities in haplotype assignment must be resolved by typing for HLA-C, and/or DQ and/or DP. When appropriate, high resolution typing must be used to resolve ambiguities.

D2.130 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

E. 0.100 For HLA typing by complement-dependent 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 (0,1,2,4,6,8) should be used. Other numerical codes can also be used.

E1.000 HLA-A, -B, -DR locus antigens.

E1.100 The laboratory must be able to type for the HLA-A, -B and/or -DR specificities, which are officially recognised by the WHO and for those deemed relevant by EFI.

E2.000 HLA Class I and II typing techniques.

E2.100 Techniques used must be those, which have been established to define HLA Class I and II specificities optimally.

E2.200 **Control reagents.**

E2.210 Each typing tray must contain at least one positive control antibody, previously shown to react with cells expressing class I and class II antigens.

E2.220 If the positive control fails to react as expected, there must be a procedure in place as whether to accept or reject the test.

E2.230 Each typing tray must include at least one negative control serum. The negative control should be one previously shown to lack leukocyte reactive antibodies.

E2.240 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.

E2.250 Procedures that deal with control serum failures in typing or crossmatch trays must be described in the laboratory manual.

E2.300 **Target cells.**

E2.310 Separation of B lymphocytes is not required if a technique is used which distinguishes between T and B lymphocytes or in assays in which antibodies with well-defined specificity are used which only define HLA class II molecules.

E2.400 **Antigen assignments.**

E2.410 Each HLA-A, B antigen must be defined by at least two sera when available, if both 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.

E2.420 Each monoclonal antibody used for alloantigen assignment must be used at a dilution and with a technique in which it demonstrates specificity comparable to antigen assignment by alloantisera on a well-defined cell panel.

E2.430 Each HLA Class II antigen should be defined by at least three sera, if all 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.

E2.440 Criteria for antigen assignment must be described in the laboratory manual.

E2.450 Ambiguity in antigen definition by serological typing must be referred for confirmation by DNA based methods.

E2.500 **Control of antibody specificity.**

E2.510 Cell panels of known HLA type must be used to prove the specificity of new antibodies. The panel cells should include at least one example of each HLA antigen the laboratory should be able to define.

E2.600 **Typing sera.**

E2.610 A reagent grade typing serum is validated only after confirmation of specificity. Specificity determinations must include supporting statistical analysis.

E2.620 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.

E2.630 Each lot of typing trays must be evaluated by testing either with at least five different cells of known phenotype representing major specificities or in parallel with previously evaluated trays. Each new shipment of previously evaluated typing trays must be verified with at least one cell of known phenotype.

E2.700 **Complement.**

E2.710 Each lot of complement must be tested to determine that it mediates cytotoxicity in the presence of specific HLA antibody but is not cytotoxic in the absence of HLA specific antibody. The complement must be kept at the recommended temperature.

E2.720 The test must employ multiple dilutions of complement to ensure that it is maximally active at least one dilution beyond that intended for use.

E2.730 Each lot and shipment of complement must be evaluated by either i) testing with at least 3 previously evaluated trays for every application for which it is intended for or ii) testing a combination of at least 3 sera and 2 cells selected to include negative, weak positive and strong positive reactions.

E2.740 Complement must be tested separately for use with each type of target cell.

F - ANTIBODY SCREENING AND CROSSMATCHING

F1.000 Techniques.

F1.100 A complement-dependent cytotoxic technique must be used for the detection of antibody to HLA antigens unless the laboratory has performed and documented testing to validate that another technique identifies alloantibody to HLA antigens at a 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 used that distinguishes them from antibodies to HLA class I antigens.

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

F1.200 **Sera.**

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

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

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

F1.300 **Panels.**

F1.310 The panel of HLA antigens must include sufficient specificities to ensure that they are appropriate for the population served and the use of the test results.

F1.320 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.

F2.000 Antibody screening by complement-dependent cytotoxicity.

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

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

F2.300 Laboratories using a CDC technique must also conform to standard E2.700 Complement.

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

F3.100 Laboratories performing assays using flow cytometry must also conform to the 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 relevant parts of section M4.000.

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 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 should be used in addition to this.

F6.110 The screening technique used must be at least as sensitive as the routine crossmatch technique.

F6.120 For crossmatching each serum must be tested undiluted and in duplicate.

F6.130 Crossmatches must be performed with unseparated lymphocytes or with T lymphocytes from the potential donor. B-cell crossmatches must be performed if 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 dithiothreitol, IgG and IgM positive and negative controls must be included.

F6.160 Laboratories using a CDC technique must also conform to standard E2.700 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 3 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 tests.

G2.200 Testing must be performed to distinguish HLA specific antibodies from non HLA antibodies and autoantibodies. The specificity of detected HLA antibodies must be defined and recorded

G3.000 Crossmatching.

G3.100 Crossmatching must be performed prospectively unless G3.110 to G3.140 are fulfilled.

G3.110 A prospective crossmatch may be omitted in recipients that have shown to be consistently negative for the presence of HLA-specific antibodies, as relevant for the

transplant protocol. Sera must have been collected as defined in G2.110, and must have been tested on at least two different samples. Screening data must include at least one result obtained within the previous 3 months, using a technique of equivalent sensitivity to that used for the crossmatching.

G3.120 If a prospective crossmatch is not systematically performed, there must be evidence that the laboratory maintains a record of potentially sensitising events for each patient.

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 this 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, they 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 a final crossmatch.

G3.220 Final crossmatches performed prior to transplantation must utilise a recipient serum sample collected within the previous 48 hours before transplant if the recipient 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 sera that are used in the final crossmatch procedure.

G3.240 Serum samples stored for crossmatching must be retained in the frozen state.

G4.000 HLA typing.

G4.100 Prospective typing of donor and recipient for HLA-A, B and DR antigens is mandatory. If required by national regulations, additional loci must be typed.

G4.200 Every effort must be made to perform verification typing for recipients prior to transplantation.

G4.300 Verification typing must be performed on potential living donors prior to transplantation.

H - OTHER ORGAN TRANSPLANTATION

H1.000 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.

H2.000 Cardiothoracic patients must be screened for the presence of HLA alloantibodies and unacceptable specificities must be defined unless a prospective crossmatch is to be performed. For these patients, G2.100 and G2.110 apply.

H3.000 Crossmatching.

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 crossmatched. Crossmatch results should be available prior to transplantation of a presensitised patient.

H3.300 Final crossmatches performed prior to transplantation should utilise a recipient serum sample collected within the previous 48 hours before transplant if the recipient 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 final crossmatch.

H3.500 Whenever possible, non-renal organs and tissues for recipients at high risk for allograft rejection should come from crossmatch 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 testing to definitively establish HLA identity by descent (D2.120 applies), or use 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.

I1.120 HLA typing for recipient and potential intra-familial donors who are not HLA identical siblings must include high resolution Class I and Class II typing by DNA methods as documented in 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 EFI or ASHI accredited laboratory to perform these tests at the required level of resolution.

I1.200 Donor typing (related cord blood unit)

I1.210 The Cord Blood Unit must be typed using DNA methods for HLA-A, B and DRB1, at a minimum of low resolution (e.g. *A*02, B*44, DRB1*11*).

I1.220 Extended typing must be included if required by the transplant protocol. (I1.100 and I1.110 also apply)

I1.230 Prior to transplantation, a verification typing must be performed for HLA-A, B and DRB1 at a minimum of low resolution. Typing must be performed on a segment of the tubing integrally attached to the unit, if available, or otherwise, on a satellite vial.

I1.240 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.

I2.000 Histocompatibility testing for unrelated transplants.

I2.100 **Volunteer bone marrow donor registries**.

I2.110 Typing of the donors must be performed by serology or DNA methods at a minimum of low resolution (e.g. A2 or *A*02*, DR11 or *DRB1*11*).

I2.200 Typing of Units for Cord Blood Banks

I2.210 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*).

I2.220 Typing of additional loci or high resolution typing must be included if required by the policy of the registry, or if requested. Laboratories not able to perform high resolution typing by DNA methods must arrange for an EFI or ASHI accredited laboratory to perform these as required.

I2.230 The identity of the Cord Blood Unit must be confirmed by HLA typing on a separate sample to demonstrate concordance of results. The verification of identity and the nature of the sample tested must be reported back to the registry. Additional typing may be performed using any stored DNA sample, provided that the identity of the unit has previously been verified.

I2.300 **Histocompatibility testing for transplants from unrelated donors.**

I2.310 HLA typing for recipient and unrelated donors must include as a minimum requirement: low resolution HLA-A/B/C typing and high resolution DRB1 typing by DNA methods. Class I typing must, as a minimum, be at a resolution which allows assignment of all serologically defined antigens. Additional loci must be included if required by the transplant protocol.

I2.320 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 documented. If all ambiguities are not included on the report, a comment must be added stating that other ambiguous HLA (define loci) results have not been excluded and that this information is available upon request.

I2.330 Laboratories 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 as required.

I2.340 Prior to transplantation using an unrelated donor, HLA typing of the recipient must be repeated using a different typing sample such that typing is verified for HLA-A, -B, and -DR, as a minimal requirement.

I2.350 For unrelated donors, requirements of I2.310, I2.320, and I2.330 must be met by typing on a sample obtained by the laboratory affiliated with the transplant centre. Registry data cannot be used for this purpose.

I2.360 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.

I2.370 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 I2.330.

I2.400 Unrelated Cord Blood Unit Typing at Transplant Centre for Donor Selection

I2.410 Verification typing must be performed by the transplant centre or if not possible, by the laboratory designated by the Cord Blood Bank.

I2.420 The cord unit must be typed for HLA-A and -B at a minimum of low resolution, and for -DRB1 at high resolution.

I2.430 Extended typing must be performed if required by the transplant protocol.

I2.500 Unrelated Cord Blood Unit Typing Prior to Transplantation

I2.510 Prior to the conditioning regimen of the recipient, a verification typing of at least HLA-A, -B, and -DRB1 at a minimum level of low resolution must be performed upon reception of the shipped unit. Typing must be performed on a segment of the tubing integrally attached to the unit, if available; otherwise a satellite vial shipped with the unit may be used. If no segment is available, this step can be performed after transplantation and must be initiated as soon as possible after thawing the unit.

I3.000 Crossmatching.

I3.110 If required by the local transplant protocol, crossmatching must be performed prior to related and unrelated transplantation.

I3.120 Crossmatching must be performed according to standard F6.000.

I4.000 Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

I4.100 Standards in L1.0000, L1.3400, L2.1100, L2.1200, L2.2000, L2.3100 and L2.3300 also apply.

I4.110 The polymorphic gene system(s) used for HCE monitoring must be identified and documented with regards to allelic variability

I4.120 Where locally developed PCR primers/probes are used, their sequence and specificity must be documented.

I4.130 Donor and patient specific alleles must be determined using appropriate reference material and documented.

I4.140 Optimal ranges of DNA quantity and purity must be defined and documented. If a sample falls outside these optimal ranges, a statement must be included on the report.

I4.150 Criteria for assignment of HCE results, on a qualitative or quantitative basis, must be defined.

I4.160 The sensitivity of the HCE assay must be validated.

I4.170 When multiple PCR primers are used in the same tube (multiplex PCR), results must take into account possible amplification bias.

I4.180 Results must be validated using DNA mixtures from two individuals at defined ratios/concentrations, before implementation into clinical use.

I4.190 When HCE testing is performed on cellular subsets isolated by cell sorting, the purity of the sorted population must be documented and taken into account in the analysis of the results. If this is not possible it must be clearly stated in the report.

I4.200 For quantitative HCE monitoring by quantitative PCR (Q-PCR), the chemistry, internal control gene and thresholds for positive or negative results of each reaction must be defined. All steps of locally developed Q-PCR assays must be validated.

I4.210 In addition to the requirements from standard C5.400, the report must contain, a description of the specimen used for testing (bone marrow, peripheral blood, cellular subsets isolated by cell sorting etc.), the date of transplant and any other information if deemed relevant for HCE interpretation (i.e. limited informative markers or clinical condition of the patient).

J - HLA/HPA/HNA and Transfusion

J0.010 Laboratories providing diagnostic testing for all Standards covered in J must follow documented protocols of laboratory investigations for each service (HLA/HPA/HNA testing) provided.

J0.020 Current HPA Nomenclature (PNC Platelet Nomenclature Committee*) must be used for recording and reporting HPA alloantigen and HPA alloantibody specificities.

J0.030 Current HNA Nomenclature (ISBT Working Party**) must be used for recording and reporting HNA alloantigen and HNA alloantibody specificities.

J1.000 HLA and Transfusion

J1.100 Platelet refractoriness

J1.120 Platelet refractory patients who require HLA matched platelets must be typed for HLA-A and HLA-B.

J1.130 For the laboratory investigation of suspected alloimmune platelet refractoriness the patient must be tested for HLA class I antibodies.

J1.140 The specificity of detected HLA antibodies must be defined and recorded or crossmatching must be performed to provide compatible platelets. For crossmatching using lymphocytes standards in section F6.000 apply.

J1.150 All selected plateletpheresis donors used for the provision of HLA matched platelets must be typed for HLA-A and HLA-B.

J1.200 Transfusion Related Acute Lung Injury (TRALI)

J1.210 For the laboratory investigation of TRALI the sera from implicated donor(s) must be tested for both HLA class I and class II antibodies.

J1.220 The specificity of detected HLA antibodies must be defined and recorded.

J1.230 If HLA specific antibodies are identified, the patient and donor must be typed at least for the relevant loci.

J1.300 Transfusion Associated Graft Versus Host Disease (TAGVHD)

J1.310 The patient and donor(s) must be typed at least for HLA-A, B and DRB1.

J1.400 Febrile Non Haemolytic Transfusion Reactions (FNHTR)

J1.410 The patient's serum must be tested for the presence of HLA antibodies.

J2.000 Human Platelet Antigens (HPA) and Transfusion

J2.100 HPA Testing

J2.200 HPA typing must be performed by a validated HPA typing technique. If DNA typing is performed then relevant standards in section L also apply.

J2.210 The clinically significant HPA specificities must be defined and documented locally.

J2.300 Donor HPA Testing

J2.310 If HPA alloimmunisation is identified in a patient, verification typing must be performed on donors whose products may be used.

J2.400 Composition of platelet panel for HPA alloantibody detection

J2.410 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.

J2.500 Investigation of HPA antibodies

J2.510 The antibody screening technique must be validated before use.

J2.520 Positive and negative controls must be included in each assay.

J2.530 In glycoprotein specific assays, a positive control for each glycoprotein used should be included. For ELISA based assays section N also applies. For bead array techniques section M 2.000 also applies.

J2.540 The specificity of detected HPA alloantibodies must be defined and recorded.

J2.600 Neonatal Alloimmune Thrombocytopenia (NAIT)

J2.610 The maternal serum sample must be investigated for the presence of HPA antibodies.

J2.620 HPA typing of the mother, father and neonate should be performed.

J2.700 Post Transfusion Purpura (PTP)

J2.710 The patient must be HPA typed and their serum investigated for HPA antibodies.

J3.000 Human Neutrophil Antigens (HNA) and Transfusion

J3.100 HNA Testing

J3.110 The clinically significant HNA specificities must be defined and documented.

J3.200 HNA Typing

J3.210 HNA typing must be performed by a validated HNA typing technique. If DNA typing is performed then relevant standards in section L also apply.

J3.300 **Composition of neutrophil /granulocyte panel for HNA alloantibody detection**

J3.310 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.

J3.400 Investigation of HNA antibodies

J3.410 The antibody screening technique must be validated before routine use.

J3.420 Positive and negative controls must be included in each assay.

J3.430 In glycoprotein specific assays, laboratories must make all reasonable efforts to include a positive control for each glycoprotein used. For ELISA based assays section N also applies. For bead array techniques section M2.000 also applies. For flow cytometry section M2.000 to M5.000 also apply.

J3.440 The specificity of detected HNA alloantibodies must be defined and recorded

J3.500 Neonatal Alloimmune Neutropenia (NAIN)

J3.510 The maternal serum sample must be investigated for the presence of HNA antibodies.

J3.520 HNA typing of the mother, father and neonate should be performed.

** Vox Sanguinis 2003 85, 240-245* *** Vox Sanguinis 2008 94, 277-285*

K - DISEASE ASSOCIATION

K1.000 If complete HLA typing is performed by serology standards in section E must be followed.

K1.100 Typing may also be limited to all products of a single or limited number of HLA loci.

K2.000 Typing for a single antigen by CDC

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 specified antigen.

K2.120 The control cells must also include two cells for each cross reacting antigen, which might be confused with the specific antigen.

K2.130 The control cells must also include at least two cells lacking the specific and cross reacting 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 cross reacts with the specified antigen (if available).

K2.300 Sera to define each antigen must meet requirements of Section E as appropriate.

K3.000 If HLA typing is performed by DNA techniques standards in section L must be followed.

K3.100 **Typing for a single allele-group by molecular techniques**

K3.110 Where typing for a single allele-group is performed a positive control DNA known to encode the allele-group of interest must be included in each test.

K3.120 Where typing for a single allele-group is performed a negative control DNA known not to encode an allele belonging to the allele-group of interest must be included in each test.

L – NUCLEIC ACID ANALYSIS

L1.0000 General laboratory design, equipment and reagents.

L1.1000 Laboratory design.

L1.1100 Laboratories performing amplification of nucleic acids must use a dedicated work area with restricted traffic flow and physical barriers to prevent DNA contamination.

L1.1200 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. Pre-amplification physical containment must include use of dedicated equipment, laboratory coats, and disposable supplies.

L1.1300 All activities occurring from and including thermal cycling must take place in the 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 amplifications 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-test″).**

L1.2100 Contamination must be monitored for amplification products that are produced in the laboratory.

L1.2200 Routine wipe-tests of pre-amplification work areas and equipment 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 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 by maintenance according to the manufacturer or annual thermal verification of the block using a calibrated device designed specifically for this purpose.

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 if documented to be free of contamination at each use. When reagents are combined to create a master mix, it is recommended that one critical component (e.g. DNA polymerase) be left out of the mixture.

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

L1.3500 For commercial kits, the source, lot number, expiry date, and storage conditions must 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 in 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 lot or shipment of primers. The specificity and quantity of amplified product must be confirmed with reference material. For commercial kits, each lot or shipment must be tested against at least one DNA sample of known type.

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

L1.4400 Each lot of local primers must be tested with reference DNA for appropriate sensitivity and specificity.

L2.0000 Nucleic acid extraction, electrophoresis and analysis.

L2.1000 **Nucleic acid extraction.**

L2.1100 Nucleic acids must be extracted and purified by a published 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 test results. DNA purity and concentration should be determined for each sample; if there is 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 gel 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 SSOP and 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 under justified special emergency situations.

L2.3300 The method of allele assignment must be designated and the allele database must be documented.

L2.3310 Databases of HLA sequences used for allele assignment must be accurate and updated at least once a year with the most current version of the IMGT/HLA database.

L2.3400 If the typing result is ambiguous, all possible combinations must be documented. If ambiguities are not included on the report, a comment that additional data are available in the laboratory must be added.

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. an 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. Non-specific and weak amplifications as well as a tendency to primer-dimer formation 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 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).

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 (HLA 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 non-polymorphic positions).

L3.2420 The signal to noise ratio must be sufficient to ensure reliable nucleotide assignments.

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 resolve (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 not considered in the assignment of alleles.

L3.2530 Criteria for allele assignment must be established. Established sequence-specific artefacts 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, routine sequencing of both strands is recommended. If a sequence suggests a novel allele or a rare combination of alleles, the sequences of both strands must be determined.

L3.2550 The laboratory must document the sequence database utilized to interpret the primary data. Records of the databases used must be maintained according to any regulation by which the laboratory is obliged to abide, but for a minimum of four years.

L3.2560 If a determined sequence is ambiguous, all possible allelic combinations must be documented. If ambiguities are not included on the report, a comment that additional data are available in the laboratory must be added.

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 lot must be tested with reference DNA so that each probe is tested for specificity and signal intensity at least once. For commercial kits each lot and shipment must be tested in parallel against at least one DNA sample of known type. The specificity and signal intensity for each probe must be defined and monitored.

L3.3140 Probes must be utilised under empirically determined conditions that achieve the 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 the hybridization is performed.

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

L3.3230 Each assay must include appropriate controls to validate the hybridization and the detection steps 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 steps of the assay. For reverse SSOP the negative control must either be included in the hybridization and detection step of the assay or monitored by gel electrophoresis.

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

L3.3260 Standards in L1.3200 must be followed for the incubators and water baths and for heated reagents.

L3.3270 For automated hybridization devices, the calibration of the pumps and of the heating 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 at least annually according to the manufacturer's specifications, and monthly functional checks of dispensing/aspirating must be performed.

L3.3271 Where a scanner is used for acquisition of the raw data, a second visual reading must 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 flow 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 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.

L3.3281 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. 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

M1.000 Application

M1.100 Section M2.100 applies to flow cytometry and flow analysis using equipment designed for beads only (fluoroanalyzer). Sections M2.200 to M5.000 apply to flow cytometry.

M2.000 Instrument Standardisation and Maintenance

M2.100 General Instrument Standardisation and Maintenance

M2.110 For instruments that perform an automated integrated multi-parameter standardisation (e.g. Luminex), this function may be used instead of individual optical alignment and fluorescence standardisation described in M2.200, M2.300 and M2.400. The reagents specified by the manufacturer to perform this test must be used.

M2.120 The result of the standardisation must be recorded.

M2.130 The instrument must only be used if the test has passed.

M2.140 The frequency of standardisation must conform to manufacturer's instructions, and must be performed at any time that the temperature delta check is not correct, or as specified in M2.200 to M2.400 if there are no manufacturer's instructions.

M2.150 There must be a procedure for regular cleaning of the instrument which must conform to manufacturer's instructions. Cleaning must be documented.

M2.160 The instrument must be maintained according to manufacturer's instructions but at least once a year.

M2.200 Optical Standardisation

M2.210 The optical standard must be run every day of instrument use unless otherwise specified by the manufacturer.

M2.220 The optical standard must also be run at any time when maintenance or adjustment of the instrument during operation is likely to have altered optical alignment.

M2.230 The optical standard must consist of latex beads or other uniform particles and a threshold value for acceptable optical standardisation must be established for all relevant signals.

M2.240 The results of optical focusing/alignment must be recorded and fall within the defined acceptable range.

M2.300 Fluorescence Standardisation

M2.310 The fluorescence standard must be run every day of instrument use unless otherwise specified by the manufacturer and must be run any time maintenance or adjustment of the instrument during operation is likely to have altered settings.

M2.320 A fluorescence standard must be used for each fluorochrome employed in analytical procedures.

M2.330 The results of fluorescence standardisation must be recorded and fall within the defined acceptable range.

M2.400 Compensation

M2.410 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.

M2.420 Compensation settings must be determined every day of use and at any time maintenance or adjustment of the instrument during operation is likely to have altered them.

M2.430 Compensation must be carried out for all fluorochromes used.

M2.440 Acceptable compensation values must be defined and recorded.

M2.500 Equipment Maintenance

M2.510 For laser-based instruments, the laboratory must record the current input (amps) and laser light output (milliwatts), at the normal operating wavelength. Readings must be measured after the laser has peaked and with normal operating power set. Other pertinent regular functional checks must be defined and documented.

M3.000 General Reagents

M3.100 The specificity of labelling reagents for the identification of cell subset must be verified using a published method and/or the manufacturer's documentation and/or local documented quality control testing.

M3.200 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.

M3.300 Secondary labelling reagents must be titred to determine the dilution with optimal signal to noise ratio.

M3.400 If a multicolour technique is employed, the reagent must not cross-react with the other immunoglobulin reagents used to label the cells.

M3.500 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.

M3.600 Each lot and shipment of labelling reagents must be tested for proper performance and thresholds for adequate intensity must be defined and documented.

M3.700 The quantities of reagents used for each test sample must be determined by the manufacturers or from published data and verified locally by appropriate titration procedures.

M4.000 Antibody screening and cross-matching

M4.100 Cell based testing

M4.110 For cell based antibody screening and crossmatching an individual fluorochrome per subset should be used for the identification of each population subset (multicolour technique).

M4.120 If a single colour technique is used, the purity of the isolated cell population must be sufficient to define the population for analysis and must be documented.

M4.130 The target sub-populations must be defined and identified by appropriate labelling antibodies (e.g. T lymphocytes: CD3, B lymphocytes: CD19) and must include a sufficient number of events per sub-population, relevant to the test performed.

M4.140 The binding of human immunoglobulin must be assessed with a fluorochrome labelled F(ab') anti-human IgG specific for the Fc region of the heavy chain.

M4.200 Cell and bead based antibody screening.

M4.210 For the detection of anti-HLA antibodies or assignment of antibody specificity using cell and bead based antibody screening the composition of the panel must conform to the standards in section F1.300.

M4.300 Controls

M4.310 A negative control must be used. This must be a serum from a non-alloimmunised human donor(s) which has been screened and found negative by flow cytometry.

M4.320 A positive control must be used. This must be human serum with antibodies of the appropriate isotype, specific for HLA antigens.

M4.330 Control sera must be tested at the same time and under the same conditions as the sera under test.

M4.400 Policies and procedures:

M4.410 There must be policies and procedures to address at least antibody screening and cross-matching technical instructions including reagent standardisation and optimisation, reagent validation, incubation times and temperatures.

M4.420 Interpretation instructions must include details of the threshold for significant levels of antibody binding (positivity) and the mechanism for reporting positive results (mean, mode or median channel shifts, relative mean fluorescence, or number of molecules of fluorescent marker).

M4.430 Acceptable reactivity required for negative, positive and secondary control reagents, for the test to be valid must be defined and documented.

M5.000 Cell-based HLA typing by flow cytometry (e.g. HLA B27*)*

M5.110 The specificity of each lot of labelling reagents for the identification of HLA specificities must be determined by testing (1) at least five cells known to express the target antigen, (2) at least two cells for each cross-reacting antigen, and (3) at least two cells which lack the specific and cross-reacting antigens. Acceptable criteria for validation must be defined and results must be recorded.

M5.120 The specificity of each lot and shipment of labelling reagents for the identification of HLA specificities must be shown to have comparable reactivity to the previously validated lot or shipment.

M5.200 Controls

M5.210 Controls for HLA typing by flow cytometry must be run for each test cell preparation.

M5.220 For direct labelling, a negative control must be conjugated with the same fluorochrome.

M5.230 For indirect labelling a negative control (eg. an irrelevant primary antibody), if available, should be used in conjunction with the same secondary antibody conjugated with the same fluorochrome as used for the specific antibody under test.

M5.250 Positive controls must include a pan-reacting anti-HLA monoclonal antibody which must be tested against each cell under the same conditions as the specific antibodies used in the test.

M5.260 A control cell known to express the HLA specificity under test must be included in each run.

M5.300 Policies and procedures:

M5.310 There must be policies and procedures in place to address at least HLA typing technical instructions including reagent standardisation and optimisation, reagent validation, incubation times and temperatures.

M5.320 Interpretation instructions must define the required reaction criteria in the negative and positive control samples for the test results to be valid, as well as criteria for positivity of the HLA antigen under test.

M5.330 A documented procedure must be followed for monoclonal antibodies which react with antigens other than those expected.

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 Microplate washer performance must be checked monthly and acceptable performance documented.

N2.000 ELISA technique.

N2.100 If commercial kits are used, the manufacturer's instructions must be followed unless the laboratory has performed and documented testing to support a deviation in 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(s).

N2.220 The positive control must be a human serum specific for HLA antigens and of the 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 *Panels*.

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 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.