Standards for Histocompatibility Testing

Notice and Disclaimer

These standards set forth only the minimum requirements for accredited histocompatibility laboratories. These standards do not set forth all that may be required of a facility to conform to federal or state laws or regulations (or non US equivalent) or the standard of care prevailing in the relevant community. Each facility must determine whether additional practices and procedures should be used in their particular locale. UNOS expressly disclaims any warranty that compliance with these standards meets all federal or state laws or regulations (or non US equivalent) or the standard of care that may prevail in any relevant community.

A General Policies
B Personnel Qualifications
C Quality Assurance
D HLA Antigens/Alleles
E HLA Typing
F Mixed Leukocyte Culture Tests
G Antibody Screening
H Renal and Pancreas Organ Transplantation
I Other Organ Transplantation
J Red Cell Typing for Organ Transplantation
K Immune Function/Response Monitoring
L Chimerism Analysis
M Nucleic Acid Analysis
N Flow Cytometry
O Enzyme Linked Immuno Sorbent Assay (ELISA)

E- HLA Typing

E1.000 Analysis

E1.100 The level of resolution of HLA typing must be appropriate for the clinical application.

E1.200 The method of assignment of HLA types must be documented for each technique used.

E1.300 The laboratory must have and adhere to a written policy that establishes when antigen redefinition and retyping are required.

E1.400 The laboratory must maintain a list of antigens and/or alleles defined by each test used in the laboratory.

E2.000 HLA Typing by Microcytotoxicity

E2.100 HLA-Class I Antigens

E2.110 If HLA-A,B, and/or C locus typing is done the laboratory must be able to type for all HLA-A,B and/or C specificities that are officially recognized by the W.H.O. Committee and for which reagents are readily available.

E2.200 HLA-Class II Antigens

E2.210 If DR, DQ and/or DP locus typing is done, the laboratory must be able to type for all HLA-DR, DQ and/or DP specificities that are officially recognized by the WHO Committee and for which reagents are readily available.

E2.300 Techniques

E2.310 Techniques used must be those that have been validated to optimally define HLA Class I and/or HLA Class II antigens.

E2.320 For each cell-serum combination, the results must be recorded in a manner that indicates the approximate percent of cells killed. The numerical codes used in the most recent edition of the ASHI Laboratory Procedure Manual are recommended.

E2.400 Controls

E2.410 Each typing tray must include at least one positive control serum known to react with all cells expressing the Class of antigens being tested at a comparable titer to that of the test typing reagents.

E2.420 Each typing must include at least one negative control serum. The negative control must be documented to be non-reactive under the specified test conditions.

E2.430 Cell viability in the negative control well at the end of incubation must be sufficient to ensure accurate interpretation of results.
E2.440 In assays in which cell viability is not required, results of positive and negative controls must be sufficiently discriminatory to ensure accurate interpretation of results.

E2.500 Target Cells

E2.510 If the cell donor has been transfused within the previous seven days, results are acceptable only if antigens are unequivocally defined. If a reliable typing cannot be obtained for a potential recipient, the typing must be repeated at a later date or by a different technique. If a reliable typing cannot be obtained for a deceased donor, or if there is extraneous reactivity (above background), the typing must be repeated with cells obtained from lymph node or spleen or by molecular methods.

E2.520 When typing for HLA-class II antigens with B-lymphocyte enriched preparations, the proportion of B lymphocytes in each preparation must be documented and must be sufficient to ensure accurate interpretation of results.

E2.600 Typing Assignments

E2.610 Each HLA antigen must be defined by a sufficient number of antisera or monoclonal antibodies to clearly define all the antigens for which the laboratory tests.

E2.620 It is recommended that each HLA class I antigen be defined by at least two operationally monospecific antisera or monoclonal antibodies or at least three partially non-overlapping multispecific antisera or monoclonal antibodies.

E2.630 It is recommended that each HLA class II antigen be defined by at least three operationally monospecific antisera or monoclonal antibodies or at least five partially non-overlapping multispecific antisera or monoclonal antibodies.

E2.700 Reagent Validation

E2.710 Cell panels of known HLA Class I and class II type must be available to validate new typing reagents. It is recommended that panel cells include at least one example of each HLA antigen the laboratory is able to define, and be from ethnic groups representative of the population served by the laboratory.

E2.720 The specificity of typing reagents obtained locally must be documented by external and/or internal QC testing.

E2.730 The specificity of individual reagents (used for preparation of local trays) received from other laboratories or commercial sources must be confirmed to ensure that they reveal the same specificities in the receiving laboratory.

E2.740 Each lot and/or shipment of new commercial typing trays must be evaluated by testing either with at least five different cells with different phenotypes or in parallel with previously evaluated trays.

E2.750 Complement

E2.751 Each lot and/or shipment 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. Optimal performance must be established and documented.

E2.752 Complement must be tested separately for use with each type of target cell (i.e., T-cells, B-cells, CLL cells) and with each test method used, since a different dilution or preparation may be required for optimal performance.

E3.000 HLA Typing by Nucleic Acid Analysis

E3.010 Laboratories performing HLA typing using nucleic acid analysis must conform to all pertinent standards in Section M- Nucleic Acid Analysis.

E3.100 Typing by Restriction Fragment Length Polymorphism (RFLP)
E3.110 Probes

E3.111 Each DNA probe utilized must be validated by family studies demonstrating Mendelian inheritance of the polymorphism detected and by extensive population studies.

E3.112 Each probe used must give a signal adequate to detect a single copy gene. Whenever possible, it is recommended that locus-specific probes be used.

E3.113 The HLA locus and allele(s) designation(s) must be defined for each RFLP type pattern.

E3.200 Amplification Based Typing

E3.220 Typing by Amplification Followed by Hybridization with Sequence-Specific Oligonuleotide Probes (SSOP)

E3.221 The specificity and target sequence of primers must be defined. The HLA locus and allele(s) designation(s) must be defined for each primer.

E3.222 The HLA locus and allele(s) designation(s) must be defined for each probe and template primer combination.

E3.223 Positive or negative probe hybridization must be defined for each probe with all alleles that are recognized by the W.H.O. Nomenclature Committee for Factors of the HLA System provided that nucleotide sequences and reference DNA are readily available.

E3.230 Typing by Sequenced Based Typing (SBT)

E3.231 Templates must have sufficient specificity for a locus or allele to provide interpretable primary sequencing data.

E3.232 The HLA locus and allele(s) designation(s) must be defined for each template/primer combination. Each unknown sequence must be compared with the sequences of all alleles that are recognized by the W.H.O. Nomenclature Committee for Factors of the HLA System provided that the nucleotide sequences are readily available.

E3.233 The laboratory must have a process to recognize and document ambiguous combination(s) of alleles for each template/primer combination.

E3.234 The laboratory must maintain records that define the sequence database utilized to interpret the primary data. This database must be updated at least annually. If a determined sequence is ambiguous (i.e., more than one possible interpretation of available data) the report must indicate all possible allelic combinations.

E3.240 Typing by Amplification with Sequence Specific Primers Followed by Analysis of Restriction Fragment Length Polymorphisms (SSP-RFLP).

E3.241 The HLA locus and allele(s) designation(s) must be defined for each RFLP type.

E3.250 Typing by Amplification with Sequence-Specific Primers (SSP)

E3.251 The HLA locus (loci) and allele(s) designation(s) must be defined for each primer combination.

E3.252 Positive or negative amplification must be defined for each primer mixture with all possible combinations of alleles that are recognized by the W.H.O. Nomenclature Committee for Factors of the HLA System provided that nucleotide sequences and reference DNA are readily available.

E3.300 Typing by Other Methods

E3.310 If alternative methods are used for HLA typing, procedures must be defined and validated, and must include sufficient controls to ensure accurate assignment of types. All relevant standards from the above sections and Section P must be applied.