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
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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)

N- Flow Cytometry

These standards apply to histocompatibility testing and leukocyte phenotyping by flow cytometry.

N1.000 Instrument Standardization/Calibration

N1.100 An optical standard, consisting of latex beads or other uniform particles, must be run to ensure proper focusing and alignment of all lenses in the path for both the exciting light source and signal (light scatter, fluorescence, etc.) detectors.

N1.110 A fluorescent standard(s) for each fluorochrome to be used must be run to ensure adequate amplification of the fluorescent signal(s). These fluorescent standards may be incorporated in the beads or other particles used for optical standardization (ref. Q1.100) or may be a separate bead or fixed cell preparation.

N1.120 Both the optical and fluorescent standards must be run each time the instrument is turned on and any time maintenance, adjustments, or problems have occurred during operation that could potentially affect instrument function.

N1.120 The results of optical focusing/ alignment must be recorded in a daily quality control log.

N1.130 Threshold values for acceptable optical and fluorescent standardization results must be established for all relevant signals for each instrument used.

N1.140 In the event a particular threshold value cannot be attained, there must be a written protocol detailing the corrective action required.

N1.200 If performing analyses that require the simultaneous use of two or more fluorochromes, an appropriate procedure must be used to compensate for overlap in their emission spectra.

N1.300 Laser power output and current input (amps) must be recorded daily for each instrument. Acceptable thresholds and corrective action protocols must be documented.

N2.000 Flow Cytometric Crossmatch Technique

N2.100 The laboratory must ensure the appropriate definition and purity of cell populations by the use of either a multi-color technique or other documented method.

N2.110 The laboratory must assess the binding of human immunoglobulin using a fluorochrome labeled reagent such as either an $F(ab')_2$ anti-human IgG that is specific for the Fc region of the heavy chain or other documented method.

N2.120 Crossmatch results for a specific cell population (e.g., T-cells, B-cells and/or monocytes) must be based on the use of a monoclonal antibody that detects an appropriate cluster designated antigen (e.g., CD3 for T cells, CD19 or CD20 for B cells and CD14 for monocytes).

N2.130 Each laboratory must establish and document the optimum serum/cell ratio.

N2.200 Controls

N2.210 The negative control must be human serum documented to be non-reactive against the crossmatch target cells.

N2.220 The positive control must be human antibody of the appropriate isotype for the assays and specific for the antigens that are targeted in the crossmatch. Positive controls must be used at a titer appropriate for the assay (i.e., a titer at which moderate changes in assay sensitivity are likely to be detected) and must react with appropriate target cells from all humans.

N2.230 The anti-human immunoglobulin reagent must be titered to determine the dilution with optimal activity (signal to noise ratio). If a multicolor technique is employed, the reagent must not demonstrate crossreactivity with the other immunoglobulin reagents used to mark the cells.

N2.240 Regardless of the method used for reporting raw data (mean, median, mode channel shifts or quantitative fluorescence measurements), each lab must establish its own threshold for discriminating positive reactions. Any significant change in protocol, reagents, or instrumentation requires repeat determination of the positive threshold.

N2.300 Interpretation

N2.310 Each laboratory must define the criteria used to define positive and negative crossmatches.

N3.000 Immunophenotyping By Flow Cytometry

N3.100 Terminology used must conform to the most recent publication of the International Workshop of Differentiation Antigens of Human Leucocytes or other appropriate scientific organizations.

N3.200 Cell Preparation

N3.210 The method used for cell preparation must be documented to yield appropriate preparations of viable cells sufficient to ensure accurate test results.

N3.220 For internal labeling, the method used to allow fluorochrome labeled antibodies to penetrate the cell membrane must be documented to be effective.

N3.300 Quality Control

N3.310 Specificity controls, consisting of appropriate cell types known to be positive for selected standard antibodies must be run within laboratory-defined intervals sufficiently short to assure the proper performance of reagents.

N3.320 A negative reagent control(s) must be identified for each test cell preparation. It is recommended that this control consist of monoclonal antibody(ies) of the same species and subclass and be prepared/purified in the same way as the monoclonal(s) used for phenotyping.

N3.330 For indirect labeling, it is recommended that the negative control reagent be an irrelevant primary antibody and the same secondary antibody(ies) conjugated with the same fluorochrome(s) used.

N3.340 For direct labeling, it is recommended that the negative control reagent be an irrelevant antibody conjugated with the same fluorochrome and at the same fluorochrome: protein ratio used in all relevant test combinations.

N3.350 Each laboratory must define acceptable time periods between processing, labeling and analysis of samples. Control samples must be treated in the same manner.

N3.360 Gating strategies must be employed to assure that the population of interest is being selected without significant contamination.

N3.370 Conclusions about abnormal proportions or abnormal numbers of cells bearing particular internal or cell surface markers must only be drawn in comparison with local `control' data obtained with the same instrument, reagents and techniques.
N3.380 Determination of percent positives must take into consideration the results of the negative control reagent.

N3.400 Reagents

N3.410 The laboratory must have a policy to validate the specificity of monoclonal antibodies. It is recommended that initial validation use appropriate control cells. Subsequent quality control may consist of testing in parallel with previous lots.
N3.420 The quantities of reagents used for each test sample must be determined by the manufacturers or from published data and whenever possible be verified locally by titration.

N3.430 Monoclonal antibodies that have been reconstituted from lyophilized powder form for storage at $4^{o}C$ must be processed according to the manufacturer's instructions or locally documented procedures to remove microaggregates prior to use in preparation of working stains.