

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.

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- M Nucleic Acid Analysis
- N Flow Cytometry
- O Enzyme Linked Immuno Sorbent Assay (ELISA)

M- Nucleic Acid Analysis

Section M applies to testing related to histocompatibility, transplantation, and detection of chimerism.

M1.000 Universal Standards (The standards in M1 apply to all nucleic acid testing).

M1.100 Nucleic Acid Extraction

M1.110 Nucleic acids must be purified by standard methods that are documented and have been validated in the laboratory. For each method, written guidelines must specify the minimal acceptable sample (e.g., volume, number of cells, type of cells). If tests are performed without prior purification of nucleic acids, written guidelines must specify the minimal acceptable sample and the test method must be documented and validated in the laboratory.

M1.120 Specimens must be handled and stored under conditions that maintain sufficient integrity of nucleic acids to ensure reliable test results.

M1.130 If nucleic acids are not used immediately after purification, samples must be stored under conditions that preserve the integrity of the nucleic acids that will be tested.

M1.140 Nucleic acids must be of sufficient quality (e.g., purity, concentration) to ensure reliable test results.

M1.200 Electrophoresis

M1.210 Each electrophoretic run must include negative and positive controls that are processed with each assay.

M1.220 If the size of a nucleic acid is a critical factor in any aspect of the analysis of the data, size markers that produce discrete electrophoretic bands spanning and flanking the entire range of expected fragment sizes must be included in each gel.

M1.230 If the size of a nucleic acid is a critical factor in the analysis of the data, the amount of DNA loaded in each lane must be within a range that ensures equivalent migration of DNA in all samples, including size markers.

M1.240 Acceptable electrophoretic conditions must be validated, documented, and used for each assay.

M1.250 The laboratory must establish criteria for accepting the validity of each gel and of each lane of the gel.

M1.300 Analysis

M1.310 Acceptable limits of signal intensity must be specified for positive and negative results. If these are not achieved, corrective action is required.

M1.320 Two independent interpretations of primary data are recommended.

M1.330 Automated systems and computer programs must be validated prior to use and tested routinely for accuracy and reproducibility of manipulations.

M2.000 Template Amplification

M2.100 Laboratory Design For Assays Using Template Amplification

M2.110 Use of physical and/or biochemical barriers to prevent nucleic acid contamination (carry-over) is required. Pre-amplification procedures must be performed in a work area that excludes amplified nucleic acid that has the potential to serve as a template in any amplification assays performed in the laboratory (e.g., PCR product, plasmids containing HLA genes or VNTR/STR loci). Physical separation and restricted traffic flow are recommended. Use of a static air hood or a class II biological safety cabinet is recommended. Biochemical procedures can be used to inactivate amplified products.

M2.120 Pre-amplification physical containment must include use of dedicated lab coats, gloves and disposable supplies.

M2.130 The laboratory must perform procedures to remove carry-over contamination from work areas used for manipulation of pre-amplification reagents or samples. Frequent cleaning with dilute acid or bleach and/or UV treatment of work surfaces is recommended.

M2.140 Methods that utilize two consecutive steps of 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-amplification work area and post-amplification work areas.

M2.200 Equipment and Reagents

M2.210 Equipment

M2.211 Use of equipment that is dedicated to pre-amplification procedures is recommended.

M2.212 Each work area (i.e., pre-amplification, secondary amplification, and post-amplification) must have dedicated pipettors. Positive displacement pipettes or filter-barrier tips are recommended for pre-amplification and secondary amplification work areas.

M2.213 Thermal cycling instruments must precisely and reproducibly maintain the appropriate temperature of samples. Accuracy of temperature control for samples must be verified at least every 6 months.

M2.214 Incubators and water baths must be monitored for accurate temperature maintenance every time the assay is performed.

M2.220 Reagents

M2.221 All reagents (solutions containing one or multiple components) utilized in the amplification assay 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., Mg++) be left out of the mixture.

M2.222 Reagents used for initial amplification must not be exposed to post-amplification work areas. Reagents used for secondary amplification must be stored in an area that prevents carry-over contamination. The appropriate performance of each lot and shipment of reagent must be documented before results using these reagents are reported.

M2.223 Reagents from different lots of commercial kits must not be mixed without appropriate quality control.

M2.300 Primers

M2.310 Primers must be stored under conditions that maintain specificity and sensitivity.

M2.320. Conditions that influence the specificity or quantity of amplified product must be demonstrated to be satisfactory for each set of primers.

M2.330 Laboratories must have a policy for quality control of each lot and shipment of primers. It is recommended that reference material be used for quality control whenever possible.

M2.340 For labeled primers the specificity and sensitivity of the labeling and detection method must be validated. The performance of each lot or shipment of labeled primers must be periodically confirmed.

M2.400 Amplification Templates

M2.410 Samples containing nucleic acids that will be amplified (e.g., blood, DNA isolates) must be stored under conditions that do not result in artifacts, inhibition of the amplification reaction, and exposure to post-amplification work areas or any other sources of carry-over contamination.

M2.420. The acceptable range for the amount of target must be specified and validated.

M2.430 DNA from any source or RNA from any cells expressing the target gene may be used. If reverse transcription is used, appropriate controls must be included for this step.

M2.500 Contamination

M2.510 Nucleic acid contamination must be monitored for the most common amplification products that are produced in the laboratory. Routine wipe tests of pre-amplification work areas must be performed. Monitoring must be performed using a method that is at least as sensitive as routine test methods. If amplified product is detected, the area must be cleaned to eliminate the contamination and measures must be taken to prevent future contamination.

M2.520 At least one negative control (no nucleic acid) must be included in each amplification assay. Testing of open tubes in the work area is recommended.

M2.530 To minimize the detection of minor contaminants and the occurrence of stochastic fluctuation during thermal cycling, it is recommended that the number of cycles be set at a level sufficient to detect the target nucleic acid but insufficient to detect small amounts (e.g., <10 molecules) of contaminating template.

M2.600 Controls and Quality Assurance

M2.610 The quantity of specific amplification products must be monitored (e.g., gel electrophoresis, hybridization).

M2.620 Criteria for accepting or rejecting an amplification assay must be specified.

M2.630 If presence of an amplified product is used as the end result, controls must be included to detect amplification in every amplification mixture. Amplification specificity must be monitored on a periodic basis.

M2.640 If an amplified product is used as a nucleic acid target, variation in the amount of amplified product must be monitored (e.g., hybridization with a consensus probe, gel electrophoresis). The acceptable range for the amount of target must be specified.

M3.000 Technique-Specific Standards

M3.100 Oligonucleotide Probe Assays

M3.110 Oligonucleotide Probes

M3.111 The specificity and target sequence of oligonucleotide probes must be defined.

M3.112 Oligonucleotide probes must be stored under conditions that maintain specificity and sensitivity.

M3.113 Oligonucleotide probes must be utilized under empirically determined conditions that achieve the defined specificity. Laboratories must perform quality control testing to confirm specificity for each lot and shipment of probe. Reference material must be used for quality control whenever possible.

M3.120 Labeling of Nucleic Acids and Detection

M3.121 The specificity and sensitivity of the labeling and detection method must be established, reproducible, and documented in the laboratory before results are reported.

M3.130 Hybridization

M3.131 Hybridization must be carried out under empirically determined conditions that achieve the defined specificity.

M3.132 It is recommended that the specificity of hybridization be confirmed using positive and negative controls for hybridization with each probe and that the controls be capable of detecting cross-hybridization with closely related sequences.

M3.140 The laboratory must have a validated procedure for reuse of nucleic acids (probes or targets) bound to solid supports.

M3.150 If nucleic acids in solution (probes or targets) are reused, controls must be included to ensure that the sensitivity and specificity of the assay are unaltered.

M3.200 Nucleotide Sequencing

M3.210 Sequencing templates must have sufficient purity, specificity (e.g., locus or allele-specificity), quantity and quality to provide interpretable primary sequencing data. The method for preparing sequencing templates must reliably generate appropriate length sequencing templates that are free of inhibitors of subsequent reactions (e.g., residual primer extension) and free of contaminants that cause sequencing artifacts. Methods must ensure that preparation of sequencing templates does not alter the accuracy of the final sequence (e.g., mutations created during cloning, preferential amplification).

M3.220 The expected sequence of the template (e.g., library of HLA alleles) must be documented.

M3.230 Conditions for primer extension (e.g., polymerase type, polymerase concentration, primer concentration, concentration of nucleotide triphosphates, concentration of terminators) must be appropriate for the template (e.g., length of sequence, GC content).

M3.240 The specificity and sensitivity of the labeling and detection methods must be documented (e.g., demonstrating correct signal strength for a control sequence) in the laboratory before results are reported.

M3.250 Nucleotide and Allele Assignments

M3.251 Criteria for acceptance of primary data must be established (e.g., correct assignments for nonpolymorphic positions, definition of sequencing region, criteria for peak intensity, baseline fluctuation, signal-to-noise ratio and peak shapes). Validation must test for sequence-specific artifacts (e.g., sequencing of both strands of samples representative of all polymorphic motifs that are frequently encountered in the routine sample population). Established sequence-specific artifacts must be documented and utilized in routine interpretation of data.

M3.252. For heterozygous templates, sequencing of sense and anti-sense strands is strongly recommended. If only one strand is sequenced, validation must show that sequencing of only one strand consistently yields accurate

sequence assignments. If assignments are routinely based upon data from one strand of DNA, periodic confirmation of complementary strands is recommended. If a sequence suggests a novel allele or a rare combination of alleles, the sequences of sense and anti-sense strands must be determined.

M2.253 A scientifically and technically sound method must be established for interpretation, acceptance, and/or rejection of sequences, especially regions that are technically difficult (e.g., compression ends).

M2.254 Methods must ensure that sequences contributed by amplification primers are not considered in the assignment of alleles.

M3.300 Restriction Fragment Length Polymorphism

M3.310. Restriction Enzymes

M3.311. The appropriate performance of each lot of restriction enzyme must be documented before results using these reagents are reported. Each lot or shipment of restriction nucleases must be demonstrated to produce fragments of established sizes.

M3.312 Enzymes must be stored and utilized under conditions recommended by the manufacturer (i.e., test temperature, buffer) to ensure proper DNA digestion.

M3.320 Amplified DNA

M3.321 When amplified DNA is digested, controls of amplified DNA that will produce fragments of known sizes must also be digested in parallel to monitor complete digestion.

M3.322 It is recommended that amplified DNA be incubated without restriction enzyme and analyzed by gel electrophoresis to monitor target integrity

M3.323 Appropriate migration patterns of control DNA and size markers are required for each run.

M3.330 Genomic DNA

M3.331 When a DNA test sample is digested for analysis, human DNA that will produce fragments of established sizes must be digested in parallel to ensure complete endonuclease digestion.

M3.332 Probes must be used in the form as reported in the scientific literature and/or as used to determine the inheritance pattern and population distribution of the polymorphism.

M3.333 Probes must be labeled by a method appropriate for the testing procedure.

M3.334 Prehybridization, hybridization, and detection must be carried out under empirically determined conditions of concentration and stringency *that* are determined by the length or composition of the probe and that achieve the defined specificity.

M3.335 It is recommended that stringency conditions be selected to minimize the possibility of cross-hybridization.

M3.336 Re-probing of the same membrane must be performed only after demonstrating that complete stripping of the first probe has occurred.

M3.337 Assays that are reported as acceptable must reveal the appropriate patterns of the human control DNA and size markers.

M3.338 The laboratory report for each fragment detected must specify the probe, restriction endonuclease used, fragment size (kb) and the chromosomal location as defined by relevant scientific literature.

M3.400 Sequence Specific Amplification

M3.410 Each amplification reaction must include procedures 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).

M3.420 It is recommended that in each amplification assay (i.e., set up of amplification mixtures for one or more samples) controls be used to detect contamination with previously amplified products (e.g., a special primer pair internal to all amplification products or a combination of primers to detect any DNA that could confound the typing result).

M3.500 Other Techniques

M3.510 The method must be validated in the laboratory. If the test detects polymorphism with Mendelian inheritance, validation must include demonstration of expected inheritance.

M3.520 Appropriate controls must be included for each component of the test.