

Histocompatibility		
Tag #	Standard	Guidance
	The following specialty sustaining standards of practices shall be incorporated into the laboratory's quality management system, where applicable to the scope of services provided.	
HC1	<p>Histocompatibility Standard 1</p> <p>Reports shall use terminology for HLA antigens which conforms to the latest report of the HLA Nomenclature Committee (WHO).</p>	<p>The World Health Organization Regional Office for the Americas/Pan Health Organization 525 23rd Street, N. W. Washington, D.C. 20037</p> <p>Telephone: (202) 974-3000 Fax: (202) 974-3663 E mail: postmaster@paho.org Website: http://www.paho.org</p>
HC2	<p>Histocompatibility Standard 2</p> <p>At least annually, the ability of testing personnel to reproduce results shall be assessed, using a previously tested specimen as an unknown. Records of the results for each individual shall include the review date and specimen identification and shall be reviewed and approved by a supervisor.</p>	<p>Approval should be documented on the record.</p> <p>Competency assessment should evaluate each testing personnel for each assay by each methods. The assessment may occur on the same day the specimen was initially tested.</p>
HC3	<p>Histocompatibility Standard 3</p> <p>The laboratory's quality control program shall include participation in at least one national or regional cell or DNA exchange program, such as the Southeast Organ Procurement Foundation (SEOPF), the International Cell Exchange sponsored by the UCLA Immunogenetics Center, the College of American Pathologists (CAP) and the American Society of Histocompatibility and Immunogenetics (ASHI), or other as approved by the Department.</p>	<p>If performance in cell or DNA exchange is poor, the laboratory should identify the reason for poor performance, resolve the problem, and document action taken.</p>

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HC4	<p>Histocompatibility Standard 4</p> <p>If ABO and Rh typing is performed on site and reported, the laboratory shall have a New York State permit in immunohematology.</p>	
HC5	<p>Histocompatibility Standard 5</p> <p>If the laboratory utilizes ABO agglutinins to remove red blood cells during lymphocyte isolation, the specificity of the ABO reagents shall be verified with control cells for each lot used.</p>	
HC6a HC6b HC6c	<p>Histocompatibility Standard 6</p> <p>If a laboratory provides histocompatibility testing for a transplant center, the laboratory shall:</p> <p>a) establish and document the HLA Class I and II specificities that should be identified for each type of transplant ;</p> <p>b) HLA type cells from organ donors referred to the laboratory; and</p> <p>c) use HLA antigen terminology that conforms to the latest report of the World Health Organization (W.H.O.) Committee on Nomenclature. Potential new antigens not yet approved by this committee must have a designation that cannot be confused with W.H.O. terminology.</p>	<p>HLA Class I specificities include HLA-A, HLA-B and Cw. HLA Class II specificities include HLA-DR, DQ and DP antigens. For some transplant protocols, identification of only Class I specificities may be sufficient. Other protocols may require identification of both Class I and Class II specificities.</p> <p>This standard now recognizes that laboratories may be using serological or DNA or a combination of methods to perform HLA typing. The laboratory must decide in conjunction with the transplant program(s) that they support, what level of antigen definition is required for the type of transplant being performed. In some cases, low resolution typing using serological methods may be adequate while in other cases, such as bone marrow transplant, high resolution typing by DNA analysis may be required. The laboratory should document discussions with transplant programs.</p> <p>a) The laboratory should be an active participant in the transplant center's clinical program when establishing the specificities needed.</p> <p>b) Laboratories should retype organ donors referred to the lab even if testing was previously done in another histocompatibility testing lab.</p>

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	SEROLOGICAL HLA TYPING	
	Histocompatibility Standard 7	
	A complement dependent lymphocytotoxic method shall be used for cell typing and shall incorporate controls; and,	
HC7a	a) each typing tray shall minimally include one complement-dependent positive serum control known to react with all cells and one negative serum (or serum pool) control known to lack HLA antibody; and,	
HC7b	b) cell viability in the negative control at the end of incubation shall be that value established by the laboratory to be sufficient to permit accurate interpretation of results.	

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	<p>Histocompatibility Standard 8</p> <p>The laboratory shall validate the specificity of antisera using cells of known type; and,</p>	
HC8a	a) specificity of sera obtained locally shall be validated using a cell panel from a minimum of 40 subjects from various ethnic groups, which includes cells with antigens and common splits to which HLA antibodies are directed, cells possessing only one defined antigen at a locus, and additional cells as needed to identify an antibody with certainty.	
HC8b	b) specificity of individual sera obtained from commercial or other laboratory sources shall be verified using a method which includes a positive and negative control for each serum tested with each cell panel;	
HC8c	c) reactivity of each lot of commercial typing trays shall be validated by pre-test against at least five different cells representing major specificities or by testing in parallel with previously validated trays; and,	
HC8d	d) typing sera reactions shall be recorded, reviewed by a supervisor, and shall be used to modify locally prepared typing trays and applied to all tray interpretations.	
	<p>Histocompatibility Standard 9</p> <p>Each lot of complement shall be tested to verify that it induces cytotoxicity in the presence of specific antibody but is not cytotoxic in the absence of specific antibody.</p>	
HC9		

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HC10	Histocompatibility Standard 10 When typing trays are locally prepared, the laboratory shall maintain sera inventory records which document the source, bleeding date, identification number, specificity and volume remaining for each serum lot.	

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	<p>Histocompatibility Standard 11</p> <p>In addition to applicable requirements in the Standard Operating Procedures and Compliance section of these Standards, the SOPM shall contain:</p>	
HC11a	a) the protocols for preparation and/or selection of typing reagents, whether locally or commercially prepared, and verification of reactivity;	
HC11b	b) if the laboratory uses locally prepared cell panels, a list of individuals for fresh panel bleeding;	
HC11c	c) the protocol for the preparation of lymphocytes;	
HC11d	d) the scoring system protocol used for antigen assignment, including, where applicable, literature references and/or instrument calibration documentation;	
HC11e	e) the policy for antigen redefinition and retyping, including, where applicable, the updating of results and issuance of amended reports;	
HC11f	f) the policy for remediation for those individuals not meeting the laboratory's established level of performance for reproducibility of test results;	
HC11g	g) a protocol for ensuring that reagents used for typing are adequate to define all HLA-A, B and DR specificities that are officially recognized by the most recent W.H.O. Committee on Nomenclature and for which reagents are readily available; and,	
HC11h	h) have available and follow written criteria for the assignment of HLA antigens.	

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<p>HC12a</p> <p>HC12b</p>	<p>Histocompatibility Standard 12</p> <p>For the assignment of HLA antigens:</p> <p>a) an established scoring system shall be used; and,</p> <p>b) testing personnel shall be trained in the use of the scoring system and such training shall be documented.</p>	
<p>HC13a</p> <p>HC13b</p>	<p>Histocompatibility Standard 13</p> <p>The laboratory shall:</p> <p>a) for monospecific sera:</p> <p style="padding-left: 40px;">i) at least two sera should be used to identify each class I antigen (A, B, and C); and,</p> <p style="padding-left: 40px;">ii) at least three sera shall be used to define each class II antigen (DR and DQ).</p> <p>b) for multispecific sera:</p> <p style="padding-left: 40px;">i) at least three partially non-overlapping sera shall be used to define each class I antigen (A, B, and C); and,</p> <p style="padding-left: 40px;">ii) at least five partially non-overlapping sera shall be used to define each class II antigen (DR and DQ).</p>	
<p>HC14</p>	<p>Histocompatibility Standard 14</p> <p>If monoclonal antibodies are used for class II typing (DR and DQ), each antigen shall be defined by at least two antibodies with private epitope specificity and one antibody with public epitope specificity OR at least three partially non-overlapping antibodies with public specificities.</p>	

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HC15	<p>Histocompatibility Standard 15</p> <p>If the laboratory uses B lymphocyte-enriched preparation for class II typing, the proportion of B lymphocytes shall be that which has been determined by the laboratory to clearly define class II alleles.</p>	
HC16	<p>Histocompatibility Standard 16</p> <p>Refrigerators and freezers must be monitored to ensure maintenance of optimal temperatures for storage of each type of specimen or reagent. The laboratory's storage of both critical reagents and relevant specimens must use an audible alarm system or centrally monitored temperature alarm system.</p> <ul style="list-style-type: none"> a) The laboratory must have an emergency plan for alternative storage. b) For samples that may be required for future testing, the laboratory must have a system to retrieve specimens for further testing in a timely manner. 	
	MIXED LYMPHOCYTE CULTURE (MLC) TESTS	
HC17	<p>Histocompatibility Standard 17</p> <p>The laboratory shall have a protocol to establish a minimum acceptable level of cell viability.</p>	
HC18	<p>Histocompatibility Standard 18</p> <p>The laboratory shall establish acceptable limits for background counts using an appropriate autologous cell control in each MLC test run.</p>	
HC19	<p>Histocompatibility Standard 19</p> <p>For each MLC test run, three unrelated stimulator cell controls for each responder cell shall be included.</p>	

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HC20	<p>Histocompatibility Standard 20</p> <p>The laboratory shall use a properly functioning biological safety cabinet or other appropriately aseptic work area.</p>	Refer to Safety Sustaining Standard of Practice 9 (SSP9) for operational guidelines.
HC21	<p>ANTIBODY SCREEN AND CROSSMATCH</p>	<p>These techniques are developed primarily for kidney transplants.</p> <p>The technique used in the laboratory is developed with the initial validation studies. These studies should use the complement-dependent microlymphocytotoxicity assay as the basis for deciding the conditions necessary to detect HLA antibodies at the specificity required.</p> <p>Initial validation studies should establish the concentration of the patient sera to be used by testing anti-sera of known concentration and the appropriate incubation times.</p>
	<p>Histocompatibility Standard 21</p> <p>The laboratory shall use a technique that detects HLA-specific antibody with a specificity alternate or superior to that of the basic complement-dependent microlymphocytotoxicity assay.</p>	
HC22	<p>Histocompatibility Standard 22</p> <p>The laboratory shall use well characterized panels of both T and B lymphocytes to identify antibodies to differentiate Class II antibodies from Class I.</p>	All cells have Class I antigens. B-lymphocytes have also express Class II antigens and are used to distinguish antibodies to HLA Class II antigens from antibodies to Class I antigens.
HC23	<p>Histocompatibility Standard 23</p> <p>The laboratory shall use crossmatching techniques that are documented to have increased sensitivity in comparison with the basic microlymphocytotoxicity test.</p>	The technique used in the laboratory is developed with the initial validation studies. Laboratories may use increased incubation times or the addition of antiglobulin to increase sensitivity.

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	<p>Histocompatibility Standard 24</p> <p>For testing performed for the purpose of transplantation, the laboratory shall have available and follow written policies and protocols specifying the histocompatibility testing (HLA typing, antibody screening, compatibility testing and crossmatching) to be performed for each type of cell, tissue or organs to be transfused or transplanted. The policies must include, as applicable:</p>	
HC24a	a) timing of collection of specimens for final crossmatch done prior to transplantation;	d) High risk patients are those that have had previous transplants, infections, transfusions, etc. These patients may need more frequent testing.
HC24b	b) timing and acceptable reactivity of specimens for crossmatch post transfusion or following a sensitizing event;	
HC24c	c) testing protocols for cadaver donor, living, living-related and combined organ and tissue transplants	
HC24d	d) testing protocols for patients at high risk for allograft rejection;	
HC24e	e) the circumstances under which MLC's will be performed; and,	
HC24f	f) the sensitivity and specificity of the test system required to support clinical transplant protocols (for example, antigen or allele-level typing).	

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<p>HC25a</p> <p>HC25b</p> <p>HC25c</p> <p>HC25d</p>	<p>Histocompatibility Standard 25</p> <p>The laboratory shall maintain serum specimens and related records for potential transplant recipients for the purpose of:</p> <p>a) initial typing;</p> <p>b) periodic screening;</p> <p>c) pretransplantation crossmatch; and,</p> <p>d) screening following a sensitizing event.</p>	<p>This standard assumes there is collaboration between the laboratory and transplant facility.</p>
<p>HC26a</p> <p>HC26b</p>	<p>Histocompatibility Standard 26</p> <p>For renal transplants or for combined organ transplant in which one of the organs is a kidney, the laboratory shall have available results of the final crossmatches before the transplant.</p> <p>For nonrenal transplantation, if HLA testing and final crossmatching were not performed prospectively because of an emergency situation, records must reflect any information concerning the transplant provided to the laboratory by the transplant candidate's physician.</p>	<p>Crossmatch results for non-renal transplants may be reviewed retrospectively.</p>

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	DNA-BASED TESTING	
	Histocompatibility Standard 27 All polymorphic loci shall: a) be validated by family studies demonstrating Mendelian inheritance; and, b) be documented in the literature, including identification of the restriction endonucleases and probes or primers used to detect the polymorphism.	The validation studies and literature references should be documented during the initial validation of the assay.
HC27a HC27b		
HC28	Histocompatibility Standard 28 All records demonstrating polymorphisms shall be retained including autoradiographs, computer generated images or recordings as applicable.	The laboratory should have a system for maintaining and retrieving the original image. This also applies to image analysis software.
	Histocompatibility Standard 29 In addition to the information required in the Reporting section of these Standards, the report shall contain: a) name of the test DNA locus; b) name of the probe, where applicable; c) name of the restriction endonuclease, where applicable; and, d) size or alphanumeric description of reported alleles.	
HC29a HC29b HC29c HC29d		

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	DNA-BASED TESTING USING RESTRICTION ENZYMES	
HC30	<p>Histocompatibility Standard 30</p> <p>The laboratory shall use a known human DNA control and, where appropriate, test gels in every run to monitor restriction enzyme activity, fragment production and electrophoretic separation.</p>	The cell line K562 is commonly used as the human DNA control.
	DNA-BASED TESTING USING PROBES	
HC31	<p>Histocompatibility Standard 31</p> <p>For in-house developed assays, pre-hybridization, hybridization and autoradiography shall be carried out under empirically determined, periodically verified and documented conditions of concentration and temperature determined by the nature of the probe as determined during initial validation process.</p> <p>Hybridization conditions shall minimize the possibility of cross-hybridization and maximize the specificity of binding between probe and test DNA; and,</p>	
HC32	<p>Histocompatibility Standard 32</p> <p>Only those autoradiographs and membranes where the control DNA and size marker patterns meet the laboratory's pre-established criteria for acceptance shall be analyzed.</p>	

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	DNA-BASED TESTING USING AMPLIFICATION	
HC33	<p>Histocompatibility Standard 33</p> <p>For in-house developed methods, primers shall be of known specificity and sequence. Conditions of time, temperature and concentration which optimize amplification product specificity or quantity shall be empirically determined and documented for each set of primers during the initial validation. The conditions shall be periodically verified.</p>	
HC34	<p>Histocompatibility Standard 34</p> <p>For in-house developed methods, the number of amplification cycles determined during the initial validation studies shall be set at a level that minimizes the synthesis of extraneous DNA but is sufficient to synthesize detectable levels of test DNA.</p>	
HC35	<p>Histocompatibility Standard 35</p> <p>For those laboratories performing HLA typing for the purpose of disease association studies, the laboratory must check each typing using materials to monitor the test components and each phase of the test system to ensure acceptable performance.</p>	
HC36	<p>Histocompatibility Standard 36</p> <p>The laboratory must document all control procedures performed, as specified in this section.</p>	

Engraftment Monitoring		
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	The following specialty sustaining standards of practices shall be incorporated into the laboratory's quality management system, where applicable to the scope of services provided.	
EM1	<p>Engraftment Monitoring Standard 1</p> <p>The laboratory shall include a sensitivity control in each patient run.</p>	For this control, it is suggested that a small amount of a positive sample be mixed with an excess of a negative sample, e.g., 1:20 for a 5% sensitivity.
EM2	<p>Engraftment Monitoring Standard 2</p> <p>The final report shall include, at minimum, a summary of the method that was used, the DNA loci tested, and the objective findings in a readily interpretable format.</p>	