ASHI University

Title: HLA Typing by Serology

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Section 2: Objectives

To provide the technologist with a better understanding of the theoretical and technical aspects involved in serologic HLA typing. This module will include a discussion of the following topics:

1. A brief history and introduction to the development of microlymphocytotoxicity assay and its use in serologic HLA typing.
2. The clinical importance of appropriate use of serologic HLA typing.
3. An understanding of specific equipment used in serologic testing.
4. The choices made by laboratories in selecting commercially-prepared serologic typing trays vs. the preparation of in-house typing trays.
Section 3: Introduction

Interest in histocompatibility research began in the early 1900s with tumor transplantation in mice and continued into the 1940s and 1950s prior to the discovery of tumor resistance genes in mice, which became termed H or histocompatibility genes. With the research papers of Jean Dausset, Jon van Rood and Rose Payne published in 1958, histocompatibility in humans came to the forefront. These researchers worked with assays such as leukoagglutination, complement fixation and lymphocytotoxicity along with milliliters of serum from multiparous women and isolated leukocytes and gradually began to determine the polymorphic nature of HLA gene loci. Their work led to the very beginning of HLA nomenclature as we know it today, with the identification of Bw4 and Bw6 as well as first three HLA-A locus specificities: A1, A2, A3. The miniaturized version of the lymphocytotoxicity testing, the microlymphocytotoxicity assay introduced by Paul Terasaki and James McClelland in 1964 became the standard procedure for histocompatibility testing at the National Institutes of Health (NIH). This assay is also referred to as the complement-dependent cytotoxicity (CDC) assay because it relies on the addition of rabbit complement and not endogenous complement present in reagent sera to mediate test reactivity.

With the recognition of the complexity of the HLA gene system, leading histocompatibility researchers recognized a group effort would produce much greater progress and allow for the sharing of reagents and technology. This lead to the first International Histocompatibility Workshop (IHWS) in 1964. This meeting compared the techniques being used, including leukoagglutination, complement fixation and the microlymphocytotoxicity assay in use today. It also laid the groundwork for the development of a standardized HLA nomenclature, and with the availability of standardized reagents from the NIH, allowed laboratories to begin to perform serologic HLA typing. With improved techniques and increased inter-laboratory sharing of antisera and information, more standardized serologic typing reagents became available, especially from some of the initial commercial companies that began to produce serologic HLA typing trays.

The microlymphocytotoxicity assay for serologic HLA typing is a simple, reproducible and sufficiently sensitive assay to detect and identify the specificity of HLA-A, B, C, DR and DQ antigens on the surface of lymphocytes. The basis of the microlymphocytotoxicity assay involves using very small amounts of purified lymphocytes and antisera along with rabbit complement to generate a cytotoxic or positive reaction that can be visualized by staining. A standardized scoring system was developed to interpret the amount of cytotoxic cell death and allowed inter-laboratory comparison of antisera and test results, especially among laboratories that shared or exchanged antisera used to prepare serologic typing trays.

To perform this assay, viable lymphocytes of unknown HLA phenotype are added to test wells containing well-characterized reagent antisera diluted to optimal working dilution. A room temperature incubation is performed in the wells of the microtiter HLA typing tray. If antibodies present in the antisera recognize HLA antigens on the surface of the lymphocytes added to the test well, they bind and form antibody-antigen complexes.

The serum present in the test well does not contain sufficient complement reactivity to lead to this cytotoxic cell death. At the end of the cell-serum incubation, rabbit complement is added to the test well and a second room temperature incubation is performed. Components of the rabbit complement recognize the antibody-antigen complexes bound to the surface of the lymphocytes and bind. This initial binding leads to a proteolytic enzyme
cascade that cleaves the sequence of components of the complement system, leading to cell membrane damage and cell death. Rabbit complement is chosen with sufficient cytotoxicity to cause this membrane damage and cell death however, excessive cytotoxicity would lead to cell lysis. Cell lysis would prevent intact cells from being stained and visualized to determine the amount of cell death in the cytotoxicity assay so the complement activity is attenuated to prevent lysis.

At the end of rabbit complement incubation, the cells are stained with the addition of vital stains, such as eosin Y, the negative fluorescent stains, acridine orange (AO) or carboxyfluorescein diacetate (CFDA), and the positive stains, ethidium bromide (EB) or propidium iodide (PI). Since there is little present in test wells to sustain the live cells remaining after testing and no further cell death is desired in order to provide an accurate picture of the amount of cytotoxic cell death that occurs through testing, the cell suspension is preserved with the addition of fixative agents, such as formaldehyde or glutaraldehyde, sodium azide or ethylenediaminetetraacetic acid (EDTA). Stain/quench combination reagents are available that add fluorescent stains, a fixative agent and a quenching or background darkening agent such as bovine hemoglobin or India ink. Stain/quench reagents are ideal for use when lymphocytes are isolated using immunomagnetic beads.

Serologic HLA typing results are read on an inverted phase-contrast microscope. A bright-light system is used when eosin Y and formaldehyde/glutaraldehyde are used to stain and fix the cells. A fluorescent light system employing a mercury vapor lamp is used when fluorescent stains, fixative and quenching agents are added. Test wells are visualized and reaction scores are determined using a standardized scoring system based on the amount of cytotoxic cell death. Individual test wells are compared against the reactivity seen in the negative control to confirm negative or positive reactivity. The positive control is reviewed to confirm an acceptable level of expected cytotoxic positive reactivity was seen in testing. Clusters of positive reactions are examined to determine which corresponding positive antibody specificities may identify the unknown HLA antigens.
Section 4: Methodologies and Instrumentation

A. Samples:
1. Whole blood samples collected in the anticoagulant, acid-citrate-dextrose (ACD-A, yellow top tubes) or heparin (green top tubes) are used to isolate lymphocytes for serologic HLA typing.
2. Samples should be processed with 24-48 hours of collecting since one of the key components of this assay is the availability of excellent cell viability, usually greater than 80% of the cells present in the sample.
3. Lymphocytes are separated from whole blood by commonly-used methods, such as density-gradient centrifugation using a separation medium or by the use of immunomagnetic beads.
   a. Density-gradient centrifugation will provide a cell suspension with a mixed population of T and B lymphocytes, which are acceptable for use in serologic Class I typing since both T and B cells express HLA Class I molecules on their surface. Separate T and B cell suspensions can be prepared from a mixed cell population using monoclonal antibody preparations specific for one type of cell or the other.
   b. The use of immunomagnetic beads is helpful to obtain isolated T cell and B cell suspensions, allowing the T cells to be used to perform serologic HLA Class I typing and B cells to be used to perform serologic HLA Class II typing. T cells are not used to perform serologic HLA Class II typing since they do not express HLA Class II molecules on their surface.
4. Prior to test setup, a pre-plating viability check must be performed to confirm acceptable cell viability prior to testing.

B. Microsyringes:
1. Test setup is performed using microsyringes, which as specially designed pipettors that accurately dispense microliter (ul) volumes of cells, antisera and other test reagents.
2. Single-channel microsyringes are designed for use when limited or very small volumes are available. Since these syringes dispense one volume at a time and are somewhat labor-intensive to work with, they are more useful when plating a smaller amount of testing.
3. Multi-channel microsyringes are designed for larger volumes and workloads. 6- or 8-channel microsyringes are available in many different dispensing volumes, including 1 ul, 2 ul and 5 ul per channel. These syringes are ideal for routine test setup involving dispensing cell suspensions, antisera, rabbit complement and other reagents used in the serologic HLA typing.

C. Commercial vs. In-House Prepared Serologic Typing Trays:
1. The selection of serologic HLA typing trays used is a matter of choice based on experience of the histocompatibility laboratory.
   a. Some laboratories prefer to use typing trays from a single vendor, others may choose trays from several different vendors that
complement each other and resolve difficult HLA antigen combinations.

b. Some laboratories prefer to use single typing trays vs. typing tray sets of two or more trays.

c. Budget issues are also a concern for the laboratory with a range from relatively good, inexpensive trays up to very extensive but expensive two-tray sets, with and without rabbit complement.

2. Commercially-prepared typing trays are most commonly used by laboratories that perform serologic HLA typing however, some laboratories may choose to prepare in-house or home-brewed HLA typing trays.

a. Preparing in-house trays can be less expensive despite being a labor-intensive process that requires the availability and maintenance of a frozen HLA antisera inventory, testing with the lot of rabbit complement in use to determine acceptable working dilutions and definition of antibody specificities.

b. Preparation of in-house typing trays does provide the laboratory with a total control over their process from designing test trays, the choice of antisera used and its working dilution, the antigen specificities defined by the trays and the arrangement of the antisera and controls.

c. The selection of antisera for serologic typing trays can be like putting together a puzzle to insure that as many specificities as possible can be defined within a specific number of tray wells.

i. Combinations of operationally monospecific and multi-specific antisera are determined to provide a sufficient number of antisera to define both Class I and II antigens.

ii. Negative and positive controls are important on both trays.

iii. Cell-specific positive controls may be incorporated onto the Class II typing tray to improve the detection of contaminating cells that can interfere with reading and interpretation of test results.

iv. Since many Class II antisera also have Class I antibody reactivity, antisera for placement on Class II typing trays should be adsorb with platelets to remove Class I antibodies. A T cell positive control is important on Class II typing trays to identify when an insufficient amount of B cells and too many T cells were isolated in the B cell suspension used for Class II typing.
Section 5: Click here to link to the web based ASHI U Procedure Manual
Section 6: Frequently Asked Questions

1. Should serologic typing trays be purchased with or without rabbit complement?

This is entirely up to the laboratory’s preferences. Some laboratories like to purchase bulk rabbit complement that can be used for all serologic testing: HLA typing, antibody testing and crossmatches. This can provide a baseline of reactivity for all serologic testing in order to compare reactivity.

If separate rabbit complement is used:

a. It is important to extensively evaluate the typing trays prior to use to confirm the proper working dilution of the complement.

b. It is also important to monitor that the specificities listed on the typing tray worksheets from the manufacturer provide similar reactivity to that seen with the lot of rabbit complement. It may be important to monitor and document differences in antibody reactivity seen to insure the proper HLA antigens are being identified and reported.

c. It is also important to recognize that if Class I and II typings are being performed, T cell complement has different reactivity than B cell complement and the proper type of complement needs to be maintained for both types of trays.

Commercially manufacturers extensively test the antisera present on their typing trays to identify specificities, determine working dilutions and confirm the function of their product prior to release to laboratories. The rabbit complement included with the typing trays is normally complement that has been extensively evaluated with the trays during quality control.

2. What is the best way to choose a typing tray for use in serologic HLA typing?

This is also up to the laboratory’s preference. The types of things that should be kept in mind when selecting a typing tray are:

a. What is the typing being used for?

   i. For important clinical purposes, such as testing for transplantation or transfusion, it may be important to work with one or more reliable trays or tray set to insure proper antigen identification in a timely manner.

   ii. If it is being used for disease association testing, such as B27, it is important to insure there are a sufficient number of antisera to define the desired specificity, without significant overlap or crossreactivity that will interfere with interpretation or necessitate additional testing. It may
be helpful to have a tray that is specifically designed for this type of testing.

b. What are the racial backgrounds of the majority of the samples being tested?

i. If the racial background has a high prevalence of Asian, Black, Hispanic, etc., it may be important to select a typing tray that is able to identify antigens less commonly expressed in the general population, such as A36 and A74 in Blacks, B54 and B46 in Asians and less common alleles of antigens such as B38 and B39 in Hispanics.

c. Prior experience with a specific vendor may provide information to help with the decision and many manufacturers are happy to provide as much help with the decision-making process as necessary.
Section 7: Applicable ASHI Standards

The following ASHI Standards for Accredited Laboratories (2013) apply to the reading and scoring of serologic test reactions:

C. Proficiency Testing

C.1 Enrollment, Testing and Evaluation of Samples
C.1.1 For each analyte or test method reported and for which the laboratory is ASHI-accredited, the laboratory must participate in proficiency testing. The laboratory must satisfy the first in the following sequence of proficiency testing requirements that is available.
   C.1.1.1 Participate in at least one graded external proficiency testing program that is approved by CMS for CMS-regulated analytes tested in CLIA-certified laboratories, or approved by the ASHI Accreditation Review Board for non-regulated analytes.
C.1.2 Laboratories performing proficiency testing must not engage in any inter-laboratory communications pertaining to the results of proficiency testing sample(s) until after the reporting deadline has passed. This includes situations in which one Director oversees multiple laboratories.
C.1.3 Laboratories must not send their proficiency testing results or their proficiency testing samples to another laboratory for analysis.
C.1.4 Proficiency test samples must be:
   C.1.4.1 incorporated into the regular workload
   C.1.4.2 tested in a manner comparable to, and not more extensively than, routine clinical samples
   C.1.4.3 rotated among all testing personnel
C.1.5 The laboratory must document the handling, preparation, processing, examination, and each step in the testing and reporting of results for all proficiency testing samples. A copy of all records related to proficiency testing must be retained by the laboratory for a minimum of two years.

D. Quality Systems

D.1 Introduction

D.1.1 Each laboratory that performs testing must establish and maintain written policies and procedures that implement and monitor a quality system for all phases of the total testing process (that is, preanalytic, analytic, and postanalytic) as well as for general laboratory systems.
D.1.2 The laboratory’s quality systems must include a quality assessment component that ensures continuous improvement of the laboratory’s performance and services through ongoing monitoring that identifies, evaluates and resolves problems. This component must include revision of policies and procedures necessary to prevent recurrence of problems, and documented discussion of assessment review results with appropriate staff.

D.2.6 Personnel technical competency assessment
D.2.6.1 The Laboratory Director, Technical Supervisor or designee must establish and follow written policies and procedures to assess and document technical competency of staff and, if applicable, consultant competency at least annually.
D.2.6.2 The Laboratory Director, Technical Supervisor or designee must document the performance of individuals responsible for testing patient specimens.

D.2.6.2.1 at least semiannually during the first year.

D.2.6.2.2 at least annually thereafter.

D.2.6.2.3 whenever test methodology or instrumentation changes.

D.2.6.3 The Laboratory Director, Technical Supervisor or designee must periodically give each individual who performs clinical tests a specimen with characterized analytes designated as an Unknown to verify his or her ability to reproduce test results for those analytes. The laboratory must maintain records of these results for each individual for a minimum of two years. At least once per year, each individual must test an Unknown for each clinical test that he/she performs.

D.2.6.4 The evaluation must include documentation of competency to include the following as applicable:

D.2.6.4.1 Direct observations of routine test performance, including sample preparation, specimen handling, processing and testing.

D.2.6.4.2 Monitoring of the recording, interpretation and reporting of test results.

D.2.6.4.3 Review of quality control records, proficiency testing results, and preventative maintenance records.

D.4.1.3 Reagents

The laboratory must define and follow criteria that are essential for proper storage of reagents for accurate and reliable test system operation. The criteria must be consistent with manufacturer’s instructions and recommendations, if provided. These conditions must be monitored and documented and, if applicable, include the following: (1) Water quality, (2) Temperature, (3) Humidity, (4) Protection of equipment and instruments from fluctuations and interruptions in electrical current that adversely affect patient test results and test reports.

D.4.1.3.1 Reagents, solutions, culture media, control materials, calibration materials, and other supplies, as appropriate, must be labeled to indicate the following:

D.4.1.3.1.1 Identity and when significant, titer, strength or concentration

D.4.1.3.1.2 Storage requirements

D.4.1.3.1.3 Preparation dates and expiration dates where applicable.

D.4.1.3.1.4 National Fire Protective Agency (NFPA) codes [Health, Flammability and Reactivity] or non-USA equivalent

D.4.1.3.1.5 Other pertinent information required for proper use.

D.4.1.3.2 Reagents, water, solutions, culture media, control materials, calibration materials, and other supplies whether commercially purchased or prepared in-house must not be used when they have exceeded their expiration date, have deteriorated or are of substandard quality.

D.4.1.3.3 There must be a documented system in place for identifying which lots and shipments of reagents were used for each assay.

D.4.1.3.4 Reagents received from the manufacturer without a specified expiration date must be subject to quality control protocols to determine an appropriate expiration date that ensure optimum performance.
D.4.1.3.5 Prior to reporting results obtained with new lots or shipments of reagents, satisfactory performance must be verified and documented.

D.4.1.3.6 Components of reagent kits of different lot numbers must not be interchanged unless otherwise specified by the manufacturer.

D.4.1.3.7 If commercial kits are used, the manufacturer’s instructions must be followed unless the laboratory has performed and documented validation testing to support a deviation in technique or analysis.

D.4.1.3.8 In-house reagent sera inventory must indicate source, bleeding date and identification number, reagent specificity, and volume remaining.

D.4.1.3.9 The laboratory must validate the specificity of locally procured human reagent sera and monoclonal antibodies prepared in-house using the same method employed for routine clinical testing in the laboratory. The cell control panel used for specificity validation must include cells known to express the specified antigen, cells negative for the specified antigen and cells known to express crossreacting antigens.

D.4.1.3.10 The laboratory must validate the specificity of locally procured human reagent sera and monoclonal antibodies using appropriate control cells. Subsequent quality control may consist of testing in parallel with previous lots.

D.4.1.4 Computer Programs

D.4.1.4.1 All computer software programs and version upgrades used for analyses must be validated for accuracy and this validation documented, prior to release of test results.

D.4.1.4.2 The laboratory must have an ongoing process (at least annually) to ensure that all computer-assisted analyses are accurate.

D.4.1.5 Methods Validation

D.4.1.5.3 Each laboratory that modifies an FDA-cleared or approved test system, or introduces a test system not subject to FDA clearance or approval (including methods developed in-house and standardized methods such as text book procedures) or uses a test system in which performance specifications are not provided by the manufacturer must, before reporting patient test results, establish for each test system the performance specifications for the following performance characteristics, as applicable:

D.4.1.5.3.1 Accuracy.

D.4.1.5.3.2 Precision.

D.4.1.5.3.3 Analytical sensitivity.

D.4.1.5.3.4 Analytical specificity including interfering substances

D.4.1.5.3.5 Reportable range of test results for the test system

D.4.1.5.3.6 Reference intervals (normal values)

D.4.1.5.3.7 Any other performance characteristic required for test performance

D.4.1.5.4 The laboratory must determine the test system’s calibration procedures and control procedures based upon the performance specifications.

D.4.1.5.5 The laboratory must document that any modifications to an existing procedure do not adversely alter the performance characteristics of the assay.

D.4.1.7 Instrument calibration and calibration verification procedures
D.4.1.7.1 For each applicable testing procedure which requires equipment to provide a quantitative measurement, the laboratory must perform and document instrument calibration procedures. These calibration procedures must:

D.4.1.7.1.1 Follow the manufacturer’s test system instructions, when provided.

D.4.1.7.1.2 Use calibration materials provided or specified as appropriate for the test system and, if possible, traceable to a reference method or reference material of known value.

D.4.1.7.1.3 Be performed with at least the frequency recommended by the manufacturer.

D.4.1.7.1.4 Use the criteria verified or established by the laboratory during validation.

D.4.1.7.1.5 Include the number, type, and concentration of calibration materials, as well as acceptable limits for and the frequency of calibration as established by the lab.

D.4.1.7.1.6 Require repeat calibration and documentation if verification fails to meet acceptable limits.

D.4.1.7.2 Calibration verification procedures must:

D.4.1.7.2.1 Be performed following manufacturer’s calibration instructions, when provided.

D.4.1.7.2.2 Meet the criteria verified or established by the laboratory, including the number, type and concentration of the materials, as well as acceptable limits for calibration verification.

D.4.1.7.2.3 Include at least a minimal (or zero) value, a mid-point value, and a maximum value near the upper limit of the range to verify the laboratory’s reportable range of test results for the test system.

D.4.1.7.2.4 Be performed at least once every 6 months and whenever any of the following occur:

D.4.1.7.2.4.1 A complete change of reagents for a procedure is introduced, unless the laboratory can demonstrate that changing reagent lot numbers does not affect the range used to report patient test results, and control values are not adversely affected by reagent lot number changes.

D.4.1.7.2.4.2 There is major preventive maintenance or replacement of critical parts that may influence test performance.

D.4.1.7.2.4.3 Control materials reflect an unusual trend or shift, or are outside of the laboratory’s acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem.

D.4.1.7.2.4.4 The laboratory’s established schedule for verifying the reportable range for patient test results requires more frequent calibration verification.

D.4.1.7.3 For volumetric dispensers such as Hamilton syringes which cannot be calibrated, volume dispensed must be verified and documented every six months.

D.4.1.8 Control Procedures
D.4.1.8.1 For each test system, the laboratory must have control procedures that monitor the accuracy and precision of the complete analytical process.

D.4.1.8.2 The laboratory must establish the number, type and frequency of testing control materials using, if applicable, the performance specifications verified or established by the laboratory.

D.4.1.8.3 The control procedures must:
   D.4.1.8.3.1 Detect immediate errors that occur due to test system failure, adverse environmental conditions, and operator performance.
   D.4.1.8.3.2 Monitor over time the accuracy and precision of test performance that may be influenced by changes in test system performance, environmental conditions, and variance in operator performance.

D.4.1.8.4 The laboratory must:
   D.4.1.8.4.1 For each test system, perform control procedures using the number and frequency specified by the manufacturer or established by the laboratory when they meet or exceed the requirements of this section.
   D.4.1.8.4.2 Perform the following at least once each day that specimens are assayed or examined:
      D.4.1.8.4.2.1 For each quantitative procedure, include two control materials of different concentrations.
      D.4.1.8.4.2.2 For each qualitative procedure, include a negative and positive control material

D.5 Applications and Test Systems
D.5.1 General Standards
   D.5.1.1 Test Systems
      D.5.1.1.1 Test systems selected by the laboratory must be performed:
         D.5.1.1.1.1 following the manufacturer’s instructions or as modified and validated by the laboratory and/or
         D.5.1.1.1.2 as developed and validated by the laboratory and
         D.5.1.1.1.3 in a manner that provides test results that are within the laboratory’s stated performance specifications for each test system.

   D.5.1.2 Evaluation of Test Systems
      D.5.1.2.1 The laboratory must have a system to identify, assess, and document patient test results that appear inconsistent with the following relevant criteria, when applicable:
         D.5.1.2.1 Patient age
         D.5.1.2.2 Sex
         D.5.1.2.3 Diagnosis or pertinent clinical data
         D.5.1.2.4 Distribution of patient test results
         D.5.1.2.5 Relationship with other test results

D.5.2 Methodology Standards
   D.5.2.1 Laboratories performing microcytotoxicity assays must:
D.5.2.1.1 Employ method for cell preparation that yields sufficient cells that meet or exceed the laboratory’s established criteria for purity and viability to ensure accurate test results.

D.5.2.1.2 Ensure that the typing reagents have appropriate specificity and that the complement has appropriate reactivity.
   D.5.2.1.2.1 Test each lot and/or shipment of complement 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.
   D.5.2.1.2.2 Test complement separately 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.

D.5.2.1.3 Run positive and negative controls for each cell preparation and on each tray.

D.5.2.1.4 When performing assays with B lymphocyte-enriched preparations, include a positive control for B cells and document the proportion of B lymphocytes in each preparation and that the purity is sufficient to ensure accurate interpretation of results.

D.5.2.1.5 Include at least one positive control serum known to react with all cells expressing the class of antigens being tested.

D.5.2.1.6 Document that the cell viability in the negative control is sufficient to ensure accurate interpretation of results.

D.5.2.1.7 Record the result of each cell-serum combination in a manner that indicates the approximate percentage of cells killed. Use of the numerical scores in the latest edition of the ASHI Laboratory Manual is recommended.

D.5.2.6 Laboratories performing HLA typing must:
   D.5.2.6.1 Ensure that the level of resolution of HLA typing is appropriate for the clinical application and is based on established criteria.
   D.5.2.6.2 Have written criteria or protocols for:
      D.5.2.6.2.1 Preparation of cells or cellular component isolations (for example, solubilized antigens and nucleic acids), as applicable to the HLA typing technique(s) performed.
      D.5.2.6.2.2 Selection, quality control, and usage of all typing reagents and components.
      D.5.2.6.2.3 The assignment of HLA antigens and alleles and for distinguishing common null alleles as appropriate for the clinical use of the test results.
      D.5.2.6.2.4 Determining when antigen or allele redefinition and retyping are required.
      D.5.2.6.2.5 Assignment of haplotypes, if reported:
         D.5.2.6.2.5.1 If haplotypes are assigned based upon population frequencies, this must be clearly indicated on the report and relevant references or sources must be stated.
         D.5.2.6.2.5.2 Reports must include an explanation of recombination when this occurs.
D.5.2.6.3 Ensure that typing for class I or class II antigens or alleles employs a sufficient number of antisera, monoclonal antibodies, and/or DNA markers to clearly define all antigens/alleles for which the laboratory tests.

D.5.2.6.4 Use HLA typing terminology that conforms to the latest report of the World Health Organization (W.H.O.) Nomenclature Committee for factors of the HLA System. Potential new antigens and/or alleles not yet approved by this committee must have a designation that cannot be confused with W.H.O. terminology.

D.5.3.2 Renal and/or pancreas transplantation

D.5.3.2.1 Laboratories performing testing for renal transplantation must:

D.5.3.2.1.1 Prospectively type donor and transplant candidates for HLA-A, -B and –DR. It is highly recommended that laboratories also type for Bw4/w6, -C, -DQ, DR51, 52, and 53, and DP.

D.5.3.2.2 Laboratories performing testing for renal and/or pancreas transplantation from deceased donors must also:

D.5.3.2.2.1 Prospectively type donor and transplant candidate for HLA-Bw4/w6.

D.5.3.3 Blood, Bone Marrow and Stem Cell Transplantation

D.5.3.3.1 Laboratories performing testing for blood, bone marrow and stem cell transplantation must:

D.5.3.3.1.1 Perform HLA typing at a level of resolution and including the loci that are required by the hematopoietic stem cell donor registry and/or the Transplant Program.

D.5.3.6 Platelet and Granulocyte Transfusion

D.5.3.6.1 Laboratories performing testing for platelet and granulocyte transfusion support must:

D.5.3.6.1.1 Type the recipient, and potential transfusion donor, if applicable, for HLA-A and –B antigens.

D.5.3.7 Disease Risk, Drug Hypersensitivity Reaction Risk and Vaccine Eligibility Assessment

D.5.3.7.1 Laboratories performing HLA typing for disease risk/drug hypersensitivity risk and/or vaccine eligibility assessment must perform HLA typing at the appropriate level of resolution for HLA antigens.

D.5.3.8 Parentage/relationship testing

D.5.3.8.3 Laboratories performing parentage/relationship testing by microcytotoxicity must:

D.5.3.8.3.1 Conform to all relevant Standards for microcytotoxicity

D.5.3.8.3.2 Plate each test sample on two separate trays or tray sets each containing a minimum of one monospecific or two multispecific sera defining each HLA-A and –B locus antigen tested.

D.5.3.8.3.3 Read each tray or tray set independently

The ASHI standards sited in this document are from the version noted at the top of the page. Revisions to the ASHI standards may have taken place since the development of this document. Laboratories and individuals should always reference the most current ASHI-approved standards.
Section 8: Literature Cited / Recommended Reading

Recommended Reading:


Standards for Accredited Laboratories. American Society of Histocompatibility and Immunogenetics (ASHI), Current Edition


References:


Chapter 2. Reagents, Equipment and Cell Preparation
Chapter 3. Lymphocytotoxicity Test for Typing
Chapter 4. HLA Crossmatching
Chapter 6. Serum Analysis

