Statements of Competence for Histocompatibility Personnel

Revised 1998

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This document addresses all levels of competence for Histocompatibility personnel. The designation of "SPEC" to the left of a competence statement indicates the Histocompatibility Specialist level only. The absence of a designation indicates that the statement is appropriate for all levels. Certification levels will be determined by test item difficulty.

1. Histocompatibility Testing Request – Specimen Collection
   - 1.A Provide test request information to outside sources:
     - 1.A.1 Understand types of specimens appropriate for requested test.
     - 1.A.2 Understand collection specifics: appropriate container(s), aseptic technique, proper anticoagulants/ preservatives.
     - 1.A.3 Understand proper labelling procedures as specified in laboratory procedure manual.
     - 1.A.4 Understand time and temperature limitations for transport, proper packaging and labelling for safe transport.
     - 1.A.5 Understand appropriate sources for verification of request: written or electronic request from authorized source (e.g., M.D., legal council).
   - 1.B Inspect specimens received for testing:
     - 1.B.1 Verify for completeness and accuracy of requisition.
     - 1.B.2 Check for proper patient identification, volume, appropriate anticoagulant, date of collection.
     - 1.B.3 Use universal precautions when handling specimens.
     - 1.B.4 Assess appropriateness of specimen for test requested (e.g., serum for antibody screening).
     - 1.B.5 Assess quality of specimens submitted, e.g., clotting, hemolysis, age of specimen, adverse storage or shipping condition; request new specimen if original is unacceptable.
     - 1.B.6 Identify and log specimens into laboratory records.
     - 1.B.7 Prioritize testing by understanding the necessity for and performance of STAT procedures in appropriate instances.
     - 1.B.8 Follow legal and institutional requirements regarding security and confidentiality of patient/client records.
     - SPEC 1.B.9 Assess the need for referral services as appropriate to limitations of the laboratory resources and/or priorities.
   - 1.C Venipuncture
     - 1.C.1 Determine volume appropriate for testing.
     - 1.C.2 Determine appropriate anticoagulant/preservative to maintain viability and preserve antigens and distribution of markers/characteristics of cells to be tested.
     - 1.C.3 Label specimens as specified in laboratory procedure manual.
     - 1.C.4 Draw specimens using aseptic techniques and conform to guidelines in handling potentially biohazardous specimens.
     - 1.C.5 When appropriate, obtain informed consent from donor prior to venipuncture.
     - SPEC 1.C.6 Recognize and manage adverse reactions.

2. Test Principles
   - 2.A Serological assays:
     - 2.A.1 Understand structure and nature of the HLA antigens, their tissue distribution and expression on different cell types, soluble HLA antigens, and interactions with HLA antibodies.
     - 2.A.2 Understand structure and nature of monoclonal and polyclonal HLA antibodies, their sources and how they interact with HLA antigens; know principles of alloimmunizations through pregnancy , blood transfusions and prior transplantation.
     - 2.A.3 Understand structure and nature of immunoglobulins and reducing reagents.
     - 2.A.4 Understand nature, source, action and instability of complement; recognize sources of error encountered with the use of complement in a test system.
     - 2.A.5 Understand theory of complement dependent reactions.
     - 2.A.6 Understand theory and use of vital stains in the assessment of cell injury.
     - 2.A.7 Understand use of technical variations in methodology to alter sensitivity of assay.
     - 2.A.8 Understand use of trouble-shooting procedures to overcome contamination problems encountered in assay.
     - 2.A.9 Understand use of fluorescent stains in various methodologies.
   - 2.B Cellular assays:
     - 2.B.1 Understand theory of the mixed leukocyte reaction (MLR) test (e.g., inactivation of stimulator cells, use of radioactive isotopes to measure cellular proliferation, kinetics of MLR reactions, scintillation counting, stimulatory determinants leading to cellular activation, clinical applications of the MLR as a "crossmatch" assay).
     - 2.B.2 Understand functional properties of the participating cells in MLR tests.
     - 2.B.3 Understand basic cell culture techniques, including knowledge of cell growth requirements.
     - SPEC 2.B.4 Understand theory of HLA-Dw typing utilizing HLA-Dw homozygous typing cells (HTC), including HTC selection.
     - SPEC 2.B.5 Understand theory of primed lymphocyte (secondary) MLR responses including kinetics.
     - SPEC 2.B.6 Understand theory of cell mediated lympholysis (CML) assays, including participating cell types, target antigens, killer cells vs. natural killer cells, target cell labelling, target cell lysis and gamma counting.
     - SPEC 2.B.7 Understand theory of lymphocyte transformation assays, including cell types involved, kinetics, and the use of non-specific agents, e.g., PHA, PWM, ConA and specific antigens (e.g., soluble antigens).
     - SPEC 2.B.8 Understand theory for establishment of EBV transformed B-cell lines, establishment of clones and specific cell growth requirements.
   - 2.C Flow Cytometry Assays:
     - 2.C.1 Understand the basic principles of flow cytometry including the fluidics system, light source, sensors (i.e., PMT) and excitation/emission spectra of the more commonly used fluorochromes, e.g., fluorescein and phycoerythrin.
     - 2.C.2 Understand the principles of cell surface marker analysis including methods of sample preparation, cell staining and direct and indirect immunofluorescence.
     - 2.C.3 Understand cellular properties (e.g., size and cytoplasmic granularity), in relation to light scatter and the concept of "gating" on particular cell populations.
     - 2.C.4 Understand theory of non-complement binding antibodies vs. complement-dependent antibodies, and the use of flow cytometry to detect them.
   - 2.D Molecular Biology Assays:
     - 2.D.1 Understand the basics of molecular biology, including DNA structure, the genetic code, replication, transcription and translation.
     - 2.D.2 Understand principles of methods used to amplify a target sequence of DNA, e.g., polymerase chain reaction (PCR), ligase chain reaction amplification.
     - 2.D.3 Understand principles of sequence specific oligonucleotide probe techniques, including the theory of hybridization, stringency requirements, nature of the probes utilized (unique, shared), probe design and application of the techniques, e.g., SSOPH, reverse SSOPH.
     - 2.D.4 Understand principles of sequence specific priming (SSP) techniques, including primer design and the need for internal control primers.
     - 2.D.5 Understand theory of restriction fragment length polymorphism (RFLP) technique, including DNA extraction, endonuclease digestions, applications for HLA and non-HLA polymorphisms.
     - 2.D.6 Understand principles of sequence specific conformational polymorphisms (SSCP) and its use in HLA typing.
     - 2.D.7 Understand basic principles of direct DNA sequencing and gene cloning techniques.
   - SPEC 2.E Enzyme Linked Immunoadsorbant Assays (ELISA):
     - 2.E.1 Understand basic principles of ELISA testing, including antibody-antigen interactions, specificity, kinetic properties and catalytic power of enzymes, and use of labelled enzymes to indicate antigen-antibody binding.

2.E.2 Understand types and sources of antigens that can be used in ELISA testing, and their effects on testing.
2.E.3 Understand methods of antigen isolation and antigen adherence to plates used in ELISA testing; understand effects of solid-phase immobilization of antigen that may affect enzyme activity.

3. Methodology
3.A Cell preparation:
3.A.1 Select appropriate isolation technique for desired target cells; maintain sterility during procedure when required.
3.A.2 Understand the properties of cells which influence isolation (e.g., cell density, surface immunoglobulin, adherence, cell surface receptors).
3.A.3 Recognize and eliminate/neutralize components which may interfere with an assay, (e.g., RBC, platelets, PMNs, anticoagulants).
3.A.4 Determine acceptability of target cell preparations, (e.g., viability staining, purity assessment).
3.A.5 Perform accurate cell counts and calculations.
3.A.6 Cryopreserve and thaw specimens appropriately to ensure cell function and integrity.
3.B Serum preparation:
3.B.1 Obtain, aliquot and store serum properly to maintain biologic activity.
3.B.2 Prepare serum for testing (e.g., use of reducing reagents, cell adsorption or concentration).
3.B.3 Include appropriate controls when preparing patient serum screening assays.
3.C Serological assays:
3.C.1 Understand differences in methods used for serological detection of class I and class II HLA antigens (e.g., direct cytotoxicity assays, direct/indirect fluorescence).
3.C.1.1 Select appropriate reagents according to regulatory guidelines for class I and class II HLA antigen identification; include appropriate controls.
3.C.1.2 Perform test according to established procedures regarding cell purity, addition of reagents, complement, wash steps, incubation times, temperature, vital staining.
3.C.1.3 Read and record results utilizing consistent scoring criteria as per ASHI standards.
3.C.1.4 Assess test for potential problems, including viability, carryover, false positive and false negative reactivity.
3.C.2 Understand differences in serological methods (e.g., direct cytotoxicity assays, antiglobulin-augmentation) used to detect lymphocytotoxic antibodies.
3.C.2.1 Select appropriate screening or crossmatch method (e.g., to detect HLA vs. non-HLA antibodies, IgG vs. IgM isotype, T- vs. B-cell specific) and perform test according to established procedures regarding addition of reagents, complement, wash steps, incubation times, temperature, vital staining.
3.C.2.2 Select appropriate patient specimens for testing based on laboratory's established protocol, taking into account historical PRA information and sensitization events.
3.C.2.3 Select appropriate target cells, taking into account type of antibody to be detected, panel size, frequency of HLA antigens and linkage disequilibrium.
3.C.2.4 For patients awaiting renal transplantation, determine frequency of antibody screening testing.
3.C.2.5 Read and record results utilizing consistent scoring criteria as per ASHI standards.
3.D Cellular assays:
3.D.1 Utilize appropriate techniques to prepare cellular assays for clinical and research applications.
3.D.1.1 Utilize appropriate responder cells and inactivated stimulator cells.
3.D.1.2 Select appropriate control combinations.
3.D.1.3 Pipette cell mixtures into appropriate culture vessels according to established procedures and experimental design.
3.D.1.4 Incubate cultures, utilizing correct CO_2 level, incubator temperature and humidity and total incubation time.
3.D.1.5 Label cultures with appropriate radioisotope, considering type, amount and specific activity of isotope and duration and timing of pulse.
3.D.1.6 Harvest cultures according to established procedure.
3.D.1.7 Carry out scintillation counting procedure, utilizing appropriate scintillation fluid and counting time.
3.E Flow cytometry assays:
3.E.1 Perform appropriate methods (e.g., for immunophenotyping, HLA antigen identification, antibody screening, crossmatching), according to established procedures regarding optimum cell concentration, serum/cell ratio, wash steps, incubation times.
3.E.1.1 Utilize appropriate reagents/fluorescent stains.
3.E.1.2 Utilize appropriate controls.
3.E.1.3 Select target cell panels to cover appropriate antigen specificities and/or major CREG groups.
3.E.1.4 Assess test for potential problems, including poor cell viability, high background fluorescence, autoantibodies, false positive/false negative reactions.
3.F. Molecular biology assays:
3.F.1 Determine the level of molecular typing required (low, medium or high resolution).
3.F.2 Verify specificity of test reagents.
3.F.3 Extract nucleic acid material using methods that have been referenced in scientific literature and validated in the laboratory.
3.F.3.1 Select appropriate isolation technique to provide sample of adequate quantity and quality for testing.
3.F.3.2 Assess sample for integrity, quantity and quality.
3.F.3.3 Prepare and store purified nucleic acid material appropriately; develop methods to validate integrity of sample under short- and long-term storage conditions.
3.F.3.4 Ensure that specimens are isolated from post-amplification work areas.
3.F.3.5 Understand effect of anticoagulants on extraction of nucleic acid material.
3.F.4 Perform nucleic acid amplification according to established methods.
3.F.4.1 Follow established guidelines for physical/biochemical barriers to prevent DNA contamination (e.g., dedicated work areas, supplies and equipment for pre- and post-amplification procedures).
3.F.4.2 Monitor nucleic acid contamination within each amplification assay.
3.F.4.3 Monitor quantity of specific amplification products by gel electrophoresis, hybridization with consensus probe, etc.
3.F.4.4 Include controls to detect amplification failure in every amplification mixture if presence of amplified product is used as end result (i.e., SSP method).
3.F.4.5 Understand how to optimize the PCR reaction to produce desired sensitivity and specificity required for testing.
3.F.5 Perform gel electrophoresis of nucleic acid material using established procedures:
3.F.5.1 Select appropriate-sized markers of known sequences that give discrete electrophoretic bands that span and flank entire range of system being tested.
3.F.5.2 Include known human control DNA when restriction endonucleases have been used.
3.F.5.3 Dispense equal amounts (mg/ml) of nucleic acid material into each lane.
3.F.5.4 Optimize concentration of materials in gels used for detection of PCR products.

3.F.6 Perform appropriate established procedure(s) necessary for allele identification (e.g., typing by sequence specific primers (SSP), sequence specific oligonucleotide probes (SSOP), restriction fragment length polymorphism (RFLP), direct sequencing) regarding specificity of primers and probes, temperature, hybridization conditions, specificity and sensitivity of labelling and detection methods.
3.F.6.1 Utilize appropriate controls.
3.F.6.2 Assess test for potential problems, including contamination, cross-hybridization with closely-related alleles, amplification failure, false negative and false positive amplification, specification of acceptable limits of signal intensity for positive and negative results, weak internal control fragments and unusual amplification proteins.
3.F.7 Read and record results according to established guidelines.

SPEC 3.G ELISA:
3.G.1 Perform appropriate methods (e.g., for antibody screening or crossmatching), according to established procedures regarding reagent and serum concentrations, control sera, wash steps, incubation times, pH, temperature.
3.G.1.1 Select target cell panels to cover appropriate specificities.
3.G.1.2 Utilize appropriate controls.
3.G.1.3 Assess test for potential problems, including low or high background optical density.

4. Test Result Interpretation
4.A Serological assays
4.A.1 Interpret results of HLA class I and class II serological typing:
4.A.1.1 Review controls (e.g., positive, negative cell viability, complement) to evaluate validity of data; recognize the need to repeat an assay based on inappropriate control results.
4.A.1.2 Determine antigen assignments based on patterns of serologic reactivity from manual or computer generated data.
4.A.1.2.1 Recognize appropriate antigens using monospecific and multispecific antisera.
4.A.1.2.2 Recognize cross reactive groups for class I and class II HLA antigens.
4.A.1.2.3 Recognize split specificities.
4.A.1.2.4 Recognize common antigen combinations possibly due to linkage disequilibrium.
4.A.1.2.5 Understand and interpret reactivity patterns of “public determinants” which are shared among the “private” specificities, e.g., Bw4, Bw6, DR51,DR52, DR53.
4.A.1.3 Evaluate the validity of antigen assignments and assess the need to perform additional testing; resolve any ambiguities.
4.A.1.4 Assign haplotypes/genotypes, as appropriate in family studies; consider possibility of recombination.
4.A.2 Interpret crossmatch results:
4.A.2.1 Review controls to evaluate validity of data.
4.A.2.2 Evaluate need for further testing when appropriate, (e.g., poor viability, autoantibody, weak reactivity).
4.A.2.3 Review donor/recipient match and patient’s PRA and sensitization history in light of crossmatch results; resolve any ambiguities.
4.A.3 Interpret results of patient antibody screening:
4.A.3.1 Evaluate antibody screening data from manual or computer generated data considering linkage disequilibrium, crossreactivity of antigens and racial distribution of the panel used for testing.
4.A.3.2 Calculate panel reactive antibody (PRA).
4.A.3.3 Evaluate antibody specificity using statistical indices (e.g., chi square, correlation coefficient, tail analysis).
4.A.3.4 Review patient’s sensitization history in light of positive results.
4.A.4 Interpret parentage testing results:
4.A.4.1 Identify possible maternal and paternal haplotypes in the child.
4.A.4.2 Determine presence of exclusion, if any, and order, e.g., first, second order.
SPEC 4.A.4.3 Calculate relevant inclusionary values for the HLA system at various prior probability levels and determine the need for more testing.
4.A.5 Interpret compatibility of ABO test results.
4.A.6 Identify potential HLA-compatible non-solid organ donors:
4.A.6.1 Identify platelet donors based on patient HLA type, antibody screening results and knowledge of crossreactive antigen groups and “public” determinants.
4.A.6.2 Identify potential bone marrow donors based on patient HLA type.
4.B Cellular assays:
4.B.1 Evaluate the validity of test results, taking into account stimulation and response capacity and reproducibility of replicate cultures.
SPEC 4.B.2 Understand how to reduce and normalize MLR and mitogen assay test results to allow for analysis and interpretation.
SPEC 4.B.3 Determine subsequent course of action if patient cells, control cells and/or medium controls do not give acceptable values and implement appropriate corrections.
SPEC 4.B.4 MLR interpretation:
4.B.4.1 Review reactivity of all MLR combinations with respect to HLA-Dw, DR, DQ type and identify identical, similar and disparate combinations.
4.B.4.2 Determine haplotype segregation within families and review for possible recombination and for parental haplotypes that demonstrate shared stimulatory determinants.
4.C Flow cytometry assays:
4.C.1 Review controls to evaluate validity of data.
4.C.2 Establish criteria for reporting and evaluating test results based on laboratory’s established threshold for positive reactions.
4.C.3 Distinguish between calculations of linear vs. log amplification in determining fluorescence intensity.
4.C.4 Evaluate need for further testing when appropriate.
4.C.5 For crossmatching, review donor/recipient match, and patient PRA and sensitization history, in light of crossmatch results.
4.D Molecular biology assays:
4.D.1 Establish criteria for accepting or rejecting an amplification assay.
4.D.2 Establish criteria for identification of contamination of amplified nucleic acid material.
4.D.3 Establish acceptable limits of signal intensity for each primer mixture, probe and positive and negative controls; initiate corrective actions when these criteria are not met.
4.D.4 Evaluate controls and size markers, and determine if repeat testing is required.
4.D.5 Recognize primer-dimer products and differentiate bands based on size.
4.D.6 Use two independent interpretations of primary data whenever possible.
4.D.7 Establish procedures for assignment of types; be able to recognize reaction patterns that may have more than one possible interpretation.
4.D.8 Establish procedures for incorporating criteria for assignment of new alleles.
4.D.9 Determine appropriate high resolution testing to be performed based on low resolution results.
4.D.10 Identify and address discrepancies between molecular and serological testing results.
4.D.11 Recognize unusual antigen association patterns and initiate additional testing if needed.
4.D.12 Establish criteria for nucleotide assignments by direct sequencing methods.

4.D.12.1 Determine criteria for sequencing one or both DNA strands.
4.D.12.2 Determine criteria for sequencing of both DNA strands when unexpected test results are found.
4.D.13 Assign haplotypes/genotypes, as appropriate, in family studies; consider possiblity of recombination.
SPEC 4.E. ELISA:
4.E.1 Review controls to evaluate validity of data.
4.E.2 Define criteria for reporting and evaluating of test results based on laboratory's established threshold for positive reactions.
4.E.3 Evaluate need for further testing when appropriate.
4.F Reports:
4.F.1 Prepare technical reports for review and signature of director using appropriate recognized nomenclature (i.e., most recent WHO publication).
4.F.2 Report results appropriately to authorized personnel in emergency situations.
SPEC 4.F.3 Review results of testing and issue reports to appropriate personnel; follow regulatory guidelines for inclusion of minimum required information.
4.F.4 For parentage testing, determine legal recipient(s) of report and ascertain proper authorization for release of report.

5. Reagent Selection and Preparation
5.A Select and/or prepare reagents appropriate for serological assays:
5.A.1 Choose appropriate media (e.g., HBSS, PBS, RPMI, McCoys, etc.), with required additives and supplements (e.g., sera, antibiotics, antimycotics, buffers, heparin).
5.A.2 Prepare and/or standardize blood cell isolation gradients, e.g., ficoll-hypaque/isopaque and percoll.
5.A.3 Prepare control serum reagents utilizing appropriate methods of collection, screening, pooling, heat-inactivation, storage conditions and methods of plasma conversion.
5.A.4 Prepare cryopreservation solutions to correct concentration (e.g., DMSO).
5.A.5 Handle serum complement appropriately:
5.A.5.1 Determine for each lot/batch number the titer and strength of reactions against each type of target cells to be tested.
5.A.5.2 Follow precautions in the handling and storage of complement: aliquot size, maintenance temperature, thawing, dispensing, lability.
5.A.6 Handle antiglobulin reagent appropriately:
5.A.6.1 Determine for each lot/batch number the titer and strength of reactions against each type of target cells to be tested.
5.A.6.2 Follow precautions in the handling and storage of antiglobulin reagent: aliquot size, maintenance temperature, thawing, dispensing, lability.
5.A.7 Prepare vital stains for cell viability determination, e.g., trypan blue, eosin, ethidium bromide, etc.
5.B Identify HLA serological reagents:
5.B.1 Determine methods to obtain, preserve and inventory serum reagents.
5.B.2 Develop a well defined panel of cells, taking into account racial distribution, crossreactive antigens, antigen frequency and linkage disequilibrium.
5.B.3 Characterize serum with appropriate cells.
5.B.4 Understand and utilize methods for the identification of reagent grade sera using methods of serum analysis (e.g., chi square, tail analysis, correlation coefficient), with or without computer assistance.
5.B.5 Request confirmation of antibody specificity for locally procured serum reagents from at least one other laboratory whenever possible.
5.B.6 Perform testing to confirm specificity of serum reagents procured from other laboratories.
5.C Prepare reagents for cellular testing:
5.C.1 Choose appropriate media (e.g., HBSS, PBS, RPMI, McCoys, etc.), with required additives and supplements (e.g., sera, antibiotics, antimycotics, buffers, growth factors, heparin).
5.C.2 Prepare and/or standardize blood cell isolation gradients, e.g., ficoll-hypaque/isopaque and percoll.
5.C.3 Prepare control serum reagents utilizing appropriate methods of collection, screening, pooling, heat-inactivation, storage conditions and methods of plasma conversion.
5.C.4 Prepare a panel or pool of stimulating cells, selecting individual cell donors based on HLA type.
5.C.5 Prepare appropriate type of scintillation cocktail with attention to safe use and storage.
5.C.6 Prepare radioisotope labelling reagents (e.g., tritiated thymidine), with consideration of specific activity, concentration, appropriate storage, use and disposal.
5.D Select and/or prepare reagents appropriate for flow cytometry assays:
5.D.1 Prepare appropriate negative control serum/reagents.
5.D.2 Prepare appropriate positive control sera (pool of high PRA sera) and positive control reagents.
5.D.3 Verify specificity of monoclonal antibodies.
5.D.4 Select appropriate reagents/fluorescent stains specific for anti-human immunoglobulin isotypes and for cell surface markers.
5.D.5 Determine quantities of reagents to be used for each test sample.
5.E Prepare reagents for molecular assays:
5.E.1 Prepare and/or standardize reagents for nucleic acid extraction.
5.E.2 Define specificity and sequence of all primers used.
5.E.3 Prepare and/or standardize primer mixtures to achieve the defined specificity for template used in testing; store under conditions that maintain specificity and sensitivity.
5.E.4 Test each set of primers periodically for specificity, using reference nucleic acid material.
5.E.5 Prepare appropriate controls to detect technical failure and contamination with previously amplified products.
5.E.6 Aliquot reagents used for amplification procedures into volumes appropriate for single use unless materials have been documented to be free of contamination at each use.
5.E.7 Validate probes used in RFLP studies by family studies and population studies to demonstrate Mendelian inheritance of polymorphism detected.
5.E.8 Prepare and/or standardize size markers that reflect range of expected fragment sizes.
5.E.9 Define for each oligonucleotide probe and template the HLA locus and allele specificity.
SPEC 5.F Prepare reagents for ELISA:
5.F.1 Prepare reagent controls.
5.F.2 Prepare positive control sera from pooled highly alloimmunized individuals, ensuring that the antibodies are of the appropriate isotype.
5.F.3 Prepare serum from non-alloimmunized human donor(s) for use as negative control.

6. Instrumentation and Equipment
6.A Select equipment and instruments appropriate to specific testing.
6.A.1 Calibrate instruments and equipment by applying criteria established by the laboratory, manufacturer and/or accrediting and regulatory agencies.
6.A.1.1 Calibrate flow cytometer.
6.A.1.1.1 Use standards for each fluorochrome.
6.A.1.1.2 Compensate machine for "spill-over" of the fluorochrome signal.
SPEC 6.A.1.1.3 Focus and align the laser.
6.A.1.1.4 Establish threshold values for acceptable optical standardization for all relevant signals.
6.A.1.2 For nucleic acid amplification, set number of cycles for amplification at a level sufficient to detect target nucleic acid but insufficient to detect small amounts of minor contaminants or occurrence of stochastic fluctuation.
6.A.1.3 Monitor temperature of thermal cycling instruments used for DNA amplification.
SPEC 6.A.1.4 Perform periodic calibration of ELISA reader.
6.A.1.4.1 Verify that the light source produces intensity and wavelength of light required for the test system.
6.A.1.4.2 Verify that movement of plate reader is precise.

6.A.1.5 Perform periodic performance checks on microplate washer.

6.A.2 Operate equipment and instruments utilizing established protocols and in accordance with criteria established by manufacturers and/or accrediting and regulatory agencies.

6.A.3 Conduct and document validation and preventive maintenance programs for all instruments and equipment.

6.A.4 Evaluate equipment and instrument performance at prescribed intervals; recognize malfunctions, initiate and document corrective actions.

6.A.5 Adjust and/or repair simple instruments and equipment; obtain external services when appropriate.

6.A.6 Initiate contingency plan when malfunctions occur (e.g., power or equipment failure).

6.A.7 Monitor and document operating conditions (e.g., temperature, CO_2 levels, liquid nitrogen levels).

7. Quality Assurance

7.A Reagents/Equipment

7.A.1 Develop and maintain quality control procedures on equipment, instruments, reagents and products.

SPEC 7.A.2 Develop corrective action plan on quality control procedures; implement corrective action when necessary.

7.A.3 Choose and standardize controls appropriate to the requirements of the assay in use; maintain records in accordance with regulations.

7.A.4 Maintain a reagent log and control system:

7.A.4.1 Record data on purchased chemicals, biologicals, radionucleotides.

7.A.4.2 Label reagents with name, strength, titer or concentration, date prepared/received/opened, storage requirements, expiration date.

7.A.4.3 Store, handle and dispose of all reagents properly.

7.A.4.4 Maintain log of material safety data sheets (MSDS) and manufacturers' product inserts.

7.A.5 Evaluate and choose biologicals and chemicals conforming to purity, sensitivity, specificity, potency, sterility and stability required for the intended analysis.

7.A.6 Assess the quality of reagents by reproducibility, parallel studies, titration studies, pH, etc.

7.A.7 Perform appropriate tests for nucleic acid, radioisotope, chemical and microbial contamination.

7.B Documentation

7.B.1 Validate changes in testing methods by parallel or confirmatory testing.

7.B.2 Maintain identity of specimen throughout testing and reporting of results, meeting processing time deadlines; address delays in result reporting.

7.B.3 Establish and implement policies to reject specimens when criteria of acceptability for testing are not met and to ensure that unacceptable specimens are not tested.

7.B.4 Document incidents related to quality of testing.

7.B.5 Establish a system to maintain records of testing results for all subjects tested for a period of at least 2 years, depending on local regulations..

7.B.6 Establish and implement a system to report testing results in a timely, accurate and reliable manner.

7.B.7 Establish and implement policy to perform periodic antibody screening of patients awaiting renal transplantation.

7.B.8 Maintain records of potentially sentitizing events for each patient awaiting renal transplantation.

SPEC 7.B.9 Establish and implement policy for repeat testing to be performed when necessary.

SPEC 7.B.10 Establish, implement and document corrective action procedures to deal with any inconsistency or errors in reporting of test results or problems with communication with laboratory.

SPEC 7.B.11 Develop and implement program to regularly assess abilities of laboratory personnel to reproduce test results of previously characterized specimens.

SPEC 7.B.12 Validate prior to use any automated systems and computer programs used to assist in the interpretation of reaction patterns; test routinely for accuracy and reproducibility.

7.C Compliance

7.C.1 Maintain testing guidelines set by regulatory agencies (e.g., ASHI, UNOS, CLIA, OSHA) for procedures being performed in laboratory.

7.C.2 Participate in internal and external (e.g., ASHI/CAP Surveys) quality assurance and proficiency testing programs.

7.C.3 Implement program to monitor compliance to laboratory policies.

8. General Laboratory Skills

8.A Prepare reagents according to protocols, understanding the metric system and the meaning of pH, molar, molal, % by weight or volume, normal, isotonic, picomoles, etc..

8.B Maintain an accurate inventory of supplies and reagents; utilize supplies effectively while considering shelf life and expiration dates.

8.C Validate, utilize and maintain computer databases of patient information and programs used for interpretation of data.

8.D Use appropriate cleaning, decontamination and sterilization procedures for glassware, instruments and work areas.

8.E Identify and minimize laboratory hazards:

8.E.1 Identify sources of biohazards and establish protective procedures for handling and disposal of such materials consistent with OSHA, CDC and mandated laboratory guidelines.

8.E.2 Correctly document, handle, store and discard radioactive materials per mandated laboratory guidelines and as dictated by the isotope's characteristics.

8.E.3 Correctly handle, store and dispose of flammables, volatile and toxic chemicals, liquid nitrogen and gas cylinders according to mandated guidelines.

8.E.4 Dispose of glass, syringes and needles properly.

8.E.5 Discard venipuncture materials according to established guidelines.

8.F Utilize appropriate general laboratory safety procedures:

8.F.1 Determine location and utilization of fire extinguishers, eye wash, fire blanket, fire alarm, evacuation plans, power failure procedures, etc.

8.F.2 Maintain list of personnel to be notified regarding emergencies.

8.F.3 Recognize and be prepared to act in case of personnel or patient accident/injury.

8.F.4 Document and report personal injuries to appropriate personnel.

9. General Principles

9.A Demonstrate understanding of general principles of immunology:

9.A.1 Essential features of the immune system: types of immune responses, concepts of antigenic specificity and immunologic memory.

9.A.2 Antigen: types (cellular/soluble), immunogenicity, concept of antigen vs. antigenic determinant/epitope.

9.A.3 Humoral immune response: immunoglobulin structure, classes and functions, dynamics of an antibody response and of the interaction between antigen and antibody, including the complement pathway of immune-mediated injury.

9.A.4 Cellular immune response: general features of cellular interaction [types of cells and characteristic phenotypes differentiating cell populations (T helper, cytotoxic/suppressor, etc.)] and cellular communication e.g., cytokines.

9.A.5 Transplantation immunology: application of basic immunologic principles to graft rejection/acceptance, graft vs. host disease, mechanisms of tolerance, etc.

9.B Demonstrate understanding of general principle of genetics:

9.B.1 Chromosome theory of heredity, meiosis, mitosis.

9.B.2 Mendelian inheritance: law of segregation vs. independent assortment, allelism, recessive/dominant inheritance, homozygous/heterozygous, autosomal/sex-linked, recombination, gene conversion, etc.

9.C Demonstrate understanding of general principles of immunogenetics:

9.C.1 Historical background and nomenclature of the human major histocompatibility complex (MHC).

9.C.2 Genetics of the human MHC

9.C.2.1 Location of MHC genes encoding cell surface products and principles of mapping HLA loci.

9.C.2.2 Principles of inheritance, concepts of HLA haplotype, genotype, phenotype including genetic recombination.

9.C.2.3 Principle of genetic (linkage) disequilibrium between alleles of different loci on an HLA haplotype.

9.C.2.4 Geographic and racial variations of MHC gene products, antigen and haplotype frequencies.

9.C.3 General immunochemistry of MHC products

9.C.3.1 Basic biochemical structure of HLA gene products.

9.C.3.2 Serologic phenomena related to HLA antigen-antibody interaction, e.g., crossreactivity, CYNAP reactions, antibody avidity, polyclonal nature of antisera.

9.C.3.3 Concept of multiple antigenic determinants (epitopes) on a single HLA molecule.

9.C.4 General function of MHC gene products

9.C.4.1 Principle of self-recognition (restriction) in host immune responses.

9.C.4.2 HLA and association with disease, susceptibility vs. resistance, relative risk and association vs. linkage.

9.D Demonstrate understanding of general principles of hybridoma technology:

9.D.1 Basic principles of the production, standardization and use of monoclonal antibodies.

9.D.2 Clusters of Differentiation (CD) nomenclature, CD's that are applicable to transplantation and disease states.

9.E Demonstrate understanding of general principles of flow cytometry:

9.E.1 Principles of fluorescence excitation/emission.

9.E.2 Distribution of cell markers in various cell populations, applications of cell marker analysis.

9.F Demonstrate understanding of general principles of molecular biology:

9.F.1 Theory of structural components of nucleic acids, base pairing; organization of genes, chromatin and chromosomes.

9.F.2 Theory of gene expression including transcription and translation, and null alleles..

9.F.3 Principles of primer and probe design.

9.F.4 Principles of polymerase chain reaction (PCR) and its applications.

9.F.5 Principles of molecular testing methods for major histocompatibility complex gene products (e.g., RFLP, SSOP, SSP, direct sequencing).

9.G Demonstrate understanding of general principles of ELISA:

9.G.1 Principles of ELISA testing methods for antigen or antibody detection and crossmatching.

10. Supervisory functions/Management SPEC

10.A Personnel:

10.A.1 Determine levels and types of personnel appropriate for workload.

10.A.2 Know and utilize institutional policies regarding employee selection, evaluation, benefits, councilling and grievance procedures.

10.A.3 Schedule and supervise personnel to optimize efficiency in providing necessary level of service.

10.A.4 Help provide continuing education for the technical personnel and others (students, physicians, etc.).

10.A.5 Conduct training of laboratory technologists, students and physicians in techniques and procedures used in laboratory.

10.A.6 Keep abreast of current appropriate literature and actively participate in seminars and other presentations for the instruction of laboratory and other staff.

10.A.7 Document competency of technical personnel by job function.

SPEC 10.B Financial:

10.B.1 Determine and choose cost effective methodologies, reagents and equipment for laboratory operation.

10.B.2 Assist in determination of laboratory budget, considering costs and revenues.

10.B.3 Prepare technical reports reflecting volume of work performed and procedures utilized; identify progress or adverse trends.

SPEC 10.C General:

10.C.1 Know and implement appropriate federal, state, local, institutional and accreditation agency laws, regulations, standards and policies.

10.C.2 Establish communications to interact effectively with patients and medical personnel both within and outside the laboratory at all levels.

10.C.3 Assist in laboratory space allocation and design when applicable.

10.C.4 Prepare and update laboratory manual(s) of procedures and policies.

10.C.5 Assist in preparation of laboratory certification/accreditation applications.

10.C.6 Prepare reports of quality assurance and proficiency testing results.

10.C.7 Assist in development and implementation of new procedures and technologies.

10.C.8 Validate commercial software packages for data analysis, data management, inventory systems and generation of documents and graphics.

10.C.9 Maintain appropriate security measures of computer systems