

Standards for Accredited Laboratories

American Society for Histocompatibility and Immunogenetics

~~2009-2010~~ Revised Standards ~~Approved by the ASHI Board of Directors~~

DRAFT: April 30, 2010 ~~Approved by CMS – January 2010~~

A. General Provisions

A.1 Basis and Scope

A.1.1 This document sets forth the conditions that a laboratory must satisfy in order to be accredited by the American Society for Histocompatibility and Immunogenetics (ASHI) to perform testing on human specimens. These Standards have been established by the ASHI Quality Assurance and Standards Committee following review, and response to, public comments. These Standards have been approved by the ASHI Board of Directors. These Standards have been established to help ensure accurate and dependable immunogenetics, histocompatibility, transplantation and parentage/relationship testing consistent with the current state of well-established laboratory procedures.

A.1.2 All laboratories requesting ASHI accreditation must meet the same requirements, regardless of their location in the U.S. or a foreign country and regardless of whether or not they are using ASHI accreditation for compliance with CLIA regulations.

A.2 Definitions and Abbreviations

The following definitions apply, unless the context indicates otherwise:

Accuracy means correctness or freedom from error (for example, obtaining the expected HLA-allele assignment in a Proficiency Test).

Ambiguous means a test result that may be interpreted in two or more possible ways.

Analyte means a substance or constituent for which the laboratory conducts testing.

ASHI means the American Society for Histocompatibility and Immunogenetics.

ASHI Accreditation Review Board (ARB) means the individuals who have been appointed by the ASHI Board of Directors to evaluate the compliance of laboratories seeking ASHI accreditation with ASHI Standards by developing and enforcing relevant policies, assigning laboratory inspectors and evaluating applications and inspection reports. The ARB Operations Manual is approved by the ASHI Board of Directors.

ASHI-accredited laboratory means a laboratory that has applied for and been accredited by ASHI by satisfying all applicable requirements of the accreditation process.

ASHI-approved laboratory means a laboratory outside the United States that meets ASHI requirements for accreditation, but is not required to follow CMS regulations.

Authorized person means an individual authorized under State law to order tests or receive test results or both.

Calibration means a process of testing and adjusting an instrument or test system to establish a correlation between the measurement response and an established reference standard.

CD means cluster of differentiation as applied to nomenclature for cell surface proteins.

CDC means the Centers for Disease Control and Prevention of the US Department of Health and Human Services.

CLIA means the Clinical Laboratory Improvement Amendments of 1988 and all subsequent modifications.

CLIA certificate means a certificate issued by CMS:

(1) To a laboratory after an inspection that finds the laboratory to be in compliance with all applicable requirements, or reissued before the expiration date, pending an appeal, in accordance with 42 CFR 493.49, when an inspection has found the laboratory to be out of compliance with one or more requirements.

(2) On the basis of the laboratory's accreditation by ASHI (indicating that the laboratory is deemed to meet applicable CLIA requirements) or reissued before the expiration date, pending an appeal, in accordance with 42 CFR 493.61, when a validation or complaint survey has found the laboratory to be noncompliant with one or more CLIA requirements.

(3) Or reissued before the expiration date, pending an appeal, in accordance with 42 CFR 493.45, that enables the entity to conduct histocompatibility testing until the entity is determined to be in compliance.

Clinical test means a procedure used for patient care to determine the characteristic presence, absence, or quantity of an analyte in a human specimen.

CMS means Centers for Medicare and Medicaid Services

Combined Parentage Index means the mathematical product of the parentage indices obtained for all genetic systems tested in a case study. See parentage index below.

Combined Relationship Index means the mathematical product of the relationship indices obtained for all genetic systems tested in a case study. See relationship index below.

Complaint means a **written** report made to ASHI that alleges noncompliance with ASHI Standards or with Federal and/or State laws and regulations.

CREG (Cross Reactive Group) means a group of serologically cross-reactive HLA antigens.

Designee means a qualified person or persons with documented authority from the Director and/ or Technical Supervisor to perform a particular task or set of tasks that are the responsibility of the Director and or Technical Supervisor.

ELISA means enzyme-linked immunosorbent assay.

Established means validated in the laboratory and based upon documented local data and/or published peer reviewed data.

Federal, State and local laws means laws or regulations issued by any federal, national, state, provincial, city, or other authority which has jurisdiction in the laboratory's location.

HHS means the Department of Health and Human Services, or its designee.

HIPAA means Health Insurance Portability and Accountability Act. This act requires patients to be informed about possible disclosure of their private health information and requires institutions to provide safeguards against inappropriate disclosure.

IMGT/HLA Sequence Database means a database for sequences of the human Major Histocompatibility Complex and includes the official sequences for the WHO HLA Nomenclature Committee for Factors of the HLA System. The IMGT/HLA Sequence Database is part of the international **ImMunoGeneTics** project (IMGT). It is available at <http://www.ebi.ac.uk/imgt/hla/>.

Immediate Jeopardy means a situation in which the facility's noncompliance with one or more requirements of participation has caused, or is likely to cause, serious injury, harm, impairment, or death to a patient.

Kit means all components of a test that are packaged together.

Microarray means a solid phase system using a panel of markers, such as labeled particles that are differentiated on the basis of the intensity of fluorescence at a specific wavelength or combination of wavelengths (e.g. with a Luminometer), or a set of markers placed at defined positions on a solid substrate.

MLC means mixed leukocyte culture.

NMDP means National Marrow Donor Program.

OPTN means Organ Procurement and Transplantation Network (see UNOS, a contracted OPTN).

Parentage Index means the ratio of the probability that the obligatory allele or haplotype inherited by the child came from the alleged parent to the probability that it came from a random individual of similar ethnic background.

Performance characteristic means a property of a test that is used to describe its quality, e.g., accuracy, precision, analytical sensitivity, analytical specificity, reportable range, reference range, etc.

Performance specification means a value or range of values for a performance characteristic, established or verified by the laboratory, which is used to describe the quality of patient test results.

Periodically means performed and documented at predetermined fixed intervals.

Physician means an individual with a doctor of medicine, doctor of osteopathy, or doctor of podiatric medicine degree who is appropriately licensed to practice medicine.

PRA (Panel Reactive Antibody) means the percentage (%) of a panel reacting with a patient's serum or the percentage of donors expected to react based on known antibody activities ("calculated" or "virtual" PRA).

Precision means the agreement between repeated measurements; an indication of the random error.

Primer means an oligonucleotide that binds to a specific target sequence of a gene or template by complementarities under defined conditions and is used to initiate DNA amplification.

Probe means an oligonucleotide that binds to and identifies the presence of target sequences of a gene by complementarities under defined conditions. Probes may be free in liquid phase or bound to solid substrates.

Procedure means a series of steps followed in a specific order to accomplish a given task.

Proficiency testing means testing performed on a set of specimens with a system to appropriately evaluate and score the testing results and to identify performance problems or system errors.

Record means written or electronic information regarding subjects, samples, testing, laboratory Quality Control and Quality Assurance activities.

Redefine means to reexamine or reevaluate especially with a view to change.

Referee laboratory means a laboratory currently in compliance with applicable ASHI requirements that analyzes proficiency testing specimens for the purpose of determining the correct response for the specimens in a proficiency testing program or that analyzes a specimen to resolve a discrepancy between two or more laboratories.

Reference panel means a collection of cells, DNA, antisera, or other materials the characteristics of which have been defined by consensus, testing by multiple techniques and/or in multiple laboratories or as blinded samples tested in another lab.

Registry donor means a person who has consented to be listed on a registry as a potential volunteer donor of hematopoietic progenitor cells or other blood products.

Relationship Index means the ratio of the probability that the obligatory allele or haplotype inherited by the individual tested would be present given the alleged relationship to the probability that it would be present in the absence of the alleged relationship.

Report means the test results provided to the authorized person who ordered or requested the testing and/or sent to be part of the medical record.

RFLP means restriction fragment length polymorphism.

SBT means sequence based typing

Specificity means the probability that the test will be negative when the specific analyte, sequence or protein is absent.

Sensitivity means the probability that a test will be positive when a particular analyte, sequence or protein is present.

Sentinel Event means an unexpected or unanticipated occurrence involving death or serious physical or psychological

injuries, or the risk thereof. The event must be thoroughly investigated as soon as possible.

SSO means a nucleic acid-based typing method using sequence-specific oligonucleotide hybridization.

SSP means a nucleic acid amplification-based typing method using sequence-specific priming.

Standard Precautions refers to the CDC directives to prevent spread of infections from one individual to another or personnel who come into contact with the individual or individual specimens that include the use of personal protective equipment and a strict hand washing regimen.

STR means short tandem repeat, usually used in relation to repeats of 1 to 9 nucleotides.

Survey means the set of testing events in a specific test category of external proficiency testing.

Test system means the procedure and all instrumentation, equipment, reagents, and supplies needed to perform an assay or examination and generate test results.

Unknown means a sample that has been previously or is concurrently tested by another individual and is tested by an individual who has no knowledge of the expected result.

UNOS means the United Network for Organ Sharing (see OPTN).

Unsatisfactory proficiency testing performance means failure to attain the acceptable response for an analyte or test or a testing event.

Unsuccessful participation in proficiency testing means any of the following:

- (1) Unsatisfactory performance for the same analyte in two consecutive or two out of three testing events.
- (2) Repeated unsatisfactory overall testing event scores for two consecutive or two out of three testing events for the same analyte.
- (3) An unsatisfactory testing event score for those subspecialties not graded by analyte (blood compatibility, immunohematology) for the same subspecialty for two consecutive or two out of three testing events.

Validated means a test system that has been proven to produce accurate results by comparison with (1) results from a qualified lab, (2) extensive comparative testing with currently accepted methods, (3) demonstrated correlation with clinical outcomes, or (4) other scientifically sound performance criteria established by that laboratory.

Verification typing means HLA typing performed on an independent sample (or, for a cord blood unit, from an attached segment or from the unit itself) with the purpose of verifying concordance of that typing assignment with the initial HLA typing assignment. Concordance does not require identical levels of resolution for the two sets of typing but requires the two assignments to be consistent with one another.

VNTR means **V**ariable **N**umber of **T**andem **R**epeats of a specific nucleotide sequence.

WHO means World Health Organization.

A.3 Applicability

These Standards apply to ASHI-accredited laboratories that perform testing of human specimens for purposes of reporting specific results relevant to the diagnosis, prevention or treatment of any disease or impairment, or the assessment of the health of individuals. In addition, these Standards apply to parentage/relationship testing or HLA typing for registries.

If any immunogenetics, transplantation and parentage/relationship testing not covered by specific ASHI Standards is performed, the laboratory must satisfy all applicable ASHI Standards. The laboratory must have and document appropriate expertise and must participate in appropriate proficiency testing.

B. Accreditation

B.1 Requirements

B.1.1 The ASHI Accreditation Program will issue a certificate of accreditation to a laboratory if the ASHI Accreditation Program determines that the laboratory meets the requirements of the ASHI Standards and remits the accreditation fee.

B.1.2 Laboratories issued a certificate of accreditation must:

B.1.2.1 Comply with the requirements of the ASHI Accreditation Program.

B.1.2.2 Meet the notification requirements of section B.2.

B.1.2.3 Permit random sample validation and complaint inspections.

B.1.2.4 Permit the ASHI Accreditation Program to monitor the correction of any deficiencies found through the inspection process.

B.1.2.5 For laboratories using ASHI accreditation for compliance with CLIA regulations or other organizations for which it has “deemed status”, authorize ASHI to release to HHS or other organizations, as applicable, the laboratory's inspection findings whenever HHS conducts random sample or complaint inspections.

B.1.2.6 For laboratories using ASHI accreditation for compliance with CLIA regulations or other organizations for which ASHI has “deemed status”, authorize ASHI to submit to HHS or other organizations, as applicable, the results of the laboratory's proficiency testing.

B.1.2.7 For laboratories using ASHI accreditation for compliance with CLIA regulations, have a mechanism to provide laboratory workers with information about how to file anonymous complaints.

B.1.3 A certificate of accreditation is valid for no more than 2 years. In the event of a non-compliance determination as a result of a random sample validation or complaint inspection, a laboratory will be subject to a full review by the ASHI Accreditation Program and/ or (for US laboratories) CMS.

B.1.4 A laboratory seeking to renew its certificate of accreditation must complete and return the renewal application to the ASHI Accreditation Program by the deadline specified by the ASHI Accreditation Program, meet the requirements of ASHI Standards, submit appropriate accreditation fees, and submit its CLIA certificate if applicable.

B.1.5 An ASHI-accredited laboratory failing to meet the requirements in B.1.2 may be subject to suspension, revocation or limitation of the laboratory's certificate of accreditation or certain alternative sanctions. The ASHI Accreditation Program must provide the laboratory with a written statement of the grounds on which the determination of noncompliance is based. The ASHI Accreditation Program must offer an opportunity for appeal, re-accreditation or limited accreditation.

B.1.6 If the ASHI Accreditation Program determines that an application for accreditation is to be denied or limited, the ASHI Accreditation Program must notify the laboratory in writing of the basis for denial or limitation of the application. The ASHI Accreditation Program must offer an opportunity for appeal or limited accreditation.

B.1.7 If the laboratory submits an appeal within 30 days of notification of the ASHI Accreditation Program's action to suspend, revoke, limit or deny the certificate of accreditation, the laboratory will retain its certificate of accreditation until a decision is made by the ASHI Accreditation Program unless the ASHI Accreditation Program finds that conditions at the laboratory pose an imminent and serious risk to human health.

B.2 Notification requirements

B.2.1 Laboratories issued ASHI accreditation must notify the ASHI Accreditation Program within 30 days of any changes in ownership, name, location, Director, Technical Supervisor, Clinical Consultant and/or General Supervisor. New Directors and Technical Supervisors must be approved by the ASHI Director Training Review

and Credentialing Committee (DTRC) for all areas of accreditation for which the laboratory reports results. **New Clinical Consultants and new General Supervisors must be approved by the ARB.**

B.2.2 ASHI-accredited laboratories seeking additional areas of accreditation or new technology that is not included in the laboratory's accreditation must notify the ASHI Accreditation Program in writing. The expertise of the Director and Technical Supervisor must be approved by the ASHI Director Training Review and Credentialing Committee (DTRC) prior to the addition of any new area(s) of accreditation. The ARB must approve the addition of new technologies.

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.1.1.2 If C.1.1.1 cannot be met, participate in a graded external proficiency testing program that is available from another source.

C.1.1.3 If C.1.1.1 - C.1.1.2 cannot be met, participate in an ungraded proficiency testing program that is approved by the ASHI Accreditation Review Board.

C.1.1.4 If C.1.1.1 - C.1.1.3 cannot be met, participate in an ungraded external proficiency testing program that is available from another source.

C.1.1.5 If C.1.1.1 - C.1.1.4 cannot be met, at least semiannually perform other procedures to validate test performance. This may be accomplished through blind testing of specimens with known results or reference specimens, exchange of specimens with other laboratories, or other equivalent systems that are approved by the laboratory Director and Technical Supervisor and meet CLIA requirements.

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 own proficiency testing samples or results of their own for analysis to another laboratory.

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. This includes the following:

C.1.5.1 A copy of the proficiency testing program report forms used by the laboratory to record proficiency testing results.

C.1.5.2 The attestation statement provided by the proficiency testing program and hand or password-protected, electronically signed by the technologist(s) and the laboratory director or technical supervisor, documenting that proficiency testing samples were tested in the same manner as patient specimens.

C.1.5.3 A copy of any reports or communication from the proficiency testing agency related to the proficiency testing exercise.

C.1.5.4 Records demonstrating review by the Director or Technical Supervisor of the laboratory's performance in each proficiency testing exercise and any related corrective action.

C.2 Successful Participation

C.2.1 Each laboratory must successfully participate in an available proficiency testing program as delineated in C.1 for each analyte or test method for which the laboratory is ASHI-accredited.

C.2.1.1 For all clinical testing except ABO/Rh typing, satisfactory performance requires 80% concordance with the consensus for each assessment of each analyte. (Example: For a Class I typing sendout consisting of 5 samples, the laboratory may not have a typing error on more than one sample to meet the requirement for 80% concordance)

C.2.1.2 For ABO/Rh typing, satisfactory performance is 100% concordance.

C.2.2 Unsuccessful participation in a PT program is defined as unsatisfactory performance on 2 consecutive assessments; or on 2 out of 3 assessments;. If a laboratory's performance in an external proficiency testing program is unsuccessful:

C.2.2.1 The laboratory must determine and document the cause for each unsatisfactory proficiency test result and take appropriate measures to prevent recurrence of the problem.

C.2.2.2 The laboratory must take immediate corrective action to ensure that the problem identified through proficiency testing has not resulted and will not result in release of incorrect test results.

C.2.2.3 The laboratory must successfully participate in an enhanced proficiency testing program in that category within the timeframe required by the ASHI Accreditation Review Board.

C.2.3 For ungraded proficiency tests, the laboratory must review, evaluate and document an explanation of the cause for results that are not in concordance with $\geq 60\%$ of participants.

C.2.4 If a laboratory fails to participate successfully in proficiency testing for a given, analyte or test, as defined in this section, the ASHI Accreditation Program must take action (in accordance with ASHI regulations as mandated by CLIA regulations) and may limit accreditation.

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 General Laboratory Systems

D.2.1 Introduction

D.2.1.1 Each laboratory that performs testing must meet the applicable general laboratory systems requirements. The laboratory must monitor and evaluate the overall quality of the general laboratory systems and correct identified problems for each type of test performed.

D.2.1.2 The laboratory must be in compliance with all applicable Federal, State and local laws including but not limited to, those governing laboratory employee health and safety, such as, use of equipment, fire safety, and the storage, handling and disposal of chemical, biological and radioactive materials.

D.2.1.3 The laboratory must establish and follow written procedures for standard precautions as defined by the CDC or if applicable non-US equivalent during collection, transport, storage and handling of blood and tissue specimens.

D.2.1.4 All records must be retained for a minimum of 2 years or longer, as specified by Federal/National, State and local laws, and must be maintained and stored under conditions that ensure proper preservation.

D.2.1.5 The laboratory shall have emergency operation policies, processes, and procedures to respond to the effects of internal and external disasters.

D.2.2 Facilities

D.2.2.1 Laboratory space must be sufficient so that all procedures and analyses can be carried out without crowding to the extent that errors may result and ensure that:

D.2.2.1.1 Adequate facilities to store records are available to the laboratory.

D.2.2.1.2 Active records are immediately available to the laboratory. Archived records may be stored in an offsite location, but must be easily retrievable within 48 hours or the time period specified by local, State and Federal regulations.

D.2.2.1.3 Adequate facilities for refrigerator and freezer storage of reagents and specimens are immediately available to the laboratory.

D.2.2.2 Lighting and ventilation must be adequate.

D.2.2.3 Uninterruptible or emergency power supplies must be used for essential equipment.

D.2.2.4 Laboratories performing amplification of nucleic acids must:

D.2.2.4.1 Use physical and/or biochemical barriers to prevent nucleic acid contamination (carry-over).

D.2.2.4.2 Perform pre-amplification procedures in a work area that excludes amplified nucleic acid that has the potential to serve as a template in any other amplification assays performed in the laboratory (e.g., PCR product, plasmids containing HLA genes or relevant STR/VNTR sequences). Restricted traffic flow is recommended.

D.2.2.4.3 Use dedicated lab coats, gloves and disposable supplies in the pre-amplification area.

D.2.2.4.4 Ensure that for methods that utilize two consecutive steps of amplification, addition of the template for the second amplification occurs in an area isolated by physical or chemical barriers from both the pre-amplification work area and post-amplification work areas.

D.2.3 Confidentiality of patient information

D.2.3.1 The laboratory must establish and follow a written policy to ensure confidentiality of protected health information throughout all phases of the testing process. US laboratories must be in compliance with the HIPAA Final Rule. [45CFR Parts 160 & 164].

D.2.3.2 Test results must be released only to authorized persons and to the individual responsible for using the test results and/or to the laboratory that initially requested the test.

D.2.4 Complaint investigations

The laboratory must have a system in place to ensure that it documents all complaints and problems reported to the laboratory. All complaints must be investigated and corrective action taken when necessary.

D.2.5 Client service evaluation and communication

D.2.5.1 Laboratories must have a written agreement for histocompatibility testing with each transplant program they service. Laboratories must review each agreement annually and revise as necessary.

D.2.5.2 The laboratory must have a system in place to document problems that result from breakdown in communication between the laboratory and authorized individuals who order tests or receive results.

D.2.5.3 The laboratory must, upon request, make available to clients a list of test methods employed by the laboratory, a list of performance specifications for each method (including normal ranges, if applicable) and a list of interfering factors that could affect the test results or interpretation of test results. Pertinent updates of testing information must be provided to clients whenever changes occur that affect the test results or the interpretation of test results.

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 evaluator 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 preventive maintenance records.

D.2.6.4.4 Direct observation of performance of instrument maintenance and function checks.

D.2.6.4.5 Assessment of test performance through testing previously analyzed specimens, internal blind testing samples or external proficiency testing samples.

D.2.6.4.6 Assessment of problem solving skills.

D.2.7 Evaluation of proficiency testing performance

D.2.7.1 The laboratory must review and evaluate, in a timely manner, the results obtained on all proficiency testing performed.

D.2.7.2 Every individual who participates in a proficiency test must be informed of the results of his/her performance in that proficiency test.

D.2.7.3 All proficiency testing evaluation and verification activities must be documented.

D.2.8 Laboratory systems assessment

D.2.8.1 The laboratory must establish and follow written policies and procedures for an ongoing mechanism to monitor, assess, and, when indicated, correct problems identified in the general, preanalytic, analytic, and postanalytic laboratory systems.

D.2.8.2 The general laboratory systems assessment must include a review of the effectiveness of corrective actions taken to resolve problems, revision of policies and procedures necessary to prevent recurrence of problems, and discussion of general, preanalytic, analytic, and postanalytic laboratory systems assessment reviews with appropriate staff.

D.2.8.3 The laboratory must document all general, pre-analytic, analytic, and post-analytic laboratory systems assessment activities and retain them for a minimum of two years, or longer, as specified by Federal, State and local laws.

D.2.9 Procedure manual

D.2.9.1 A written procedure manual(s) for all tests and assays performed by the laboratory must be available to, and followed by, laboratory personnel. Textbooks may supplement but not replace the laboratory's written procedures. Manufacturer's instructions or operator manuals may be used; however, any of the procedures or requirements not provided by the manufacturer must be provided by the laboratory.

D.2.9.2 The procedure manual(s) must include the following when applicable to the test procedure:

D.2.9.2.1 Requirements for:

D.2.9.2.1.1 Patient preparation.

D.2.9.2.1.2 Specimen collection, labeling, storage, preservation, transportation, processing and referral.

D.2.9.2.1.3 Specimen acceptability and criteria for rejection.

D.2.9.2.2 Step-by-step performance of the procedure, including test calculations and interpretation of results.

D.2.9.2.3 Preparation of slides, solutions, calibrators, controls, reagents, stains, and other materials used in testing.

D.2.9.2.4 Calibration and calibration verification procedures.

D.2.9.2.5 The reportable range for test results for the test system as established or verified.

D.2.9.2.6 Control procedures.

D.2.9.2.7 Corrective action procedures when calibration or control results fail to meet the laboratory's criteria for acceptability.

D.2.9.2.8 Limitations in the test methodology, including interfering substances and sample limitations.

D.2.9.2.9 Reference intervals and acceptable values.

D.2.9.2.10 Entering results in the patient record and reporting patient results including, when appropriate, the protocol for defining and reporting imminent life-threatening results or alert values.

D.2.9.2.11 Pertinent literature references.

D.2.9.2.12 Description of the course of action if a test system becomes inoperable.

D.2.9.3 New procedures and changes in procedures must be approved, signed, and dated by the current laboratory Director and relevant Technical Supervisor before use.

D.2.9.4 The laboratory must maintain a copy of each procedure with the dates of initial use and discontinuance.

D.2.9.5 The procedure manual(s) must be reviewed at least annually by a Director and relevant Technical Supervisor, and written evidence of this review must be in the manual.

D.3 Preanalytic systems

Each laboratory that performs clinical testing as described in A.1 must meet the applicable preanalytic system(s) requirements in this section. The laboratory must monitor and evaluate the overall quality of the preanalytic systems and, when indicated, correct identified problems.

D.3.1 Test request

D.3.1.1 The laboratory must perform tests only at the written or electronic request of an authorized person. Oral requests for laboratory tests from authorized individuals are permitted only if the laboratory documents efforts to obtain written authorization for testing within 30 days of the request.

D.3.1.2 The laboratory must ensure that the test requisition solicits the following information:

D.3.1.2.1 The name, address and contact information (or other suitable identifier) of the authorized person who ordered the test.

D.3.1.2.2 The test subject's name and/or unique identifier, gender, and age or date of birth.

D.3.1.2.3 Date of specimen collection

D.3.1.2.4 Time of specimen collection, when pertinent to testing

D.3.1.2.5 The test(s) ordered.

D.3.1.2.6 The source of the specimen when pertinent to testing.

D.3.1.2.7 Any relevant information, (e.g. transfusions, sensitization, primary or secondary graft, immunosuppressive therapy) to facilitate accurate and timely testing, interpretation, and reporting of results.

D.3.1.3 The laboratory must ensure the accuracy of all test request information transcribed into a record system or a laboratory information system.

D.3.2 Specimen collection and identification

D.3.2.1 The laboratory must establish and follow written policies and procedures for each of the following:

D.3.2.1.1 Specimen collection (e.g. anti-coagulant, quantity)

D.3.2.1.2 Specimen labeling, including:

D.3.2.1.2.1 Patient name and/or unique patient identifier.

D.3.2.1.2.2 Date and, if pertinent, time obtained.

D.3.2.1.2.3 Specimen source, when appropriate.

D.3.2.1.3 Conditions for specimen transportation.

D.3.2.1.4 Specimen acceptability and rejection.

D.3.2.1.5 Documentation of the date and time specimen is received.

D.3.2.2 Each primary collection container must be individually labeled.

D.3.2.3 When parentage/relationship testing is performed, the laboratory must comply with D.3.2.1 as well as the following:

D.3.2.3.1 At the time of collection, photographs of the subjects and/or legible photocopies of government-issued photo identification must be obtained from all parties in a parentage/relationship case study. If photocopies of government-issued identification cards cannot be obtained, it is recommended that the

numbers on these identifications be recorded. Consent must be obtained from each tested person (or in the case of a minor child or legally incompetent adult, from an individual with legal authority to provide consent) or there must be documentation of a court order.

D.3.2.3.2 Specimens received from an outside collecting facility must have documented positive identification.

D.3.2.3.3 A record must be kept at the testing facility of all identifying information including, but not limited to, name, date of birth, alleged relationship, race/ethnicity, and place and date of collection of sample. Information about each individual must be verified by the signature of that person or an individual legally authorized to represent that individual.

D.3.2.3.4 For each individual to be tested using a blood sample, any recent transfusion history (past three months) or any history of an allogeneic hematopoietic stem cell transplant must be recorded.

D.3.2.3.5 Each container of a blood or tissue sample must be individually labeled immediately prior to or following collection of the sample. If multiple buccal swabs are collected from one individual, one label may be secured around all the swabs before they are placed in the primary specimen container, or one label may be placed on the primary specimen container itself provided only swabs from a single individual are included.

D.3.2.3.6. The specimen label must include the first and last name of the subject and/or unique identifier, the date, and the name or initials of the sample collector.

D.3.2.3.7 The sample collector's first and last name and contact information must be part of the permanent record.

D.3.2.3.8 All specimens for parentage/relationship testing must have a documented chain of custody. Specimens must have been collected, transported, tested, and reported by individuals who have no conflict of interest in the case.

D.4. Analytic systems

D.4.1 Laboratory Systems

D.4.1.1 Specimen handling, processing, and storage

D.4.1.1.1 The laboratory must establish and follow written policies and procedures for each of the following:

D.4.1.1.1.1 Reliable specimen labeling, tracking and/or testing plate orientation throughout processing, testing and reporting

D.4.1.1.1.2 Processing of all samples appropriate for clinical application and/or test request.

D.4.1.1.1.3 Handling and storage of specimens under conditions that maintain integrity for reliable test results.

D.4.1.1.1.4 A system to retrieve specimens for further testing in a timely manner.

D.4.1.2 Testing Environment

The following conditions must be monitored and documented as applicable:

D.4.1.2.1 Temperature of the following must be recorded each working day, or in case of continuous use each shift:

D.4.1.2.1.1 Incubators and water baths.

D.4.1.2.1.2 Ambient temperature of laboratory space.

D.4.1.2.1.3 Refrigerators and freezers must also:

D.4.1.2.1.3.1 be monitored continuously.

D.4.1.2.1.3.2 use an audible or centrally monitored temperature alarm system for critical reagents and relevant transplant candidate specimens.

D.4.1.2.1.3.3 be covered under an emergency plan for alternative storage for critical reagents and relevant transplant candidate specimens.

D.4.1.2.2 If liquid nitrogen freezers are used, the level of liquid nitrogen must be monitored at intervals that will ensure an adequate supply at all times.

D.4.1.2.3 Incubator and environment humidity, as appropriate.

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 the 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 ensures 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.4.1.3.11 The laboratory must verify that media:

D.4.1.3.11.1 Are sterile, if sterility is required.

D.4.1.3.11.2 Supports growth, if used for cell culture.

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

As of April 24, 2003, all new procedures and major modifications to existing procedures or methods must be validated in the laboratory.

D.4.1.5.1 Performance specifications must be established and verified.

D.4.1.5.2 Each US laboratory that introduces an unmodified, FDA-cleared or approved test system must do the following before reporting patient test results as applicable:

D.4.1.5.2.1 Demonstrate that it can obtain performance specifications comparable to those established by the manufacturer for the following performance characteristics:

D.4.1.5.2.1.1 Accuracy.

D.4.1.5.2.1.2 Precision.

D.4.1.5.2.1.3 Reportable range of test results for an analytical test system or values for a qualitative test system.

D.4.1.5.2.2 Verify that the manufacturer's reference values are appropriate for the laboratory's patient population.

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.4.1.6 Equipment maintenance and function checks

D.4.1.6.1 When using unmodified manufacturers' equipment and instruments, the laboratory must perform and document the following:

D.4.1.6.1.1 Maintenance, as defined by the manufacturer and with at least the frequency specified by the manufacturer.

D.4.1.6.1.2 Function checks, as defined by the manufacturer and with at least the frequency specified by the manufacturer. Function checks must be within the manufacturer's established limits before patient testing is conducted.

D.4.1.6.2 When using equipment and instruments developed in-house, commercial equipment modified by the laboratory, or equipment for which maintenance and function check protocols are not provided by the manufacturer, the laboratory must do the following:

D.4.1.6.2.1 Establish, perform and document maintenance and function check protocols that ensure equipment and instrument performance necessary for accurate and reliable test results.

D.4.1.6.2.2 Function checks must be within the laboratory's established limits before test results are reported.

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.4.1.7.4 For **thermal cycling instruments**, the appropriate target temperatures must be achieved. Accuracy of these temperatures must be verified and documented at least every six months.

D.4.1.7.5 For **flow cytometry and flow analysis using equipment designed for beads only (fluoroanalyzer)**, instrument standardization and calibration the laboratory must, as applicable:

D.4.1.7.5.1 Include an optical standard, consisting of latex beads or other uniform particles, to ensure proper focusing and alignment of all lenses in the path for both the exciting light source and signal (e.g., light scatter, fluorescence) detectors.

D.4.1.7.5.2 Include a fluorescent standard for each fluorochrome to be used to ensure adequate detection of the fluorescent signal. These fluorescent standards may be incorporated in the beads or other particles used for optical standardization or may be a separate bead or fixed cell preparation.

D.4.1.7.5.3 Run both the optical and fluorescent standards each time the instrument is turned on and any time maintenance, adjustments or problems have occurred during operation that could potentially affect instrument function.

D.4.1.7.5.4 Record and monitor the results of optical focusing/alignment each day of use or each time the instrument is turned on.

D.4.1.7.5.5 Establish threshold values for acceptable optical and fluorescent standardization results for all relevant signals on each instrument used.

D.4.1.7.5.6 In the event a particular threshold value cannot be attained, have a written protocol detailing the corrective action.

D.4.1.7.5.7 If performing analyses that require the simultaneous use of two or more fluorochromes, use an appropriate procedure to compensate for overlap in their emission spectra.

D.4.1.7.5.8 Record laser power output and current input each day of use. Acceptable thresholds and corrective action protocols must be documented. If the instrument cannot report these data, the lab must obtain documentation from the manufacturer that the laser cannot operate under substandard conditions.

D.4.1.7.6 Laboratories performing **ELISA** must:

D.4.1.7.6.1 Demonstrate that the light source and filter of the plate reader produce the intensity and wavelength of light required for the test system.

D.4.1.7.6.2 Perform and document calibration/verification of plate alignment, movement and instrument linearity according to the manufacturer's instructions (at least once every six months) for the plate reader.

D.4.1.7.6.3 Check and document microplate washer performance during each month of use.

D.4.1.7.7 Laboratories performing **luminometry** must perform and document calibration/verification of plate alignment and instrument linearity according to the manufacturer's instructions (or at least once every six months if not defined by the manufacturer) for the plate reader.

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 in 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.4.1.8.4.2.3 If reaction inhibition is a significant source of false negative results, include a control material capable of detecting the inhibition.

D.4.1.8.4.3 For each electrophoretic procedure include, concurrent with patient specimens, at least one control material containing the substances being identified or measured (e.g. molecular weight markers).

D.4.1.8.4.4 Perform control material testing before resuming patient testing when a complete change of reagents is introduced, major preventive maintenance is performed, or any critical part that may influence test performance is replaced.

D.4.1.8.4.5 Over time, rotate control material testing among all operators who perform the test.

D.4.1.8.4.6 Test control materials in the same manner as patient specimens.

D.4.1.8.4.7 When using calibration material as a control material, use calibration material from a different lot number than that used to establish a cut-off value or to calibrate the test system.

D.4.1.8.4.8 Establish or verify the criteria for acceptability of all control materials.

D.4.1.8.4.9 When control materials providing quantitative results are used, statistical parameters (for example, mean and standard deviation) for each batch and lot number of control materials must be defined and available.

D.4.1.8.4.10 The laboratory may use the stated value of a commercially assayed control material provided the stated value is for the methodology and instrumentation employed by the laboratory and is verified by the laboratory.

D.4.1.8.4.11 Statistical parameters for locally obtained control materials must be established over time by the laboratory through concurrent testing of control materials having previously determined statistical parameters.

D.4.1.8.4.12 Results of control materials must meet the laboratory's and, as applicable, the

manufacturer's test system criteria for acceptability before reporting test results.

D.4.1.8.4.13 The laboratory must document all control procedures performed.

D.4.1.8.4.14 If control materials are not available, the laboratory must have an alternative mechanism to detect immediate errors and monitor test system performance over time. The performance of alternative control procedures must be documented.

D.4.1.8.4.15 Laboratories must adhere to their policy for quality control of each lot and shipment of reagents. Reference material must be used for quality control whenever possible.

D.4.1.8.4.15.1 For each new lot, perform parallel testing with a previously approved lot or use appropriate reference material. The number of tested samples must be determined by the Technical Supervisor.

D.4.1.8.4.15.2 For each new shipment, demonstrate that the reagents have not been compromised during shipment by testing at least one previously tested or reference sample to determine that the reagents perform as expected.

D.4.1.8.5 Laboratories performing **nucleic acid testing** must have written criteria or protocols for preventing DNA contamination using physical and/or biochemical barriers for assays involving amplification of templates.

D.5 Application 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 available:

D.5.1.2.1.1 Patient age.

D.5.1.2.1.2 Sex.

D.5.1.2.1.3 Diagnosis or pertinent clinical data.

D.5.1.2.1.4 Distribution of patient test results.

D.5.1.2.1.5 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 a 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.5.2.1.2.3 Store and use complement at the recommended temperatures.

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 results 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.5.2.2 Laboratories performing **Amplification-based nucleic acid** testing must:

D.5.2.2.1 Use a method to prepare DNA that provides sufficient quality (e.g., purity, concentration) and quantity to ensure reliable test results. Written protocols must specify the minimal acceptable sample in terms of volume or numbers of nucleated cells. If tests are performed without prior purification of nucleic acids, the method must be documented and validated in the laboratory.

D.5.2.2.2 Ensure that samples are stored under conditions that preserve the integrity of the nucleic acids that will be tested.

D.5.2.2.3 Ensure that template quantity and quality are sufficient to provide interpretable data for a locus (or loci) or allele(s).

D.5.2.2.4 Ensure that the amount of amplification template in each amplification reaction is in an acceptable range.

D.5.2.2.5 Ensure that aliquots of all batches of reagents (solutions containing one or multiple components) utilized in the amplification assay are demonstrated to be free of contamination.

D.5.2.2.6 Ensure that reagents used for primary amplification are not exposed to post-amplification work areas.

D.5.2.2.7 Ensure that reagents used for secondary amplification are stored in a contamination-free area.

D.5.2.2.8 Define criteria and perform quality control testing to confirm specificity for each lot and shipment of primers and probes.

D.5.2.2.9 Ensure that each lot and shipment of primers or probes is monitored to confirm stability and performance of the primers or probes.

D.5.2.2.10 Ensure that oligonucleotide probes and primers are stored under conditions that maintain specificity and sensitivity.

- D.5.2.2.11** Verify that the conditions for primer extension (e.g. polymerase type, polymerase concentration, primer concentration, concentration of nucleotide triphosphates) are appropriate for the template (e.g. length of sequence, GC content).
- D.5.2.2.12** Ensure that for each set of primers, conditions that influence the specificity or quantity of amplified product have been demonstrated to be satisfactory for the range of samples routinely tested.
- D.5.2.2.13** Set the number of cycles at a level sufficient to detect the target nucleic acid but insufficient to detect small amounts of contaminating template.
- D.5.2.2.14** Monitor the quantity of specific amplification products (e.g., gel electrophoresis, hybridization).
- D.5.2.2.15** Recognize and document ambiguous combination(s) of alleles for each template/primer or template/probe combination and have procedures available to resolve these as appropriate for the clinical use of the test results.
- D.5.2.2.16** Define and document the genetic designation (e.g., locus) of the target amplified by each set of primers or hybridized with probes.
- D.5.2.2.17** Define the specificity and sequence of each primer and/or probe.
- D.5.2.2.18** Routinely monitor for contamination of pre-amplification areas by the most common amplification products that are produced in the laboratory.
- D.5.2.2.19** Routinely monitor pre-amplification work areas with wipe tests.
- D.5.2.2.19.1** Monitor potential contamination using a method that is at least as sensitive as routine test methods and that uses appropriate testing primers. At least one negative (no nucleic acid) and one positive control must be included in each amplification assay.
- ~~**D.5.2.2.19.2** It is recommended that each wipe test amplification be run without added DNA and with added DNA as a control for wipe test inhibition.~~
- D.5.2.2.19.23** If contamination ~~and/or inhibition~~ is detected, clean the area to eliminate the contamination ~~or source of inhibition~~ and document re-testing, as well as the measures taken to prevent future contamination.
- D.5.2.2.19.34** Document acceptable electrophoretic conditions used for each gel electrophoresis.
- D.5.2.2.20** If the size of a nucleic acid is a critical factor in the analysis of the data:
- D.5.2.2.20.1** In each gel, include size markers that produce discrete electrophoretic bands spanning and flanking the entire range of expected fragment sizes.
- D.5.2.2.20.2** The amount of DNA loaded in each lane must be within a range that ensures equivalent migration of DNA in all samples, including size markers.
- D.5.2.2.21** Define and document the specificity and sequence of primer targets. The genetic designation (e.g. locus) of the target amplified by each set of primers must be defined and documented. For each locus analyzed, the laboratory must have documentation that includes the chromosome location, the approximate number of alleles, and the distinguishing characteristics (e.g. sizes, sequences) of the alleles that are amplified.
- D.5.2.2.22** Have acceptable limits of signal intensity for positive and negative results. If these are not achieved, acceptance of the results must be justified and documented.
- D.5.2.2.23** Adhere to the established criteria for accepting or rejecting an amplification assay or document the justification for acceptance of an assay when acceptance criteria are not met.
- D.5.2.2.24** Have two independent reviews and interpretations of the data.

D.5.2.2.25 When applicable, interpret data using the IMGT/HLA or other appropriate nucleotide sequence database. The database that is used must be updated at least every twelve months.

D.5.2.2.26 When applicable, document in laboratory records which version of the IMGT/HLA or other appropriate nucleotide sequence database was used for allele interpretation.

D.5.2.3 Laboratories performing **SSOP** methods must:

D.5.2.3.1 Define the specificity and critical polymorphic sequence of each primer and probe.

D.5.2.3.2 Label probes by a method appropriate for the testing procedure.

D.5.2.3.3 Ensure that hybridization conditions for maintaining sensitivity and specificity have been established.

D.5.2.3.4 Ensure that pre-hybridization, hybridization, and detection are carried out under empirically determined conditions of concentration and stringency that are determined by the length or composition of the probe and that achieve the defined specificity.

D.5.2.3.5 Establish criteria to determine positive or negative hybridization results for each probe using nucleotide sequences, reference DNA and/or manufacturers' QC data.

D.5.2.3.6 Ensure that each probe used gives an adequate signal, and allows detection of alleles in a heterozygous individual.

D.5.2.3.7 Document the specificity and sensitivity of the labeling and detection methods (e.g. demonstrate correct signal strength for a control sequence) in the laboratory before results are reported.

D.5.2.3.8 If there is reuse of nucleic acids (probes or targets) bound to solid supports, have a validated procedure for re-hybridization assays and include controls to ensure that the sensitivity and specificity of the assay are unaltered.

D.5.2.4 Laboratories performing **SSP** methods must:

D.5.2.4.1 Ensure that an internal control is included for each primer mixture that will detect technical failures and that produces a product distinguishable from the specific typing product.

D.5.2.4.2 Ensure that the amplification conditions are acceptable for the primers used.

D.5.2.4.3 Include a negative (no nucleic acid) or contamination control in each assay.

D.5.2.4.4 Ensure that primers used produce adequate amounts of amplification products to be visualized.

D.5.2.5 Laboratories performing **sequencing** methods must:

D.5.2.5.1 Ensure that the method for preparing sequencing templates reliably generates appropriate length sequencing templates that are free of inhibitors of subsequent reactions (e.g. residual primer extension) and free of contaminants that cause sequencing artifacts.

D.5.2.5.2 Ensure that the methods employed for preparation of sequencing templates do not alter the accuracy of the final sequence (e.g. mutations created during cloning, preferential amplification).

D.5.2.5.3 Ensure that the conditions for primer extension in cycle sequencing reactions (e.g. polymerase type, polymerase concentration, primer concentration, concentration of nucleotide triphosphates, concentration of terminators) are appropriate for the template (e.g. length of sequence, GC content).

D.5.2.5.4 For heterozygous templates, if only one strand is sequenced, ensure that sequencing of only one strand consistently yields accurate sequence assignments. Sequencing of sense and anti-sense strands is strongly recommended. If assignments are routinely based upon data from one strand of DNA, periodic confirmation of complementary strands is recommended.

D.5.2.5.6 Establish criteria for acceptance and interpretation of primary data (e.g. correct assignments for nonpolymorphic positions, definition of sequencing region, criteria for peak intensity, baseline fluctuation,

signal-to-noise ratio and peak shapes). Document established sequence-specific artifacts and utilize the information in routine interpretation of data.

D.5.2.5.7 Ensure the use of a scientifically and technically sound method for interpretation, acceptance, and/or rejection of sequences, especially in regions that are technically difficult (e.g. compression, ends).

D.5.2.5.8 Ensure that sequences contributed by amplification primers are not considered in the assignment of alleles.

D.5.2.5.9 Determine the sequences of both sense and anti-sense strands, if a sequence suggests a novel allele or a rare combination of alleles.

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.

(Proposed text for associated guidance: Genotypic identity can only be proven if both parents are available or if the segregation of the four haplotypes is clearly defined.)

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 the 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.5.2.7 Laboratories performing **Antibody Analysis and/or Crossmatch** testing must:

D.5.2.7.1 Test each patient serum undiluted or at a dilution that has been established to be optimal for the method used, and document the dilution(s) in the test records.

D.5.2.7.2 Have written criteria or protocols for:

D.5.2.7.2.1 Selecting appropriate patient serum samples.

D.5.2.7.2.2 Preparation of donor cells or cellular component isolations (for example, solubilized antigens) as applicable to the technique(s) performed.

D.5.2.7.3 Use a negative control of human serum documented to be non-reactive against the antigenic target.

D.5.2.7.4 Use a positive control of an appropriate isotype and specificity, known to react with the specific cell types or antigens being tested, as applicable.

D.5.2.7.5 Use the positive control at a dilution appropriate for the assay (i.e., a titer at which moderate changes in assay sensitivity are likely to be detected).

D.5.2.7.6 If a cell donor has been transfused within the previous seven days, accept the results only if there is no evidence of potential interference from cells derived from transfusion products.

D.5.2.7.7 For solid organ transplantation, use a technique(s) that detects HLA-specific antibody with a sensitivity superior to that of the basic complement-dependent microlymphocytotoxicity assay.

D.5.2.7.8 Use a panel of antigens sufficient in number and phenotypic distribution with respect to individual antigens and/or CREGs for the intended use of the test results and for the population served.

D.5.2.7.9 Document the HLA class I and/or class II phenotypes of the panel for assays intended to provide information on HLA antibody specificity.

D.5.2.7.10 Document that the pooled cells or antigens, used for a present/not present detection of antibody, include the major antigen specificities or CREGs or are derived from a population of sufficient size to ensure representation of major antigen specificities.

D.5.2.7.11 When applicable, use a method that detects antibodies to HLA class II antigens and distinguishes them from antibodies to HLA class I antigens.

D.5.2.7.12 Have a process for distinguishing HLA class I and class II antibodies from non-HLA antibodies as appropriate for clinical applications.

D.5.2.7.13 Use appropriate methods and/or controls to assess the impact of xenogeneic, chimeric, monoclonal, or other therapeutic antibodies in the assay.

D.5.2.7.14 Ensure that there is a procedure to monitor and adjust for non-specific binding of antibody.

D.5.2.7.15 Use an HLA antibody screening method that is at least as sensitive or equivalent to, and predictive of, the routine crossmatch method, and is consistent with clinical transplant protocols.

D.5.2.7.16 When CREG nomenclature is reported, maintain documentation of antigens defined by each CREG.

D.5.2.8 Laboratories performing **solid phase techniques** must:

D.5.2.8.1 Validate all calculations. Determine the positive or negative cutoffs specific for each method.

D.5.2.8.2 Establish, verify and follow criteria to ensure a sufficient number of beads or other substrates of each specificity are analyzed in each assay.

D.5.2.8.3 Validate the test method using reference human antibodies with well-characterized specificity (ies). Subsequent quality control may consist of testing in parallel with previous lots.

D.5.2.9 Laboratories performing **flow cytometry** techniques must:

D.5.2.9.1 Establish the optimum serum-to-target ratio.

D.5.2.9.2 Establish the threshold for discriminating positive reactions regardless of the method used for reporting raw data (mean, median, mode channel shifts or quantitative fluorescence measurements). Any significant change in protocol, reagents or instrumentation requires a repeat determination of the positive threshold.

D.5.2.9.3 Define acceptable time periods between processing, labeling and data acquisition. Control samples must be treated in the same manner.

D.5.2.9.4 Use the dilution and/or volume of reagents locally validated prior to use.

D.5.2.9.5 Process antibodies or other reagents from lyophilized powder in order to remove microaggregates prior to use, according to the manufacturer's instructions or locally documented procedures.

D.5.2.9.6 Assess the binding of human immunoglobulin using a fluorochrome-labeled reagent, such as an F(ab')₂ anti-human IgG specific for the Fc region of the heavy chain, or other documented method.

D.5.2.9.7 Use Anti-human immunoglobulin reagents according to manufacturer's protocol or titered to determine the dilution with optimal sensitivity (signal-to-noise ratio). If a multicolor technique is used, the reagent must not demonstrate crossreactivity with the other immunoglobulin reagents used to label the cells.

D.5.2.9.8 Laboratories performing **cell-based antibody screening and/or crossmatching by flow cytometry** must:

D.5.2.9.8.1 Document that the method used for cell preparation meets or exceeds the laboratory's established criteria for purity and viability; and is sufficient to ensure accurate test results.

D.5.2.9.8.2 Differentiate specific populations (e.g., T cells, B cells and /or monocytes) using monoclonal antibodies that detect the appropriate CD antigen(s), and that are labeled with a fluorochrome different from the one used to detect the binding of the patient's antibody.

D.5.2.9.8.3 For internal labeling, document that the method used to allow fluorochrome-labeled antibodies to penetrate the cell membrane is effective.

D.5.2.10 Immune Function Tests

D.5.2.10.1 Laboratories performing **cell culture** must:

D.5.2.10.1.1 Use a laminar flow hood or other appropriately aseptic work area for preparation of cultures incubating for > 18 hours.

D.5.2.10.1.2 Monitor incubators for appropriate temperature, CO₂ concentration and humidity.

D.5.2.10.1.3 Document that lymphocyte viability is sufficient at the start of culture to maintain cell proliferation to ensure accurate test results, if applicable.

D.5.2.10.1.4 Incubate cell cultures for the length of time shown to give appropriate cellular proliferation, if applicable.

D.5.2.10.2 Laboratories performing **MLC** or other cellular based assays must also, as applicable:

D.5.2.10.2.1 Use a negative control for each responder cell that consists of responder cells stimulated with autologous cells.

D.5.2.10.2.2 Ensure that each assay includes HLA class II-disparate stimulator cells as positive controls for responder cell proliferation.

D.5.2.10.2.3 Show that stimulator cells are capable of stimulating unrelated HLA class II-disparate cells.

D.5.2.10.2.4 Use serum in the culture medium that has been screened to ensure the ability to support cellular proliferation, lack of cytotoxic antibodies and sterility.

D.5.2.11 Laboratories performing **RFLP** methods must:

D.5.2.11.1 Ensure empirically determined conditions of concentration and stringency that achieve the defined specificity prehybridization, hybridization, and detection.

D.5.2.11.2 Ensure that each DNA probe demonstrates Mendelian inheritance of the polymorphism detected by extensive population studies and/or published data.

D.5.2.11.3 Ensure that each probe used gives a signal adequate to detect a single copy gene.

D.5.2.11.4 Document the appropriate performance of each lot, or shipment, of restriction enzymes to produce fragments of established sizes before results using these reagents are reported.

D.5.2.11.5 Ensure that assays that are reported as acceptable demonstrate the appropriate migration patterns of digested control DNA and size markers.

D.5.2.11.6 Ensure that assays that are reported as acceptable reveal the appropriate patterns of the human control DNA and size markers.

D.5.2.11.7 When amplified DNA is digested,

D.5.2.11.7.1 Use controls of amplified DNA that will produce fragments of known sizes, digested in parallel, to monitor complete digestion.

D.5.2.11.7.2 Include in each electrophoretic run negative and positive controls that are processed with each assay. Negative controls must include DNA that is incubated without restriction enzyme.

D.5.2.11.8 When genomic DNA is digested, specify, in the record for each fragment detected, the restriction endonuclease used, fragment size, and the chromosomal location, as defined by relevant scientific literature.

D.5.2.12 Laboratories performing **ABO/Rh typing** must:

D.5.2.12.1 If using serological methods:

D.5.2.12.1.1 Use established procedures and criteria when performing titration of anti-ABO antibodies.

D.5.2.12.1.2 Use reagent typing sera (Anti-A, anti-B, and anti-D) to meet or exceed appropriate FDA criteria. A and B cells may be prepared by the laboratory provided there is documentation that they are satisfactory for the intended use.

D.5.2.12.2 Determine the ABO group on red cells using anti-A and anti-B sera, and test the serum or plasma for expected antibodies with A₁ and B cells. Cord cells and blood from newborns must be tested for red cell antigens only, not for antibodies.

D.5.2.12.3 If testing for the A₁ subgroup of ABO group A, use a reagent and a technique documented not to agglutinate A₂ cells.

D.5.2.12.4 Determine the Rh type by using anti-D, if Rh typing is performed. Use a control system that is appropriate to the anti-D reagent in use.

D.5.2.12.5 Document reagent performance, with appropriate cell controls, for applicable antisera on each day of use.

D.5.2.12.6 If performed using molecular techniques, adhere to all appropriate nucleic acid Standards.

D.5.2.12.7 Compare the current ABO/Rh group, [including subgroup when applicable](#), with previous records that are readily available. Any discrepancy found between the current results and the previous record must be resolved before transplantation.

D.5.2.13 Laboratories performing **immunophenotyping and/or single antigen typing by flow cytometry** must:

D.5.2.13.1 Use specificity controls consisting of appropriate cell types known to be positive for selected standard antibodies for each lot or shipment, where applicable.

D.5.2.13.2 Use a negative reagent control(s) for each test cell population. It is recommended that this control consist of monoclonal antibody(ies) of the same species and subclass and be prepared/purified in the same way as the monoclonal(s).

D.5.2.13.3 Where indirect labeling is involved, use a negative control reagent that is an irrelevant, isotype-

matched primary antibody and the same secondary antibody(ies) conjugated with the same fluorochrome(s) used in all relevant test combinations.

D.5.2.13.4 Where direct labeling is involved, use a negative control reagent that is an irrelevant antibody conjugated with the same fluorochrome and at the same fluorochrome: protein ratio used in all relevant test combinations unless 3-or 4-color fluorescence staining is used for CD4 cell counting.

D.5.2.13.5 Employ gating strategies to assure that the population of interest is being selected without significant contamination.

D.5.2.13.6 Ensure the appropriate definition and purity of cell populations by the use of either a multi-color technique or other documented method.

D.5.2.13.7 Base conclusions about abnormal proportions or abnormal numbers of cells bearing particular internal or cell surface markers using comparison with local ‘control’ data obtained with the same instrument, reagents and techniques.

D.5.3 By Application

D.5.3.1 General Transplant Support

D.5.3.1.1 Laboratories performing histocompatibility testing for **transplantation** support must:

D.5.3.1.1.1 Have policies specifying the testing to be performed for each type of cell, tissue or organ to be transplanted. The laboratory's policies must include, as applicable:

D.5.3.1.1.1.1 Individual protocols for each type of transplant differentiated by type of donor, organ or transplanted tissue, as applicable.

D.5.3.1.1.1.2 Protocols for patients at high risk for allograft rejection.

D.5.3.1.1.1.3 The sensitivity and specificity of the test system required to support clinical transplant protocols (for example, antigen or allele-level typing).

D.5.3.1.1.2 Have a policy for storage and maintenance of relevant transplant candidate samples. The policy must define the samples to be retained and the duration of storage.

D.5.3.1.1.3 Have a policy in place to evaluate the extent of sensitization of each patient at the time of his/her initial evaluation and following potentially sensitizing events.

D.5.3.1.1.4 Have a policy to attempt to obtain and store serum samples after known sensitizing events.

D.5.3.1.1.5 Have a policy to periodically screen serum samples from each transplant candidate for antibody to HLA antigens, including the frequency of screening serum samples.

D.5.3.1.1.6 Have a policy with supporting documentation for verifying that each transplant candidate has been ABO typed on two separate occasions prior to the addition of patient to the UNET deceased donor waitlist. “Two separate occasions” is defined as two samples, taken at different times, sent to the same or different labs.

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.5.3.2.1.2 Screen transplant candidates for the presence of anti-HLA antibodies at initial evaluation, at intervals consistent with established clinical transplant protocols, and following sensitizing events.

D.5.3.2.1.3 Perform crossmatching prospectively using the samples and the sensitivity appropriate for the clinical protocols established with the transplant center. Have results of crossmatches available before transplantation for transplants using living donors.

D.5.3.2.1.4 Have a policy for selection of sera for crossmatching of allosensitized patients that addresses the impact of historic and current sensitizing events.

D.5.3.2.1.5 When feasible, incorporate a serum sample obtained post-sensitization in the final crossmatch if a transplant candidate receives a blood transfusion, has an allograft that is rejected or removed, or experiences any other potentially sensitizing event.

D.5.3.2.1.6 Use potential donor T lymphocytes for crossmatching. It is recommended that B lymphocyte crossmatches also be performed using a method that distinguishes between T and B cell reactions.

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.5.3.2.2.2 Follow policies and procedures established by a joint agreement with the transplant program to have periodic (e.g. monthly) serum samples submitted from potential transplant recipients for HLA antibody screening and crossmatching.

D.5.3.2.2.3 Have results of final crossmatches available before renal transplantation or combined organ and tissue transplants in which a kidney is to be transplanted, except for emergency situations. If emergency transplants are performed before the crossmatch test results are available, information provided by the transplant candidate's physician to the laboratory as to the reason for the emergency transplant must be documented.

D.5.3.2.2.4 All UNOS histocompatibility laboratories must review and verify the UNet Waitlist histocompatibility data for each patient for whom the laboratory performed testing. Documentation of such review must be kept for at least three years or the interval required by local, State and Federal regulations, which ever is the longer, and must be available for audit by UNOS.

D.5.3.2.2.5 All UNOS histocompatibility laboratories must use a method for antibody identification that can identify HLA antibody specificities even in very highly sensitized transplant candidates. A solid phase method must be used if unacceptable antigens based on antibody screening are listed.

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.5.3.3.1.2 Repeat HLA typing of recipient using a new sample such that the individual's HLA typing is ~~confirmed~~ **verified** prior to final donor selection for both related and unrelated donor transplantation.

D.5.3.3.1.3 Repeat HLA typing of a related or unrelated stem cell donor using a new sample such that the individual's HLA typing is ~~confirmed~~ **verified** prior to stem cell collection. For unrelated donors, registry data is acceptable as the first of these two samples.

D.5.3.3.1.4 ~~In the case of cord blood units, verify the HLA assignment using a sample obtained from a contiguous segment. Perform augmented testing (e.g. MLC, T cell precursor frequency, SNP analysis, typing of minor histocompatibility antigens) as appropriate for the transplant protocol and optimal donor selection.~~

D.5.3.3.1.4.1 ~~Repeat typing for low-intermediate resolution assignments at HLA-A, B and high resolution HLA-DRB1, is usually sufficient to~~ ~~confirm~~ **verify** the HLA type.

[D.5.3.3.1.4.2 When the laboratory performs the confirmatory verification typing, document that HLA assignments are concordant with previous HLA typing assignments.](#)

D.5.3.3.1.5 Perform adequate testing to definitely establish HLA identity of phenotypically HLA-identical siblings.

D.5.3.4 Chimerism and Engraftment Monitoring

D.5.3.4.1 Laboratories performing **chimerism testing** must:

D.5.3.4.1.1 Have reagents or testing mechanisms appropriate to identify informative recipient and donor markers among individuals tested, except monozygotic twins.

D.5.3.4.1.2 Adjust for preferential amplification in the data analysis when using amplification-based methods.

D.5.3.4.1.3 Assess and consider the stoichiometry of the reaction when more than one locus is amplified in a single amplification reaction mixture (multiplex).

D.5.3.4.1.4 Perform initial engraftment analysis using pre-transplant patient and donor samples.

D.5.3.4.1.4.1 For systems with discrete alleles (e.g., STR) run an allele ladder concurrently with patient samples collected post-transplant.

D.5.3.4.1.4.2 For systems without discrete alleles (e.g., VNTR, RFLP), for each locus tested, amplify and analyze patient and donor samples collected pre-transplant, and/or control samples demonstrated to have similar performance characteristics (e.g., sensitivity, completion in PCR) concurrently with patient samples collected post-transplant.

D.5.3.4.1.5 Include appropriate controls for the characteristic used (e.g. size, sequence polymorphism) to distinguish donor and recipient alleles in each test.

D.5.3.4.1.6 Specify criteria for accepting or rejecting the amplification of a particular genetic locus or of an individual sample.

D.5.3.4.1.7 Establish criteria for evaluating the relative amounts of recipient and donor in a mixed chimeric sample if results are reported in a quantitative or semi-quantitative manner.

D.5.3.4.1.8 Document the purity obtained if processing involves isolation of cell subsets. If purity is not assessed, this must be documented on the test report.

D.5.3.4.1.9 Laboratories performing **STR/VNTR testing** must document, for each locus analyzed, the chromosome location, the alleles known for each locus, and the distinguishing characteristics (e.g. sizes, sequences) of the alleles that are amplified.

D.5.3.5 Transplantation of Other Organs and Tissues

D.5.3.5.1 If final crossmatches required by policies were not performed prospectively, the laboratory must document the circumstances, if known.

D.5.3.5.2 Laboratories performing testing for transplantation other than renal and/or pancreas transplantation must follow policies and procedures established by a joint agreement with the transplant program to have serum samples submitted from potential transplant recipients for HLA antibody screening and crossmatching.

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.5.3.6.1.2 If the laboratory maintains a donor registry, obtain informed consent before blood and/or blood products are taken from a potential transfusion donor and before the donor is placed on a list of available donors.

D.5.3.6.1.3 Follow applicable Standards when performing crossmatch and antibody analysis tests to detect and differentiate HLA class I, platelet-and/or granulocyte-specific antibodies.

D.5.3.6.1.4 If applicable, provide recommendation regarding compatibility requirements for future transfusion support.

D.5.3.7 Disease Risk and Vaccine Assessment

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

D.5.3.8 Parentage/Relationship Testing

D.5.3.8.1 Laboratories performing parentage/relationship testing must perform tests using a sufficient number of genetically independent loci to ensure that the combined parentage index is greater than 100 for cases where the alleged parent is not excluded. In cases where an alleged parent is excluded, the laboratory must confirm, with an independent isolation and test, the phenotype of the alleged parent. In cases without one parent, the phenotype of both the child and the excluded parent must be similarly confirmed.

D.5.3.8.2 A single genetic inconsistency must not be the sole basis for a statement of exclusion.

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.

D.5.3.8.4 Laboratories performing parentage/relationship testing using **nucleic acid analysis** must:

D.5.3.8.4.1 Conform to all relevant Standards in all previous nucleic acid sections.

D.5.3.8.4.2 Test a sufficient number of genetically independent loci. The combined parentage index must be greater than 100 for cases where an alleged parent is not excluded.

D.5.3.8.4.3 Use adequate electrophoretic resolution to distinguish closely spaced alleles whenever possible. When closely spaced alleles are not resolved, it must be taken into account in a parentage/relationship index calculation.

D.5.3.8.4.4 Use a negative control to monitor for contamination, and a positive control to verify the accuracy of the procedure.

D.5.3.8.4.5 Interpret all results twice, independently by two different individuals.

D.5.3.8.4.6 The phenotype of the excluded alleged relative(s) must be confirmed with an independent isolation and in cases without a *known* parent, the child's phenotype must also be confirmed with an independent isolation.

D.5.3.8.4.7 Laboratories performing **STR/VNTR testing** must also:

D.5.3.8.4.7.1 Use adequate electrophoretic measurement of STR/VNTR size and use ladders of known size or repeat numbers which encompass the commonly reported alleles.

D.5.3.8.4.7.2 Document, for each locus analyzed, the chromosome location, the approximate number of known alleles, and the distinguishing characteristics (e.g. sizes, sequences) of the alleles that are amplified.

D.5.3.8.4.7.3 Use ladders of known size or repeat numbers which encompass the commonly reported alleles when performing electrophoretic measurement of STR/VNTR size.

D.5.3.8.4.7.4 Report STR/VNTR alleles by repeat number as determined by the International Society of Forensic Genetics.

D.5.3.8.5 After testing, an adequate sample of remaining biological materials obtained from a tested individual must be stored for a minimum of six months after the release of the relationship testing report.

D.6 Post-analytical systems

D.6.1 Introduction

D.6.1.1 Test results must be released only to authorized persons and, if applicable, the individual responsible for using the test results and the laboratory that initially requested the test.

D.6.1.2 The laboratory must immediately alert the individual or entity requesting the test, and, if applicable, the individual responsible for using the test results when any test result indicates an imminent life-threatening condition, or panic or alert values.

D.6.1.3 When the laboratory cannot report patient test results within its established time frames, the laboratory must determine, based on the urgency of the patient test(s) requested, the need to notify the appropriate individual(s) of the delayed testing.

D.6.1.4 If a laboratory refers patient specimens for testing:

D.6.1.4.1 The referring laboratory must not revise results or information directly related to the interpretation of results provided by the testing laboratory.

D.6.1.4.2 The referring laboratory may permit each testing laboratory to send the test result directly to the authorized person who initially requested the test. The referring laboratory must retain or be able to produce an exact duplicate of each testing laboratory's report.

D.6.1.4.3 The authorized person who orders a test must be notified by the referring laboratory of the name and address of each laboratory location where the test was performed.

D.6.1.5 When errors in any reported test results are detected, the laboratory must do the following:

D.6.1.5.1 Promptly notify the authorized person ordering the test and, if applicable, the individual using the test results of reporting errors.

D.6.1.5.2 Issue corrected reports promptly to the authorized person ordering the test and, if applicable, the individual using the test results.

D.6.1.5.3 Maintain a copy of the original report, as well as the corrected report.

D.6.2 Test report

D.6.2.1 The laboratory must have adequate systems in place to report results in a timely, accurate, reliable, and confidential manner and ensure subject confidentiality throughout those parts of the total testing process that are under the laboratory's control.

D.6.2.2 The report must contain:

D.6.2.2.1 The date(s) of collection of sample(s) and, when pertinent to interpretation of the test, the testing date(s).

D.6.2.2.2 The specimen source, when appropriate.

D.6.2.2.3 The Laboratory and/or Institution's unique identifier/number, name and address.

D.6.2.2.4 The name or unique identifier of each individual tested.

D.6.2.2.5 The date of the report.

D.6.2.2.6 The test method and, if applicable, the units of measurement.

D.6.2.2.7 The test results and, if applicable, interpretation.

D.6.2.2.8 The identification of the genetic loci analyzed according to standard nomenclature or published reference.

D.6.2.2.9 The level of sensitivity for chimerism testing, when appropriate.

D.6.2.2.10 The identity of any subcontracted laboratory (if applicable) and that portion of the testing for which it bears responsibility must be noted on the report.

D.6.2.2.11 All phenotype terminology using relevant internationally-approved nomenclature.

D.6.2.2.12 A list of all ambiguous allele combinations.

D.6.2.2.13 A listing of all unresolved alleles when high resolution typing is reported.

D.6.2.2.14 If NMDP allele codes are reported:

D.6.2.2.14.1 Codes used must accurately and completely define all unresolved alleles.

D.6.2.2.14.2 A complete list of all possible alleles covered by the NMDP code.

D.6.2.2.15 For U.S. laboratories using a test method and reagents that are not FDA-approved, a statement to the effect that “This test was developed in and its performance characteristics determined by [lab name]. It has not been cleared or approved by the U.S. FDA.”

D.6.2.3 Prior to release, final reports must be reviewed and approved by the Director, Technical Supervisor or designee who at least meets the requirements of a General Supervisor.

D.6.2.4 The laboratory must report any information regarding the condition and disposition of specimens that do not meet the laboratory's criteria for acceptability.

D.6.2.5 All test reports or records of the information on the reports must be maintained by the laboratory in a manner that permits ready identification and timely accessibility.

D.6.2.6 Parentage/relationship testing reports must be released only to authorized individuals and must contain:

D.6.2.6.1 The date of collection of each specimen.

D.6.2.6.2 The name, address and phone number of the laboratory.

D.6.2.6.3 The name of each individual and the alleged relationship to the child.

D.6.2.6.4 The racial/ethnic origin(s) assigned by the laboratory to parties tested for the purpose of calculations.

D.6.2.6.5 The phenotypes established for each individual in each genetic system tested.

D.6.2.6.6 The identity of any subcontracting laboratory(ies) and that portion of the report for which each bears responsibility.

D.6.2.6.7 A statement as to whether or not the alleged relationship can be excluded. A single genetic inconsistency must not be the sole basis for a statement of exclusion.

D.6.2.6.8 When there is not a statement of exclusion, the report must contain:

D.6.2.6.8.1 The individual parentage/relationship index for each genetic system reported based on validated calculations using appropriate allele or haplotype frequencies and considering mutation rates, if applicable.

D.6.2.6.8.2 The combined parentage/relationship index.

D.6.2.6.8.3 The probability of parentage/ relationship expressed as a percentage. The prior probability(ies) used to calculate the probability of parentage must be stated.

D.6.2.6.9 If the results are inconclusive, an explanation as to the nature of the problem.

D.6.2.6.10 The original or password-protected electronic signature of the laboratory Director or Technical Supervisor or designee with a doctoral degree in medical, biological, clinical laboratory sciences, or genetics and qualified by training and experience.

D.6.3 Postanalytical systems assessment

D.6.3.1 Analytic systems assessment

D.6.3.1.1 If a laboratory performs the same test using different methodologies or instruments, or performs the same test at multiple testing sites, the laboratory must have a system that twice a year evaluates and defines the relationship between test results using the different methodologies, instruments, or testing sites.

D.6.3.1.2 The laboratory must establish and employ policies and procedures, and document actions taken when 1) test systems do not meet the laboratory's established criteria including quality control results that are outside of acceptable limits; and when 2) errors are detected in the reported clinical results. In the latter instance, the laboratory must promptly a) notify the authorized person ordering or individual utilizing the test results of reporting errors; b) issue corrected reports, and c) maintain copies of the original report as well as the corrected report for a minimum of two years, or the interval required by local, state, and federal regulations.

D.6.3.1.3 If immunogenetics, histocompatibility and/or transplantation immunology testing is referred, the subcontracting laboratory must be accredited by ASHI or CLIA or ARB approved (for laboratories outside the U.S.) to perform the referred testing. The referring laboratory must keep on file the following:

D.6.3.1.3.1 A copy of the subcontracting laboratory's accreditation documentation for those test systems

D.6.3.1.3.2 A copy of the testing laboratory's report.

D.6.3.2 Corrective Actions

D.6.3.2.1 Laboratories must have a mechanism in place for addressing any testing discrepancies that occur within or between different laboratories.

D.6.3.2.2 Corrective action policies and procedures must be available and followed as necessary to maintain the laboratory's operation for testing patient specimens in a manner that ensures accurate and reliable patient test results and reports.

D.6.3.2.3 The laboratory must document all corrective actions taken when test systems do not meet the laboratory's verified or established performance specifications which include, but are not limited to:

D.6.3.2.3.1 Equipment or methodologies that perform outside of established operating parameters or performance specifications.

D.6.3.2.3.2 Patient test values that are outside of the laboratory's reportable range of test results for the test system.

D.6.3.2.3.3 The reference intervals (normal values) for a test procedure that the laboratory determines are inappropriate for the laboratory's patient population.

D.6.3.2.3.4 Results of control and/or calibration materials fail to meet the laboratory's established criteria for acceptability. All patient test results obtained since the last acceptable test run must be evaluated to determine if patient test results have been adversely affected. The laboratory must take the corrective action necessary to ensure the reporting of accurate and reliable patient test results.

D.6.3.2.3.5 The criteria for proper storage of reagents and specimens are not met.

D.6.3.2.4 Any errors detected in patient or proficiency testing results must be documented, investigated, and corrective action taken as needed to prevent recurrence.

D.6.3.2.5 Any accidents determined to be attributable to inadequate laboratory space or to staff safety conditions must be documented, investigated, and corrective action taken, as needed, to prevent recurrence.

D.6.3.2.6 The laboratory must have a policy to address sentinel events/immediate jeopardy situations that includes immediate reporting to the ASHI Accreditation Program, with appropriate and complete documentation and investigation of the event.

D.6.3.3 Test records

D.6.3.3.1 The laboratory must maintain an information or record system that documents testing on all subjects and includes the following:

D.6.3.3.1.1 The test requisition, if applicable.

D.6.3.3.1.2 The positive and/or unique identification of the specimen.

D.6.3.3.1.3 The tissue source of the specimen

D.6.3.3.1.4 The date and time of specimen receipt into the laboratory.

D.6.3.3.1.5 The condition and disposition of specimens that do not meet the laboratory's criteria for specimen acceptability.

D.6.3.3.1.6 The records, test data, results, and dates of all specimen testing, including the identity of the personnel who performed the test(s).

D.6.3.3.1.7 Legally-reproduced copies of all preliminary and final reports.

D.6.3.3.1.8 Records of instrument printouts, if applicable,

D.6.3.3.1.9 Documented review of final test reports by a Director, Technical Supervisor or designee who meets, at a minimum, the requirements of General Supervisor.

D.6.3.3.2 Records for all subjects tested and all internal and external quality control tests must be retained for a minimum of two years, or longer as required by local, State, and/or Federal regulations.

D.6.3.3.3 Records may be saved in computer files only, provided that back-up files are maintained to ensure against loss of data.

D.6.3.3.4 If the laboratory ceases operation, the laboratory must make provisions to ensure that all records and, as applicable, slides, blocks, and tissue are maintained and available for the time frames specified in section D.6.3.3.2.

E. Personnel

E.1 Requirements

The laboratory must:

E.1.1 Have a director who meets the qualification requirements of section E.2.1 and provides overall management and direction, in accordance with section E.2.2.

E.1.2 Have a technical supervisor who meets the qualification requirements of section E.3.1 and provides overall technical supervision in accordance with section E.3.2.

E.1.3 Have a clinical consultant who meets the qualification requirements of section E.4.1 and provides overall clinical consultation in accordance with section E.4.2.

E.1.4 Have a general supervisor who meets the qualification requirements of section E.5.1 and provides overall general supervision in accordance with section E.5.2.

E.1.5 Have testing personnel who meet the qualification requirements of section E.6.1 and provide testing services and reporting of results in accordance with section E.6.2.

E.1.6 Have adequate staff to carry out the volume and variety of tests required without a degree of pressure that might contribute to errors.

E.1.7 Have all personnel meet the requirements of federal, state and local laws including state licensure where required.

E.2 Laboratory Director Qualifications and Responsibilities

E.2.1 Qualifications

The Laboratory Director must:

E.2.1.1 Be qualified by education, training and experience in each area of technology, analyte, test, or procedure for which the laboratory is ASHI-accredited to provide adequate management and direction of the laboratory personnel and activities. Assessment of qualifications for each area of accreditation will be the responsibility of the ASHI Director Training Review Committee. Assessment of qualifications for each technology will be the responsibility of the Accreditation Review Board.

E.2.1.2 Meet at least one of the following educational requirements:

E.2.1.2.1 Hold an earned doctoral degree in a chemical, physical, biological or clinical laboratory science from an accredited institution.

E.2.1.2.2 Be a doctor of medicine or osteopathy licensed to practice medicine or osteopathy in the state, country, or other jurisdiction in which the laboratory is located, if such licensing is required by law.

E.2.1.3 Meet at least one of the following certification requirements for areas of accreditation regulated by CLIA:

E.2.1.3.1 Be certified and continue to be certified in clinical or combined anatomic/clinical pathology by the American Board of Pathology or the American Osteopathic Board of Pathology or other appropriate medical board.

E.2.1.3.2 Be certified and continue to be certified by a Board approved by HHS.

E.2.1.3.3 For laboratories outside of the U.S.A, be certified and continue to be certified by an appropriate professional board or other certifying agency.

E.2.1.3.4 Or, before February 24, 2003, must have served as a director of an ASHI-accredited laboratory performing human histocompatibility and immunogenetics testing and must meet the requirements in E.2.1.1, E.2.1.2, and E.2.1.3.

E.2.1.4 Meet one of the following training requirements:

E.2.1.4.1 Have at least 2 years full-time post-doctoral laboratory training or experience in immunology, histocompatibility, immunogenetics, or a related field, or a residency in clinical or combined anatomical/clinical pathology or other related medical specialty, and have at least 2 years full-time post-doctoral training in directing or supervising high complexity testing in human histocompatibility and immunogenetics in an ASHI-accredited or approved laboratory.

E.2.1.4.2 If a candidate has relevant pre-doctoral experience supervising and/or performing high complexity testing in human histocompatibility and immunogenetics in an ASHI-accredited or approved laboratory, this may be credited at a rate of 0.5 years of post-doctoral training per each year of appropriate pre-doctoral experience up to a total of 2 of 4 years of post-doctoral experience.

E.2.2 Responsibilities

The Laboratory Director must:

E.2.2.1 Be responsible for the overall operation and administration of the laboratory, including the employment of personnel who are competent to perform test procedures, record and report test results promptly, accurately and proficiently, and assure compliance with the applicable regulations.

E.2.2.1.1 The Laboratory Director, if qualified, may function as the Technical Supervisor, Clinical Consultant, General Supervisor, and/or testing personnel or document delegation of these responsibilities to personnel meeting the qualifications under sections E.3, E.4, E.5, and E.6, respectively.

E.2.2.1.2 If the Laboratory Director reapportions performance of his or her responsibilities, he or she remains responsible for ensuring that all duties are properly performed.

E.2.2.2 Be accessible to the laboratory to provide on-site direction, telephone and electronic consultation that is commensurate with the workload. Each director may direct no more than five histocompatibility and/or immunogenetics laboratories.

E.2.2.3 Ensure that testing systems developed and used for each of the tests performed in the laboratory provide quality laboratory services for all aspects of test performance, which includes the pre-analytic, analytic, and post-analytic phases of testing.

E.2.2.4 Ensure that the physical plant and environmental conditions of the laboratory are appropriate for the testing performed and provide a safe environment in which employees are protected from physical, chemical, and biological hazards.

E.2.2.5 Ensure that the test methodologies selected have the capability of providing the quality of results required for patient care.

E.2.2.6 Ensure that verification procedures used are adequate to determine the accuracy, precision, and other pertinent performance characteristics of the method.

E.2.2.7 Ensure that laboratory personnel are performing the test methods as required for accurate and reliable results.

E.2.2.8 Ensure that the laboratory is enrolled in an ASHI-approved proficiency testing program for the testing performed and that:

E.2.2.8.1 The proficiency testing samples are tested as required in section C.

E.2.2.8.2 The results are returned within the timeframes established by the proficiency testing program.

E.2.2.8.3 All proficiency testing reports are received and reviewed by the appropriate staff to evaluate the laboratory's performance and to identify any problems that require corrective action.

E.2.2.8.4 An approved corrective action plan is followed when any proficiency testing result is found to be unsuccessful or unsatisfactory.

E.2.2.9 Ensure that the quality control and quality assessment programs are established and maintained to assure the quality of laboratory services provided and to identify failures in quality as they occur.

E.2.2.10 Ensure the establishment and maintenance of acceptable levels of analytical performance for each test system.

E.2.2.11 Ensure that all necessary remedial actions are taken and documented whenever significant deviations from the laboratory's established performance characteristics are identified, and that patient test results are reported only when the system is functioning properly.

E.2.2.12 Ensure that reports of test results include pertinent information required for interpretation, according to section D.6.2 of the Standards.

E.2.2.13 Ensure that consultation is available to the laboratory's clients on matters relating to the quality of the test results reported and their interpretation concerning specific patient conditions.

E.2.2.14 Ensure that a general supervisor provides on-site supervision of high complexity test performance in accordance with ASHI Standards.

E.2.2.15 Provide appropriate consultation and supervision to ensure accurate testing and reporting of test results for all aspects of services provided by the laboratory. Ensure that the laboratory employs a sufficient number of laboratory personnel with the appropriate qualifications as described in sections E.5 and E.6 of this document.

E.2.2.16 Ensure that prior to testing patient specimens there is documentation that all personnel have the appropriate education and experience, receive the appropriate training for the type and complexity of the services offered, and have demonstrated that they can perform all testing operations reliably to provide and report accurate results.

E.2.2.17 Ensure that policies and procedures are established for monitoring individuals who conduct preanalytical, analytical, and postanalytical phases of testing to ensure that each individual is competent to process specimens, to perform test procedures and to report test results promptly and proficiently, and whenever necessary, to identify needs for remedial training or continuing education to improve skills.

E.2.2.18 Ensure that an approved procedure manual is available to all personnel responsible for all aspects of the testing process.

E.2.2.19 Document the responsibilities and duties of each consultant, supervisor, and person engaged in the performance of the pre-analytic, analytic, and post-analytic phases of testing. The documentation must identify the procedures that each individual is authorized to perform, specify the supervision that is required for specimen processing, test performance or result reporting, and delineate the supervisory or director review that is required prior to reporting test results.

E.2.2.20 Ensure that each member of the technical staff participates in continuing education relevant to his/her areas of responsibility histocompatibility and/or immunogenetics testing at least to the level of the minimum requirements outlined by the ASHI Accreditation Review Board.

E.3 Technical Supervisor Qualifications and Responsibilities

E.3.1 Qualifications

The Technical Supervisor must:

E.3.1.1 Be qualified by education, training and experience for each area of technology, analyte, test, or procedure to provide adequate technical supervision of the laboratory personnel and activities for which the laboratory is ASHI-accredited. Assessment of qualifications for each area of accreditation will be the responsibility of the ASHI Director Training Review Committee. Assessment of qualifications for each technology will be the responsibility of the Accreditation Review Board.

E.3.1.2 Meet at least one of the following educational requirements:

E.3.1.2.1 Hold an earned doctoral degree in a chemical, physical, biological or clinical laboratory science from an accredited institution.

E.3.1.2.2 Be a doctor of medicine or osteopathy licensed to practice medicine or osteopathy in the state, country, or other jurisdiction in which the laboratory is located, if such licensing is required by law.

E.3.1.3 Meet at least one of the following certification requirements for areas of accreditation regulated by CLIA:

E.3.1.3.1 Be certified and continue to be certified in clinical or combined anatomic/clinical pathology by the American Board of Pathology or the American Osteopathic Board of Pathology or other appropriate medical board.

E.3.1.3.2 Be certified and continue to be certified by a Board approved by HHS.

E.3.1.3.3 For laboratories outside of the U.S.A, be certified and continue to be certified by an appropriate professional board or other certifying agency.

E.3.1.3.4 Or, before February 24, 2003, must have served as a Technical Supervisor of an ASHI-accredited laboratory performing human histocompatibility and immunogenetics testing and must meet the requirements in E.3.1.1, E.3.1.2, and E.3.1.3.

E.3.1.4 Meet one of the following training requirements:

E.3.1.4.1 Have at least 2 years full-time post-doctoral laboratory training or experience in immunology, histocompatibility, immunogenetics, or a related field, or a residency in clinical or combined anatomic/clinical pathology or other related medical specialty, and have at least 2 years full-time post-doctoral training in directing or supervising high complexity testing in human histocompatibility and immunogenetics in an ASHI-accredited or approved laboratory.

E.3.1.4.2 If a candidate has relevant pre-doctoral experience supervising and/or performing high complexity testing in human histocompatibility and immunogenetics in an ASHI-accredited or approved laboratory, this may be credited at a rate of 0.5 years of post-doctoral training per each year of appropriate pre-doctoral experience up to a total of 2 of 4 years of post-doctoral experience.

E.3.1.5 For laboratories performing ABO/Rh testing, the Technical Supervisor must meet the CMS requirements in Immunohematology or Histocompatibility as equivalent for the limited Immunohematology (ABO/Rh testing) performed by facilities using ASHI accreditation to meet CLIA requirements.

E.3.1.6 For laboratories performing General Immunology Testing (e.g., platelet antigen typing, platelet antibody identification and crossmatching; chimerism analysis; immunophenotyping; immune function testing; non-HLA polymorphic allele typing), the Technical Supervisor must meet the CMS requirements in general immunology which include one year of laboratory training or experience in high complexity testing within the specialty of diagnostic immunology.

E.3.1.7 For laboratories performing Virology Testing (e.g., NAT testing for HIV-RNA, HCV – RNA, HBV – DNA for deceased organ donors), the Technical Supervisor must meet the CMS requirements in virology which include one year of laboratory training or experience in high complexity testing within the specialty of microbiology with a minimum of 6 months of laboratory training or experience in high complexity testing within the subspecialty of virology

E.3.1.8 For laboratories performing Syphilis Serology Testing (e.g., the RPR (flocculation test), the Treponemal Test (EIA) for deceased organ donors), the Technical Supervisor must meet the CMS

requirements in syphilis serology which include one year of laboratory training or experience in high complexity testing within the specialty of diagnostic immunology.

E.3.2 Responsibilities

E.3.2.1 The Technical Supervisor is responsible for the technical and scientific oversight of the laboratory.

E.3.2.2 The Technical Supervisor is required to be on-site commensurate with workload, be accessible for all hours of laboratory operation, and provide telephone or electronic consultation as needed.

E.3.2.3 The Technical Supervisor is responsible for:

E.3.2.3.1 Selection of the test methodology that is appropriate for the clinical use of the test results.

E.3.2.3.2 Verification of the test procedures performed and establishment of the laboratory's test performance characteristics, including the precision and accuracy of each test and test system.

E.3.2.3.3 Enrollment and participation in an ASHI-approved proficiency testing program commensurate with the services offered.

E.3.2.3.4 Establishing a quality control program appropriate for the testing performed and establishing the parameters for acceptable levels of analytic performance and ensuring that these levels are maintained throughout the entire testing process from the initial receipt of the specimen, through sample analysis and reporting of test results.

E.3.2.3.5 Resolving technical problems and ensuring that remedial actions are taken whenever test systems deviate from the laboratory's established performance specifications.

E.3.2.3.6 Ensuring that patient test results are not reported until all corrective actions have been taken and the test system is functioning properly.

E.3.2.3.7 Identifying training needs and assuring that each individual performing tests receives regular in-service training and education appropriate for the type and complexity of the laboratory services performed.

E.3.2.3.8 Evaluating and documenting the competency, as defined in D.2.6., of all individuals responsible for testing.

E.3.2.3.9 Ensuring that the technical staff participates in continuing education relevant to Histocompatibility testing at least to the level of the minimum requirements outlined by the ASHI Accreditation Review Board.

E.4 Clinical Consultant Qualifications and Responsibility

E.4.1 Qualifications

The Clinical Consultant must:

E.4.1.1 Have sufficient training and experience in the areas of the laboratory's ASHI accreditation to be qualified to consult with and render opinions to the laboratory's clients concerning the appropriateness of human immunogenetics, histocompatibility and/or transplantation immunology testing ordered and the interpretation of these test results in relation to diagnosis, treatment and management of patient care.

E.4.1.2 Meet at least one of the following requirements:

E. 4.1.2.1 Hold an earned doctoral degree in a chemical, physical, biological or clinical laboratory science

from an accredited institution and qualify as an ASHI laboratory director or technical supervisor and be board certified by a board approved by HHS or equivalent certification outside of the U.S.

E.4.1.2.2 Be a doctor of medicine or osteopathy licensed to practice medicine or osteopathy in the state or country in which the laboratory is located, if such licensing is required by law.

E.4.1.3 For laboratories performing ABO/Rh testing, meet the CMS requirements for clinical consultant in Immunohematology or Histocompatibility as equivalent for the limited Immunohematology (ABO/Rh testing) performed by facilities using ASHI accreditation to meet CLIA requirements.

E.4.2 Responsibilities

The Clinical Consultant must:

E.4.2.1 Provide consultation regarding the appropriateness of the testing ordered and clinical interpretation of test results in a timely manner.

E.4.2.2 Provide consultation to the laboratory's clients.

E.4.2.3 Assist the laboratory's clients in ensuring that appropriate tests are ordered to meet the clinical need.

E.4.2.4 Ensure that reports of test results include pertinent information required for specific patient interpretation.

E.4.2.5 Ensure that consultation is available and communicated to the laboratory's clients on matters related to the quality of the test results reported and their interpretation concerning specific patient conditions in a timely manner appropriate to the testing performed.

E.5 General Supervisor

E.5.1 Qualifications

E.5.1.1 Each General Supervisor must have sufficient training and experience to:

E.5.1.1.1 Provide day-to-day supervision of testing personnel and reporting of test results, under the direction of the Laboratory Director and supervision of the Technical Supervisor.

E.5.1.1.2 Be responsible for the proper performance of all laboratory procedures and reporting of test results, in the absence of the Laboratory Director and Technical Supervisor.

E.5.1.2 Each General Supervisor(s) must meet at least one of the following requirements:

E.5.1.2.1 Laboratory Director under section E.1

E.5.1.2.2 Technical Supervisor under E.2

E.5.1.2.3 Be a doctor of medicine, osteopathy, or podiatric medicine licensed to practice medicine, osteopathy, or podiatry in the State in which the laboratory is located and have at least three years of laboratory training or experience in human immunogenetics, human histocompatibility and/or human transplantation immunology testing under the supervision of a director and/or technical supervisor of an ASHI accredited laboratory. Credentialing by the American Board of Histocompatibility and Immunogenetics as a Certified Histocompatibility Technologist (CHT) or Certified Histocompatibility Specialist (CHS) certification is strongly recommended.

E.5.1.2.4 Have earned a doctoral, master's, or bachelor's degree in a chemical, physical, biological or clinical laboratory science or medical technology from an accredited institution; and have at least three years of laboratory training or experience in human immunogenetics, human histocompatibility and/or human transplantation immunology testing under the supervision of a director and/or technical

supervisor of an ASHI-accredited or an equivalent ARB-approved laboratory. Credentialing by the American Board of Histocompatibility and Immunogenetics as a Certified Histocompatibility Technologist (CHT) or Certified Histocompatibility Specialist (CHS) certification is strongly recommended.

E.5.1.2.5 Have served as a General Supervisor of an ASHI-accredited laboratory on or before February 28, 1992 and:

E.5.1.2.5.1 Is qualified as a laboratory technologist under E.6; and

E.5.1.2.5.1.1 After qualifying as a laboratory technologist, has had at least six years of pertinent full-time laboratory experience of which not less than two years have been spent working in the designated laboratory specialty; or

E.5.1.2.5.1.2 With respect to individuals first qualifying before July 1, 1971, has had at least 15 years of pertinent full-time laboratory experience before January 1, 1968; this required experience may be met by the substitution of education for experience.

E.5.2 Responsibilities

E.5.2.1 The General Supervisor is responsible for day-to-day supervision or oversight of the laboratory operation and personnel performing testing and reporting test results.

E.5.2.2 The General Supervisor must:

E.5.2.2.1 Be accessible to testing personnel at all times testing is performed to provide primarily on-site supervision and telephone or electronic consultation as needed to resolve technical problems in accordance with policies and procedures established either by the laboratory director or technical supervisor.

E.5.2.2.2 Be responsible for providing day-to-day supervision of test performance by testing personnel.

E.5.2.2.3 Perform a timely review (appropriate to the clinical circumstances) of all testing performed and reported by testing personnel in the absence of an on-site general supervisor.

E.5.2.2.4 Be responsible for monitoring test analyses and specimen examinations to ensure that acceptable levels of analytic performance are maintained.

E.5.2.3 The Director or Technical Supervisor may delegate to the General Supervisor the responsibility for:

E.5.2.3.1 Assuring that all remedial actions are taken whenever test systems deviate from the laboratory's established performance specifications.

E.5.2.3.2 Ensuring that patient test results are not reported until all corrective actions have been taken and the test system is properly functioning.

E.5.2.3.3 Providing orientation and training for testing personnel.

E.5.2.3.4 Annually evaluating and documenting the performance of testing personnel under their supervision.

E.6 Testing Personnel Qualifications and Responsibilities

E.6.1 Qualifications

Each individual performing high complexity testing must:

E.6.1.1 Meet one of the following requirements:

E.6.1.1.1 Be a doctor of medicine or a doctor of osteopathy licensed to practice medicine or osteopathy in the State in which the laboratory is located or have earned a doctoral, master's or bachelor's degree in a chemical, physical, biological or clinical laboratory science, or medical technology from an accredited institution.

E.6.1.1.2 Have earned an associate degree in a laboratory science, or medical laboratory technology from an accredited institution.

E.6.1.1.3 Have education and training equivalent to that specified in paragraph E.6.1.2.2 of this section that includes at least 60 semester hours, or equivalent, from an accredited institution that, at a minimum, includes either:

E.6.1.1.3.1 Twenty four semester hours of medical laboratory technology courses; or

E.6.1.1.3.2 Twenty four semester hours of science courses that include:

E.6.1.1.3.2.1 Six semester hours of chemistry.

E.6.1.1.3.2.2 Six semester hours of biology.

E.6.1.1.3.2.3 Twelve semester hours of chemistry, biology, or medical laboratory technology in any combination.

E.6.1.2 Have laboratory training that includes any of the following:

E.6.1.2.1 Completion of a clinical laboratory training program in an ASHI-accredited laboratory or an equivalent organization approved by HHS. This training may be included in the 60 semester hours listed in paragraph E.6.1.1.3 of this section.

E.6.1.2.2 At least three months documented laboratory training in each specialty in which the individual performs high complexity testing.

E.6.1.2.3 Have previously qualified or could have qualified as a technologist in an ASHI accredited laboratory on or before February 28, 1992.

E.6.1.2.4 On or before April 24, 1995 be a high school graduate or equivalent and have either:

E.6.1.2.4.1 Graduated from a medical laboratory or clinical laboratory training program approved or accredited by Accrediting Bureau of Health Education Schools (ABHES) and Christian Adult Higher Education Association (CAHEA), or other organization approved by HHS or

E.6.1.2.4.2 Successfully completed an official U.S. military medical laboratory procedures training course of at least 50 weeks duration and have held the military enlisted occupational specialty of Medical Laboratory Specialist (Laboratory Technician);

E.6.1.2.5 Until September 1, 1997, have an earned a high school diploma or equivalent; and have documentation of training appropriate for the testing performed before analyzing patient specimens. Such training must ensure that the individual has:

E.6.1.2.5.1 The skills required for proper specimen collection, including patient preparation, if applicable, labeling, handling, preservation or fixation, processing or preparation, transportation and storage of specimens.

E.6.1.2.5.2 The skills required for implementing all standard laboratory procedures.

E.6.1.2.5.3 The skills required for performing each test method and for proper instrument use.

E.6.1.2.5.4 The skills required for performing preventive maintenance, troubleshooting, and calibration procedures related to each test performed.

E.6.1.2.5.5 A working knowledge of reagent stability and storage.

E.6.1.2.5.6 The skills required to implement the quality control policies and procedures of the laboratory.

E.6.1.2.5.7 An awareness of the factors that influence test results.

E.6.1.2.5.8 The skills required to assess and verify the validity of patient test results through the evaluation of quality control values before reporting patient test results.

E.6.1.2.6 In order to qualify as high complexity testing personnel under 42 CFR 493.1489(b)(3), the individual must have met or could have met the following qualifications for technologist as they were in effect on or before February 28, 1992. Each technologist must:

E.6.1.2.6.1 Have earned a bachelor's degree in medical technology from an accredited university; or

E.6.1.2.6.1.1 Have successfully completed three years of academic study (a minimum of 90 semester hours or equivalent) in an accredited college or university, which met the specific requirements for entrance into a school of medical technology accredited by an accrediting agency approved by the Secretary, and has successfully completed a course of training of at least 12 months in such a school; or

E.6.1.2.6.1.2 Have earned a bachelor's degree in one of the chemical, physical, or biological sciences and, in addition, has at least 1 year of pertinent full-time laboratory experience or training, or both, in the area in which the individual performs tests; or

E.6.1.2.6.1.3 Have successfully completed three years (90 semester hours or equivalent) in an accredited college or university with the following distribution of courses:

E.6.1.2.6.1.3.1 For those whose training was completed before September 15, 1963, at least 24 semester hours in chemistry and biology courses of which:

E.6.1.2.6.1.3.1.1 At least 6 semester hours were in inorganic chemistry and at least 3 semester hours were in other chemistry courses; and

E.6.1.2.6.1.3.1.2 At least 12 semester hours in biology courses pertinent to the medical sciences; or

E.6.1.2.6.1.3.2 For those whose training was completed after September 14, 1963:

E.6.1.2.6.1.3.2.1 Sixteen semester hours in chemistry courses that included at least 6 semester hours in inorganic chemistry and that are acceptable toward a major in chemistry.

E.6.1.2.6.1.3.2.2 Sixteen semester hours in biology courses that are pertinent to the medical sciences and are acceptable toward a major in the biological sciences.

E.6.1.2.6.1.3.2.3 Three semester hours of mathematics.

E.6.1.2.6.1.3.2.4 Experience, training, or both, covering several fields of medical laboratory work of at least 1 year and of such quality as to provide him or her with education and training in medical technology equivalent to that described in paragraphs 42 CFR 493.1491 (b)(1) and (2); or

E.6.1.2.6.1.3.3 With respect to individuals first qualifying before July 1, 1971, the technologist:

E.6.1.2.6.1.3.3.1 Was performing the duties of a laboratory technologist at any time between July 1, 1961, and January 1, 1968; and

E.6.1.2.6.1.3.3.2 Has had at least 10 years of pertinent laboratory experience prior to January 1, 1968. (This required experience may be met by the substitution of education for experience); or

E.6.1.2.6.1.3.3.3 Achieves a satisfactory grade in a proficiency examination approved by HHS.

E.6.2 Responsibilities

E.6.2.1 The testing personnel are responsible for specimen processing, test performance and for reporting test results.

E.6.2.2 Each individual performs only those tests that are authorized by the laboratory director and require a degree of skill commensurate with the individual's education, training or experience, and technical abilities.

E.6.2.3 Each individual performing testing must:

E.6.2.3.1 Follow the laboratory's procedures for specimen handling and processing, test analyses, reporting and maintaining records of patient test results.

E.6.2.3.2 Maintain records that demonstrate that proficiency testing samples are tested in the same manner as patient specimens.

E.6.2.3.3 Adhere to the laboratory's quality control policies, document all quality control activities, instrument and procedural calibrations and maintenance performed.

E.6.2.3.4 Follow the laboratory's established policies and procedures whenever test systems are not within the laboratory's established acceptable levels of performance.

E.6.2.3.5 Be capable of identifying problems that may adversely affect test performance or reporting of test results and either must correct the problems or immediately notify the general supervisor, technical supervisor, clinical consultant or director.

E.6.2.3.6 Document all corrective actions taken when test systems deviate from the laboratory's established performance specifications.

E.6.2.3.7 All testing personnel, except as stated below, may perform testing and report results without direct supervision, provided the general supervisor, qualified under Section E.5, reviews all testing performed and reported in a timely manner appropriate to the clinical circumstances.

E.6.2.3.7.1 Testing personnel qualified under E.6.1.2.4 or E.6.1.2.5 may only perform testing under direct supervision, except for those testing personnel performing high complexity testing before 1/19/1993.

E.6.2.3.7.2 The general supervisor must be accessible to testing personnel at all times high complexity testing is performed to provide supervision and telephone or electronic consultation as needed to resolve technical problems in accordance with policies and procedures established by the laboratory director.

E.6.2.4 If deceased donor transplant testing is performed, personnel for the required histocompatibility testing must be available 24 hours a day, seven days a week.

E.7 The Director, Technical Supervisor, Clinical Consultant, Director in Training, and technical staff must participate in continuing education relevant to the areas of the laboratory accreditation, at least to the level of the minimum requirements outlined by the ASHI Accreditation Review Board.

E.8 Laboratories performing parentage/relationship testing must meet the above requirements and the following:

E.8.1 Have a Director and Technical Supervisor qualified by advanced training and/or experience in parentage/relationship testing.

E.8.2 Have a qualified individual available for legal testimony, as needed.

E.8.3 Document expertise and/or accreditation for all genetic systems that are used for parentage testing/relationship.