Standards for Accredited Laboratories

American Society for Histocompatibility and Immunogenetics

2019 Revised Standards approved by the ASHI Board of Directors

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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, and transplantation 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.	**Re: A.1.2 -** Certain rare cases in which Standards are indicated to apply only to UNOS laboratories or only to U.S. Laboratories (e.g., the requirement to include the FDA disclaimer on reports) are exceptions to Standard A.1.2
A.2 Abbreviations **ARB** Accreditation Review Board **ASHI** The American Society for Histocompatibility and Immunogenetics. **CDC** Centers for Disease Control and Prevention **CFR** US Code of Federal Regulations **CLIA** Clinical Laboratory Improvement Amendments of 1988. CLIA regulations are defined in 42 CFR 493. **CMS** US Centers for Medicare and Medicaid Services **CPRA** Calculated Panel Reactive Antibody **CREG** Cross Reactive Group **DNA** Deoxyribonucleic acid **EFI** European Federation for Immunogenetics **ELISA** Enzyme-linked immunosorbent assay **HHS** US Department of Health and Human Services **HIPAA** Health Insurance Portability and Accountability Act. HIPAA Privacy Rule defined in 45 CFR part 160 and Subparts A and E of part 164 **KIR** Killer-cell immunoglobulin-like receptor **MLC** Mixed leukocyte culture	

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NFPA National Fire Protective Agency **NGS** Next Generation Sequencing **NMDP** National Marrow Donor Program. **OPO** Organ Procurement Organization **OPTN** Organ Procurement and Transplantation Network **OSHA** Occupational Safety and Health Administration. OSHA regulations are defined in 29 CFR 1910. **PCR** Polymerase chain reaction **PRA** Panel Reactive Antibody **PT** Proficiency Testing **QA** Quality Assessment **qPCR** Quantitative PCR, aka real-time PCR **SBT** Sequencing-Based Typing **SDS** Safety Data Sheet **SSOP** Sequence Specific Oligonucleotide Probe **SSP** Sequence Specific Primer **STR** Short tandem repeat **TRALI** Transfusion Related Acute Lung Injury **UNet**[SM]: The secure Internet based transplant information database created by the United Network for Organ Sharing (UNOS). **UNOS** United Network for Organ Sharing **US / USA** United States of America **VNTR V**ariable **N**umber of **T**andem **R**epeats **WHO** World Health Organization	

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A.3 Definitions The following definitions apply, unless the context indicates otherwise: **Accuracy:** Correctness or freedom from error (for example, obtaining the expected HLA-allele assignment in a Proficiency Test). **Adapters or Adaptor Sequences (in regards to NGS):** Short oligonucleotides that are attached to the DNA to be sequenced and provide a means to capture the sequence on the sequencing support and a priming site for amplification and/or sequencing of the adjoining nucleic acid. Adapter sequences are complementary to platform-specific PCR and sequencing primers. Adapters are added by ligation or as part of a PCR enrichment step that is included in most protocols. **Ambiguous:** A test result that may be interpreted in two or more possible ways. **Analyte**: A substance or constituent for which the laboratory conducts testing. **ASHI Accreditation Review Board (ARB):** 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:** A laboratory that has applied for and been accredited by ASHI by satisfying all applicable requirements of the accreditation process. **ASHI-approved laboratory:** A laboratory outside the United States that meets ASHI requirements for accreditation, but is not required to follow CMS regulations **Authorized person:** An individual authorized under state law to order tests or receive test results or both. **Barcoding, or indexing tags (in regards to NGS):** The molecular tagging of samples with unique sequence-based codes, typically consisting of three or more base pairs (usually on the adapter sequence) allowing pooling of multiple samples. **Calibration:** A process of testing and adjusting an instrument or test system to establish a correlation between the measurement response and an established reference standard. **Category:** The type of testing performed in an accredited laboratory.	**Re: A.3 Analyte -** used in relation to Proficiency Testing refers to all Class I or Class II locus tests for a single sample for any method or combination of methods or any level of resolution that is reported and graded separately. A method is graded separately if it serves as a "stand alone test" for the analyte.

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CLIA certificate: 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:** A procedure used for patient care to determine the characteristic presence, absence, or quantity of an analyte in a human specimen. **Complaint:** A written and/or verbal report made to ASHI that alleges noncompliance with ASHI Standards **or** with federal, state and/or local laws and regulations. **Confirmatory Testing**: A second analytical procedure performed to substantiate or bring into question the results of an initial laboratory test. **Coverage (in regards to NGS):** The percentage of bases called at predetermined depth for a genomic region of interest. **CPRA:** The calculated PRA is an estimation of the likelihood that a patient will have a positive crossmatch when tested against the donor population. This estimation is based on the HLA phenotypic frequencies in the donor population and the unacceptable antigens listed for the patient. **CREG:** A group of serologically cross-reactive HLA antigens. **Depth of coverage (in regards to NGS):** The number of individual sequence reads that align to a particular nucleotide position, which is often used to define the trustworthiness or quality of the sequence. **Designee:** 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. **Distributive Testing:** Laboratory testing performed on the same specimen, or an aliquot of it, that requires sharing it between two or more laboratories to obtain all data required to complete an interpretation or calculation necessary to provide a final reportable result for the originally ordered test. When such testing occurs at multiple locations with different CLIA certificates, it is considered distributive testing. **Established:** Validated in the laboratory and based upon documented local data and/or published peer reviewed data.	

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Federal, state and local laws: Laws or regulations issued by any federal, national, state, provincial, city, or other authority which has jurisdiction in the laboratory's location. **Flowcell (in regards to NGS):** A glass slide with sample lanes etched on it and a cover slip positioned on top; tiny volumes of liquid can be pumped over the flowcell. In some platforms, the flowcell has a lawn of primers that have sequences that match the adapter sequence.	
High Resolution Typing: A high-resolution HLA typing is defined as an allele or set of alleles that encode the same protein sequence for the region of the HLA molecule called the antigen binding site. The high resolution HLA typing results must contain only one unambiguously assigned genotype or may contain multiple alternative genotypes if only one includes two common and well-documented (CWD) alleles. https://protect2.fireeye.com/url?k=48d5e2bd-14958feb-48d5c5a0-0cc47adb57f0-bf6293e266775110&u=http://igdawg.org/cwd.html **IMGT/HLA Sequence Database:** Specialist databases 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/ipd/imgt/hla/ **Immediate Jeopardy:** 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. **Informatics Pipeline (in regards to NGS-HLA):**The computational work flow through which raw sequencing reads, obtained from a particular NGS platform, are processed to obtain HLA genotyping. Specific elements of the pipeline include: (1) the individual algorithms each of which performs a particular task executed by a software in a particular order (i.e. demultiplexing or alignment of reads or HLA genotyping), (2) the work flow management framework whereby the inputs and outputs from different modules/software are properly coordinated, and (3) the complete computer infrastructure, including operating system, hardware specifications, whether local (on-premises) or the cloud and reference data bases (i.e. IMGT/HLA data) needed to process large volumes of NGS data in a scalable manner. **Kit:** All components of a test that are packaged together. **Library Preparation (in regards to NGS):** The process of creating DNA fragments, of a certain size range, with adapter sequences on both ends. For most applications/platforms, PCR amplification of the library is necessary prior to sequencing. **Low resolution typing:** A low-resolution HLA typing result is defined as sufficient to assign typing to the level of serological splits. Some alleles may require 2-field typing results to satisfy this requirement. A list of serological splits can be accessed at http://hla.alleles.org/nomenclature/index.html	

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Luminometry: The measurement of photons of light emitted by chemiluminescent reactions in the electromagnetic spectrum ranging from 360 to 700nm. **Massively parallel sequencing (in regards to NGS):** A technique in which many sequencing reactions occur and are detected simultaneously. **Mate-pair mapping (in regards to NGS):** A set of sequencing joined fragments brought together from long, known, genomic distances which can be used to identify structural rearrangements. **May:** Permissive term used primarily for clarity. **Microarray:** 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 or a set of markers placed at defined positions on a solid substrate. **Minority allele** (in regards to NGS): An allele that is less represented than another allele when preferential amplification is present. **Must:** Compliance with the standard is required at all times. **Next Generation Sequencing (NGS):** A technology that utilizes clonally-amplified or single molecule templates, which are sequenced in a massively parallel fashion resulting in increased throughput by several orders of magnitude. NGS incorporates two inextricably linked processes: (1) the analytical wet bench process of sample and library preparation (which may or may not include target amplification) and sequence generation, and (2) the informatics pipeline. **Paired-end read mapping (in regards to NGS):** A set of independent reads that are derived from the same library fragment which can be used to identify structural rearrangements. **Performance characteristic:** 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:** 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:** Performed and documented at predetermined fixed intervals. **Physician:** An individual appropriately licensed as a doctor of medicine, doctor of osteopathy, or doctor of podiatric medicine by the state or other location in which they practice. **PRA:** The Panel Reactive Antibody (PRA) measures the reactivity of a patient serum towards a panel of HLA antigens. It is expressed as a percentage that defines the likelihood of the patient having a positive crossmatch. **Precision:** The agreement between repeated measurements; an indication of the random error. **Primer:** 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.	**Re: A.3 Physician -** Some states or locations require that physicians licensed by foreign nations or by other states must submit credentials for certification prior to being recognized as a physician with all rights and privileges thereto.

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Probe: 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:** A series of steps followed in a specific order to accomplish a task. **Proficiency testing:** Testing performed on a set of specimens that includes a system to appropriately evaluate and score the testing results and to identify performance problems or system errors. **Record:** Written or electronic information regarding subjects, samples, testing, laboratory Quality Control and Quality Assurance activities. **Redefine:** To reexamine or reevaluate especially with a view to change. **Referee laboratory:** 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:** 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 laboratory. **Reflex Testing:** Confirmatory or additional laboratory testing that is automatically performed by a laboratory under its standard operating procedures for patient specimens when the laboratory's findings indicate test results that are abnormal, are outside a predetermined range, or meet other pre-established criteria for additional testing. **Registry donor:** A person who has consented to be listed on a registry as a potential volunteer donor of hematopoietic progenitor cells or other blood products. **Report:** The test results provided to the authorized person who ordered or requested the testing and/or sent to be part of the medical record. **Sensitivity:** The probability that a test will be positive when a particular analyte, sequence or protein is present. **Sentinel Event:** 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. **Shall:** Compliance with the standard is required at all times. **Should:** An activity that is recommended or advised, but for which there may be effective alternatives. **Specificity:** The probability that the test will be negative when the specific analyte, sequence or protein is absent.	
Standard Precautions: 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. **Survey:** The set of testing events in a specific test category of external proficiency testing.	**Re: A.3 Test method -** Because CMS allows a laboratory to utilize a specific test method once it has been validated and approved by the laboratory director, ASHI

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Target enrichment (in regards to NGS): The isolation of genes or regions of interest prior to sequencing. **Test method:** The specific assay utilized in determining a clinical result. In these standards, the terms method and technique are used interchangeably. **Test system:** The actual assay system utilized in determining results in a testing category. **Unknown:** 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. Proficiency testing samples may serve as unknowns for individual technical competency. **Unresolved alleles:** alleles or genotypes that have not been excluded.	will no longer require laboratories to submit validation packets for individual testing methods. Test methods include but are not limited to CDC, SSOP, SSP, SBT, Solid Phase Assays. **Re: A.3 Test system -** ASHI-defined test systems include but are not limited to high or low resolution molecular typing, serological typing, flow cytometry, cellular methods, complement dependent cytotoxicity.
Unsatisfactory proficiency testing performance: Failure to attain the acceptable response for an analyte or test or a testing event	**Re: A.3 Unsatisfactory** - Is defined by CMS for serologic ABO as <100% concordance and <80% for all other tests.
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.	**Re: A.3 Unsuccessful** - The same definition of "Unsuccessful" applies to all analytes, including ABO typing. Unsuccessful performance requires enhanced PT.
Validated: A test system that has been proven to produce accurate results by comparison with (1) results from a qualified laboratory, (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**: 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. **Virtual Crossmatch:** An assessment of immunologic compatibility based on the patient's alloantibody profile compared to the donor's histocompatibility antigens.	**Re: A.3 Validated -** ARB policies require submission of validation materials to the laboratory's Commissioner if adding a new testing category or system. New methods in existing categories / systems do not need to be submitted to the commissioner, however, the validation materials must be available to the onsite inspector. **Re: A.3 Virtual Crossmatch** - These standards apply ONLY where a physical crossmatch is not performed prospectively.
A.4 Applicability	**Re: A.4 Applicability -** Per CMS, any (ASHI purview) test results for U.S. patients that are reported to physicians with patient

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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 typing for registries. If any immunogenetics and/or transplantation 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.	identifiers cannot be called "research" tests. These tests must be included in the approved areas and technologies. All relevant Standards apply.
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.1.1.1 The laboratory's CLIA certificate must be conspicuously posted in the clinical laboratory. If applicable, the laboratory's state license must be conspicuously posted. If required for the state where the laboratory is located, the license or current renewal permit of each person performing testing must be conspicuously posted. **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.	**Re: B.1.1 -** ASHI may grant accreditation to laboratories holding a valid CLIA certificate, regardless of whether or not a laboratory is using ASHI for CLIA purposes. The inspector must verify this by asking to see the laboratory's current CLIA certificate. This applies to all accredited laboratories accepting specimens from U.S. patients. **Re: B.1.2.3** - Since ASHI has deemed status to accredit laboratories for CMS, CMS reserves the right to perform random inspections of laboratories using ASHI for CMS certification to validate ASHI's performance. Any selected laboratory would receive 2 weeks' notice.
B.1.2.4 Permit the ASHI Accreditation Program and HHS to monitor the correction of any deficiencies found through the inspection process.	**Re: B.1.2.4 -** Previous deficiencies will be reviewed during the next inspection.
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.	**Re: B.1.2.7 -** A laboratory must have evidence of a process or a policy that informs staff of the mechanism of filing an anonymous complaint, e.g., the contact information for the ASHI Ombudsperson(s) or a posted sign with appropriate contact information.

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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 and HHS if using ASHI for compliance with CLIA regulations 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.	**Re: B.2.1 -** CMS considers any laboratory that lacks an individual fulfilling the qualifications of any one of these required positions to have a "Mandatory Citation."
B.2.2 ASHI-accredited laboratories seeking additional areas of accreditation, new categories or test systems 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 categories or test systems.	
C. Proficiency Testing **C.1 Enrollment, Testing and Evaluation of Samples** **C.1.1** For each analyte 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.	**Re: C.1.1 -** When multiple primary methods are used to test a single analyte, the lab must either subscribe to separate PT for each method or rotate PT samples from a single survey amongst various methods.

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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.1.1.6** Laboratories must prospectively designate in writing one external PT provider per analyte on an annual basis for the purpose of grading**.**	A primary method is: (1) used on a routine basis and either (2) used to report results as a standalone method or (3) when used in conjunction with another method(s) is the source of the primary reported results.
C.1.2 Laboratories performing proficiency testing must not engage in any inter-laboratory communications pertaining to the results of proficiency testing sample(s) until after the reporting deadline has passed. This includes situations in which one Director oversees multiple laboratories. **C.1.3** Laboratories must not send their proficiency testing results or their proficiency testing samples to another laboratory for analysis.	**Re: C.1.2 -** There can be no participation of another laboratory in reported PT results even though that is routinely done for clinical specimens. This could be considered a situation of "Immediate Jeopardy." **Re: C.1.3** - Any laboratory that receives a proficiency testing sample from another laboratory for testing must notify CMS of the receipt of that sample regardless of whether the referral was made for reflex or confirmatory testing, or any other reason.
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:	**Re: C.1.4 -** Patient specimens tested the same day as proficiency testing specimens must use the same procedures and/or reagents. PT specimens must be tested by personnel who routinely perform similar testing for patient specimens. **Re: C.1.4.2** – If the laboratory patient specimen testing procedures normally require reflex, distributive, or confirmatory

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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.	testing at another location, the laboratory must test the proficiency testing sample as it would a patient specimen only up to the point it would refer to a second laboratory and no further. Please refer to the ARB Operations Manual section IV. Proficiency Testing (PT) Requirements (part H). **Re: C.1.5.2** – CMS considers that the laboratory's PT records must include signed (or pass-word protected, electronically signed) copies of the attestation statement, not just a printed copy with the printed names of the testing personnel and the laboratory director.
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 serologic ABO/RhD 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 serologic ABO/RhD typing, satisfactory performance is 100% concordance.	**Re: C.2.1 -** Note: For any CMS Regulated Analyte, e.g., serologic ABO/RhD Typing, CLIA certified Laboratories must have a mechanism for reporting each PT Survey's results to CMS at the time the results are available. ABO/RhD by DNA methods may be performed to predict the ABO/RhD phenotype. The use of molecular DNA based screening assays is not acceptable for ABO and RhD assignment for the purposes of transfusion or transplantation. Laboratories performing molecular ABO/RhD genotyping must participate in PT (e.g. shared samples with another laboratory or in-house comparisons) with satisfactory performance of at least 80% concordance. 100% concordance is required for serological ABO/RhD testing.
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.	**Re: C.2.2 -** If a CLIA Certified laboratory's ABO/RhD typing is unsatisfactory in 2 consecutive or 2 of 3 assessments, testing

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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 ≥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.	must be outsourced until 2 consecutive satisfactory performances have occurred **Re: C.2.2 -** If a laboratory mis-assigns one DRB1 type in one sample and one DQB1 type in another sample with 5 samples in a send-out, performance is unsatisfactory (60%) for that assessment. **Re: C.2.3 -** Ungraded PT results must have documentation of review and corrective actions taken, if warranted (e.g., if the laboratory has a discordant result when the consensus ≥ 60%). Corrective action may also be warranted when a result was not graded because not enough laboratories have reported results (e.g., for *DQA1* typing).
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.	**Re: D.1.1 -** The QA program must include indicators of quality that will be monitored for all phases of laboratory testing. Appropriate (not just easily obtained) thresholds must be established for each indicator
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.	**Re: D.1.2 -** There must be a mechanism (e.g., a QA report) to summarize findings
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.	**Re: D.2.1.1 -** There must be evidence that policies and procedures are revised to prevent recurrence of problems. Follow-up procedures must assess the effectiveness of corrective actions. Discussion with the staff of problems must be documented
D.2.1.2 The laboratory must be in compliance with all applicable federal, state and local laws including but not limited to, laboratory and personnel licensure, 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.	**Re: D.2.1.2 -** Per OSHA, U.S. laboratories must have access to an updated SDS Manual. Other local requirements are likely to include training programs to review safety

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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.	requirements for "blood-borne pathogens" including use of personal protective equipment and periodic fire drills with exit routes posted. The laboratory is expected to know what these requirements are. For laboratories in the state of California: Every person or clinical laboratory licensed or registered under this chapter shall report to the California Department of Public Health, Laboratory Field Services within 30 days of change of name or address.
D.2.1.4 All records must be retained for a minimum of two years or longer, as specified by federal, national, provincial, state, local or other authorities that have jurisdiction in the laboratory's location, and must be maintained and stored under conditions that ensure proper preservation and retrieval.	
D.2.1.5 The laboratory must have emergency operation policies, processes, and procedures to respond to the effects of internal and external disasters.	**Re: D.2.1.5 -** The laboratory must have a policy that describes its plan to respond to an internal and external disaster's impact on laboratory operation based on the type of disaster that might possibly occur in its geographical location (e.g., hurricane, tornado, earthquake).It is recommended that the laboratory develop one or more written agreements with outside laboratories capable of accepting transferred tests in the event of an internal or external disaster. This is especially important if the laboratory testing is not covered by an existing facility-wide disaster plan.
D.2.2 Facilities **D.2.2.1** Laboratory space must be sufficient such 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.	**Re: D.2.2.3 -** Uninterruptible or emergency power supplies must be available at least for equipment essential for 24 hour deceased donor testing and preservation of essential specimens and reagents, as applicable.

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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 laboratory 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 barriers from both the pre-amplification work area and post-amplification work areas.	**Re: D.2.2.4.2 -** The laboratory's floor plan and traffic flow must ensure that amplified material cannot be returned to a pre-amplification area.
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.	**Re: D.2.3.1 -** All patient identifying information must be redacted on case records submitted with an ARB accreditation application.
D.2.4 Complaint investigations **D.2.4.1** 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 or OPO they serve. Laboratories must review each agreement biennially and revise as necessary. **D.2.5.2** The laboratory must have a system in place to document problems and relevant corrective actions 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.	**Re: D.2.5.1 -** There must be agreements relating to each type of transplant program, including HPC transplant programs. Only the laboratory is required to review the agreement biennially unless substantial changes are made that require the program's review and approval. **Re: D.2.5.1-** For UNOS laboratories **Transplant Program Affiliation**

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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.2.5.4** The test results (including electronic records access and electronic record distribution) must be released only to the: tested patient; authorized persons; ordering physician and/or provider; the individual responsible for using the tests results, and the laboratory that initially requested the test.	Histocompatibility laboratories must have written agreements with every transplant program the laboratory serves, unless clinical urgency prevents such an agreement. Written agreements between histocompatibility laboratories and transplant programs must include *all* of the following: 1. The sample requirements for typing and crossmatching. 2. The loci and level of resolution typed. 3. A process for requesting extended HLA typing. 4. A process for reporting HLA typing results to the OPTN Contractor. 5. A process for resolving HLA typing discrepancies and errors. 6. The maximum turnaround time from receipt of sample to reporting of results to the transplant program. 7. A process to obtain sensitization history for each patient. 8. The frequency of periodic sample collection. 9. The frequency of antibody screenings. 10. The assay format that will be used for antibody screening and for crossmatching. 11. The criteria for determining unacceptable antigens used during organ allocation. 12. The duration for which specimens need to be stored for repeat or future testing.

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	13. If desensitization is performed, then a protocol for monitoring antibody levels. 14. The criteria for crossmatching. 15. If the laboratory registers patients for the transplant program, then a process for blood type verification according to UNOS Policy 16. If post-transplant monitoring is performed, then a protocol for monitoring antibody levels. **OPO Affiliation** Histocompatibility laboratories must have written agreements with every OPO member the laboratory serves, unless clinical urgency prevents such an agreement. Written agreements between histocompatibility laboratories and OPOs must include *all* of the following: 1. The sample requirements for typing and crossmatching. 2. The loci and level of resolution typed. 3. A process for requesting extended HLA typing. 4. A process for reporting HLA typing results to the OPTN Contractor. 5. A process for resolving HLA typing discrepancies and errors. 6. The maximum turnaround time from receipt of donor sample to reporting of results to the OPO. 7. A process for prioritizing donors for histocompatibility testing.

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	8. The length of time for which donor specimens are required to be stored for repeat or future testing. 9. If the OPO performs crossmatching, then all methods used for crossmatching and the interpretation and reporting of the results. **Re: D.2.5.4** - The laboratory must have a written policy for reporting and distributing results (including electronic distribution). Reports with results derived from more than one individual must not be released unless consent is received from all individuals reflected in the report.
D.2.6 Personnel competency assessment **D.2.6.1** The Technical Supervisor or General Supervisor designee must: **D.2.6.1.1** Establish and follow written policies and procedures to assess and document competency of staff at least annually. **D.2.6.1.2** Document the performance of individuals responsible for testing patient specimens: **D.2.6.1.2.1** At least semiannually during the first year. **D.2.6.1.2.2** At least annually thereafter. **D.2.6.1.2.3** Whenever test methodology or instrumentation changes. **D.2.6.1.3** 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.2.6.2 For testing personnel,** the evaluation must include documentation of competency to include the following as applicable: **D.2.6.2.1** Direct observations of routine test performance, including sample preparation, specimen handling, processing and testing. **D.2.6.2.2** Monitoring of the recording, interpretation and reporting of test results.	**Re: D.2.6.1 -** Annual competency documentation for each test a staff member is authorized to perform must be available for the inspector to review. **Re: D.2.6.1.3** - Proficiency testing samples may serve as unknowns. **Re: D.2.6.2** All 6 elements of competency must be assessed for all staff who perform testing on patient specimens. Documentation must include direct observations of every test category (HLA typing, antibody identification, crossmatch etc.) for which testing staff are responsible. This observation must include the performance and maintenance of instruments used in performing these tests. In addition, the ability to recognize and solve problems must be documented (for example providing written answers to a problem scenario or documentation of an actual situation).

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D.2.6.2.3 Review of quality control records, proficiency testing results, and preventive maintenance records. **D.2.6.2.4** Direct observation of performance of instrument maintenance and function checks. **D.2.6.2.5** Assessment of test performance through testing previously analyzed specimens, internal blind testing samples or external proficiency testing samples. **D.2.6.2.6** Assessment of problem solving skills. **D.2.6.3** Document the performance of individuals with responsibilities in the role of Technical Supervisor, Clinical consultant and/or General Supervisor who are not listed as the CLIA laboratory director annually. **D.2.6.3.1** All laboratory director responsibilities which are delegated to the clinical consultant, technical supervisor, or general supervisor must be in writing and included in the competency assessment. **D.2.6.3.2** Competency assessment for the Technical Supervisor must include the responsibilities listed in E.3.2 **D.2.6.3.3** Competency assessment for the Clinical Consultant must include the responsibilities listed in E.4.2 **D.2.6.3.4** Competency assessment for the General Supervisor must include the responsibilities listed in E.5.2	**Re: D.2.6.3 -** Competency assessment does not need to be performed for CLIA laboratory directors unless they perform patient testing. Additionally, if the CLIA laboratory director fulfills additional roles such as technical supervisor, clinical consultant, and/or general supervisor, no competency assessment is required for these roles unless they perform patient testing. Please note that competency assessment is required for the roles of Technical Supervisor, Clinical Consultant, and General Supervisor when someone other than the CLIA laboratory director fills these positions.
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.	**Re: D.2.7.3 -** Documentation of PT performance review by technologists can, e.g., be in the minutes of laboratory staff meetings.
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.	

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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.	**Re: D.2.9.1** – and elsewhere in these Standards: CMS considers that any manufacturer's instructions must be followed even if they use words like "should", "recommended" or "good laboratory practice" unless the laboratory validates a modified procedure per Standard **D.4.1.5.3**
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.2.9.2.13** Excerpts that summarize key information or procedural steps are acceptable for use as a quick reference at the workbench provided a complete manual is available for reference. The excerpt	**Re: D.2.9.2.10** - Laboratories are expected to define their own criteria for "alert values". Examples are an extremely low Immune function test result or a positive crossmatch for a heart transplant patient who has already been transplanted.

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must correspond and must be cross-referenced to the complete procedure. Approval by the director or supervisor must be documented at the time of procedure review.	
D.2.9.3 New procedures and changes in procedures must be approved, signed and dated by the current CLIA Laboratory Director, the ASHI 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** Every procedure must be reviewed every two years by the Director and relevant Technical Supervisor, and written or electronic evidence of this review must be readily available.	**Re: D.2.9.3 -** A new Director might not be able to review all procedures immediately but would be expected to review and sign all procedures within 6 months. Any revision that changes or alters the way results are obtained or reported requires a signature by the CLIA Laboratory Director. **Re: D.2.9.4-**Discontinued procedures must be kept for the length of time required by regulatory agencies, contract, or federal, national, state, provincial, local or other authorities which have jurisdiction in the laboratory's location, whichever is the longest. **Re: D.2.9.5** – Individual procedures must be reviewed and signed.
D.3 Preanalytic Systems **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.	**Re: D.3.1.1** - Some laboratories may need to obtain written authorization for testing within 48 hours if required by state law. The patient chart, medical record, or electronic medical record may be used as the test requisition or authorization but must be available to the laboratory at the time of testing and upon request. For NMDP contract laboratories: The contract is the authorization to perform tests for the NMDP **Re: D.3.1.2.6 -** The source of the specimen is expected to be indicated when it is NOT a conventional blood sample (e.g., spleen, lymph node). To be in compliance with Standard **D.5.2.7.13** Laboratories are expected to also solicit information about patient treatment

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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.	with antibodies that can interfere with tests (like ATG), if applicable.
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.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.	**Re: D.4.1.1.1.4 -** Archived samples must be retrievable when requested.

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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 patient specimens. **D.4.1.2.1.3.3** Be covered under an emergency plan for alternative storage for critical reagents and relevant transplant patient 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.	**Re: D.4.1.2.1.3.2** and **D.4.1.2.1.3.3** - In relation to these Standards, the intention of "as applicable" is that the laboratory defines which reagents and specimens are critical and that therefore require an audible or centrally monitored temperature alarm system (and emergency storage plan). Continuous monitoring for other reagents and specimens may use other methods (e.g., a "High/Low" Thermometer)
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.	**Re: D.4.1.3 -** CMS considers that any manufacturer's instructions must be followed even if they use words like "should", "recommended" or "good laboratory practice" unless the laboratory validates a modified procedure per Standard **D.4.1.5.3**

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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.	**Re: D.4.1.3.2 -**Expired reagents may be used for training purposes or research but the laboratory must have a mechanism to ensure they are not used for clinical testing. Bottled water that comes from a manufacturer with a quality certificate is acceptable and does not require conductivity tests or cultures. The laboratory must keep a copy of the manufacturer's certificate on file. Water that is purified locally does require conductivity tests and cultures at intervals determined by the laboratory.
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.	**Re: D.4.1.3.3 -** Documentation of which lots were used does not have to be on worksheets as long as the laboratory has a system in which that can be traced.

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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.4.1.3.12** The laboratory must document historic test result review when notified by a vendor of a lot-specific change or correction to a reagent or kit that could affect test result interpretation, and take appropriate corrective action.	**Re: D.4.1.3.12**- Upon receipt of lot-specific notice of update/revision/correction, the laboratory is required to review lot-specific historic testing data for potential impact. The laboratory must retest or reanalyze samples as required and issue corrected/updated reports as necessary to reflect result change due to the vendor notice.
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.4.1.4.3** The laboratory must document historic test result review when notified by a vendor of an update/revision/correction to analysis software or template that could yield a change, correction, or update to the original test result and take appropriate corrective action.	**Re: D.4.1.4.1 -** Laboratories can satisfy this standard by performing parallel manual analyses. **Re: D.4.1.4.3 -** Upon receipt of lot-specific notice of update/revision/correction to analysis template or database used for typing or antibody testing, the laboratory is required to review lot-specific historic testing data for potential clinical impact. The laboratory must retest or reanalyze samples as required and issue corrected/updated reports as necessary to reflect result change due to the vendor notice.
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.	**Re: D.4.1.5.2 –**These standards apply to tests not specifically covered by ASHI Standards if such testing is performed in relation to transplantation and immunogenetics testing (e.g., platelet antigen genotyping, if the laboratory is not accredited for that test by another organization).

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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.	**Re: D.4.1.5.3** -This standard also applies to tests not specifically covered by ASHI Standards if such testing is performed in relation to transplantation and immunogenetics testing (e.g., testing for polymorphisms of MICA, cytokine genes, or using Next Generation Sequencing test methods). Note that if results are reported to a USA physician with patient identifiers and may, therefore, be used by the physician in making clinical decisions, these are, per CMS, not "research" tests.
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:	

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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 laboratory. **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.	

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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.	**Re: D.4.1.7.3 -** Functional checks must be performed every six months or more frequently if recommended by manufacturer.
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.	**Re: D.4.1.7.4 -** Actual temperature checks in at least selected individual wells must be performed at least twice a year. Not every well needs to be tested. For qPCR and real time PCR instruments, perform functional checks in lieu of using temperature monitoring instruments until such time as these become readily available.
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.	

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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** For flow cytometers, have a system to assure laser power and current input each day of use (either manual or automated). Acceptable thresholds and corrective action protocols must be documented.	
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** Controls as Calibration Materials: Controls provided by manufacturers in a test kit are considered to be calibration materials if they are used to calculate the cutoff value of a test or a patient test result. **D.4.1.8.4** Testing of Additional External Controls: If the manufacturer's instructions include a formula which uses the positive and/or negative controls included in the kit to determine the cutoff, additional external positive and/or negative controls must also be tested. **D.4.1.8.5** The control procedures must: **D.4.1.8.5.1** Detect immediate errors that occur due to test system failure, adverse environmental conditions, and operator performance. **D.4.1.8.5.2** 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.	**Re: D.4.1.8.3** The Laboratory Director/Technical Supervisor is responsible for the determination of what control materials to use in the laboratory. Inspectors will ensure that the laboratory is following its own established policies, specifically its Quality Control (QC) procedures.

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D.4.1.8.6 The laboratory must:	
D.4.1.8.6.1 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.4.1.8.6.2** Perform the following at least once each day that specimens are assayed or examined: **D.4.1.8.6.2.1** For each quantitative procedure, include two control materials of different concentrations. **D.4.1.8.6.2.2** For each qualitative procedure, include a negative and positive control material. **D.4.1.8.6.2.3** If reaction inhibition is a significant source of false negative results, include a control material capable of detecting the inhibition.	**Re: D.4.1.8.6.2.3 -** For molecular amplification, a control system capable of detecting reaction inhibition such as internal controls for SSP methods must be used.
D.4.1.8.6.3 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.4.1.8.6.4** 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.4.1.8.6.5** Over time, rotate control material testing among all operators who perform the test. **D.4.1.8.6.6** Test control materials in the same manner as patient specimens. **D.4.1.8.6.7** 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.4.1.8.6.8** Establish or verify the criteria for acceptability of all control materials. **D.4.1.8.6.9** 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.4.1.8.6.10 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.4.1.8.6.11** 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.4.1.8.6.12** Results of control materials must meet the laboratory's and, as applicable, the manufacturer's test system criteria for acceptability before reporting test results.	

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D.4.1.8.6.13 The laboratory must document all control procedures performed. **D.4.1.8.6.14** 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.4.1.8.6.15 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.4.1.8.6.15.1** For each new lot, perform parallel testing with a previous lot or use appropriate reference material. The number of tests must be determined by the Technical Supervisor.	
D.4.1.8.6.15.2 For each new shipment, demonstrate that the reagents have not been compromised during shipment by testing at least one previously tested or noncritical sample to determine that the reagents perform as expected.	**Re: D.4.1.8.6.15.2 -** Note: These standards indicate that testing of new shipments of a lot previously in use does not have to be as extensive as testing of new lots.
D.4.1.8.7 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.	

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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 Methods 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.	**Re: D.5.2 -** See also Standard **D.5.2.6.2.2** which covers the laboratory's need to also use appropriate methods for quality control of all critical test components.
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.	

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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).	**Re: D.5.2.2.12** - Specific amplification products can be assessed by other means like product coverage (NGS) or internal controls of each amplified product (SSO).
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.	**Re: D.5.2.2.15** -Alternative ambiguous allele combinations must be recognized and resolved when the alternatives include more than one common and well documented type* at the resolution required for the clinical use of the test results. Laboratories

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	performing confirmatory testing for NMDP donors must follow NMDP policies for resolution of typing ambiguities. *Cano et al, Human Immunology 68, 392-417 (2007) through 2014 or Mack et al., Tissue Antigens, 81, 194-203 (2013) effective January 2015.
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 by defining the alleles amplified or by defining the probe recognition site.	
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** If contamination is detected, clean the area to eliminate the contamination and document re-testing, as well as the measures taken to prevent future contamination. **D.5.2.2.19.3** Document acceptable electrophoretic conditions used for each gel electrophoresis.	**Re: D.5.2.2.18 -** Laboratories can test pooled wipe samples from multiple areas provided that all the areas are also retested if contamination is detected and cleaned. Contamination by common non-HLA products (e.g., STR systems, KIR alleles) must also be monitored.
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.	**Re: D.5.2.2.24 -** Independent review is defined as validated software analysis or review by a qualified individual of the software output. The data output results must be reviewed by a qualified individual before release. **Re: D.5.2.2.25 –** Inspectors must look for the use of the most recent IMGT database, one less than a year old or one matched to the lot of the commercial kit in use.

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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** The database that is used for HLA or KIR testing must be updated at least every 12 months with the most recent version of the IMGT database, one less than a year old or one matched to the lot of the commercial kit in use. **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.	

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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.5.2.5.5** Establish criteria for acceptance and interpretation of primary data (e.g., correct assignments for non-polymorphic 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.5.2.5.6 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.5.2.5.7** Ensure that sequences contributed by amplification primers are not considered in the assignment of alleles. **D.5.2.5.8** 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.	**Re: D.5.2.6.1** - UNOS laboratories using low resolution DNA methods must resolve types required for serology equivalents (e.g., B62 vs. B63, 70, 75, 76,77; B60 vs. B61). **Re: D.5.2.6.2.3** – There are many null alleles on the lists in the ASHI Ad Hoc Committee's report on Common and Well

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D.5.2.6.2.2 Selection, quality control, and usage of all typing reagents and components. **D.5.2.6.2.3** The assignment of HLA antigens and alleles and for distinguishing common null alleles as appropriate for the clinical use of the test results. **D.5.2.6.2.4** Determining when antigen or allele redefinition and retyping are required. **D.5.2.6.2.5** Assignment of haplotypes, if reported: **D.5.2.6.2.5.1** If haplotypes are assigned based upon population frequencies, this must be clearly indicated on the report and relevant references or sources must be stated. **D.5.2.6.2.5.2** Reports must include an explanation of recombination when this occurs. **D.5.2.6.3** Ensure that typing for class I or class II antigens or alleles employs a sufficient number of antisera, monoclonal antibodies, and/or DNA markers to clearly define all 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.	Documented Alleles (Mack et al, *Tissue Antigens* 81: 2013). Specific requirements depend on the clinical application. Laboratories supporting NMDP Transplant Programs must distinguish specified alleles**.** Refer to the following link for the current NMDP policy: http://bioinformatics.nmdp.org/Policies/Policies.aspx. **Re: D.5.2.6.2.5.1 -**Genotypic identity can only be proven if both parents are available or if the segregation of the four haplotypes is clearly defined.
D.5.2.7 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.	**Re: D.5.2.7.13** - Laboratories that do post-transplant antibody monitoring must have a mechanism to identify patients who are

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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.	being treated with xenogeneic antibodies such as ATG or Rituximab and must have verified that their antibody identification procedure is able to distinguish patient antibodies.
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.	**Re: D.5.2.8.1 -** Note that solid phase methods may be more sensitive than the most sensitive crossmatch techniques; laboratories are expected to have a policy for determination of cutoffs based on clinical consideration.
D.5.2.9 Laboratories performing flow cytometry techniques must: **D.5.2.9.1** Establish the optimum serum-to-target ratio.	

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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 cross reactivity 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, CO_2 concentration and humidity.	

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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 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 Next Generation Sequencing methods must: **D.5.2.11.1** Define the genomic region or nucleic acid to be characterized (whole or part of an HLA gene, KIR, mRNA, etc.). Ensure that the method for target enrichment reliably captures the region(s) or nucleic acid of interest and generates appropriately sized sequencing templates. **D.5.2.11.2** Document and validate the process/method for preparing the enriched sample for sequencing, including compliance with relevant vendor specifications. The process for preparing the DNA library for sequencing may vary depending on the method for target enrichment, the need for multiplexing libraries and choice of sequencing platform. **D.5.2.11.3** Document the sequencing chemistry, reagents, chips, and flow cells used for each sequencing run, including run parameters and read configuration (e.g., bidirectional, paired-end/single-end or mate-pair). The anticipated read length, depending on the particular chip or the selected size of DNA fragments during size selection of the library, must be documented. **D.5.2.11.4** Define and document acceptable analytic performance criteria for the sequencing run (e.g., base quality per read position, average read length, average coverage, and uniformity of coverage across the length of the targeted region), incorporating vendor specifications and laboratory-generated validation data. Instrument performance measures must include data from internal control samples and/or vendor supplied quality control material**.** **D.5.2.11.5** Document the informatics process used in generating and analyzing sequencing datafiles (e.g., FASTQ, BAM), including how sequencing reads are collated by barcode, how adaptor sequences and amplification primers are trimmed, and how quality criteria are used to filter or	**Re: D.5.2.11.1** • Assay validation must establish the size of the intended targets (entire gene or sub-segments), the purity of the target following enrichment, and allelic variation present in the population. It must include sufficient representation of all pertinent allelic specificities of the locus tested in order to evaluate possible allele drop-outs. Alleles with consistently poor representation in sequencing data (drop-out) must be addressed by alternative methods for detection • Software that can detect the presence of a minority allele due to potentially biased amplification in sequencing data must be used. • For validation and quality assurance, systematic co-amplification of closely related genomic sequences must be

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exclude sequencing reads. At each step, individual applications and software versions must be validated and documented. In addition, the methods by which data is transferred between each step in the informatics process must be documented. Any scripts or configurations that deviate from standard vendor installations must be identified, versioned and validated. **D.5.2.11.6** Create a policy for the storage and transmission of primary, intermediate, and final sequencing datafiles. Retained datasets must support re-analysis of the sequencing data at a later date if indicated. **D.5.2.11.7** Document changes to any component or process within the next generation sequencing workflow and revalidate the individual step and any subsequent steps in the protocol or the entire protocol as appropriate. Modifications or upgrades to the informatics pipeline may also be validated by re-analyzing previously sequenced datasets. **D.5.2.11.8** Independently validate software programs used to generate genotyping information from next generation sequencing data. Ensure that the genotyping algorithms are appropriate for the sequencing strategy used and the error modalities (e.g., homopolymer errors, substitutions) presented by different sequencing chemistries.	excluded or taken into consideration for genotype calling by the software analysis program. • PCR artifacts, such as *in vitro* cross-over events, must be quantified due to their potentially adverse effect on genotyping results. Quantification of the cross-over events can be performed computationally. **Re: D.5.2.11.2** • During validation, laboratories must establish procedures to assess the potential impact of barcode sequences on the efficiency of the enrichment method when the barcode is part of a primer. When barcodes are incorporated after target enrichment, fidelity of the barcoding method to identify a particular sample needs to be monitored (e.g., by rotating control samples with different barcode sequences). • During validation, laboratories must establish procedures to identify potential allele dropouts and preferential amplifications, and if necessary adjust the software program to detect preferential amplification levels. **Re: D.5.2.11.3** • Laboratories wishing to run HLA and non-HLA genotyping must validate and document depths of coverage for each of the assays run simultaneously. • Laboratories must establish procedures to identify or verify the different systematic error modalities presented by each sequencing instrument due to

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	sequencing chemistry or run mode, e.g., lower base quality scores towards the end of the reads, lower accuracy of longer reads and the second read in a paired-end sequencing. • Laboratories can utilize standard DNA sequences or control samples to monitor performance of the sequencing instrument over time. **Re: D.5.2.11.5 -** Laboratories must establish procedures to identify the limitations of the software analysis program. The genotyping software must provide all the necessary quality metrics (e.g., depth of coverage, quality score for the base reads, read alignment and variant call), and the laboratory must determine the acceptable values for each quality metric in order to assure an accurate result. **Re: D.5.2.11.6 -** Laboratories must establish policies for storing unaligned, processed sequencing files (*e.g.,* FASTQ) based on the requirements. **Re: D.5.2.11.7 -** Validation of software for NGS analysis can be performed using existing sequencing datasets. For HLA genotyping, the validation dataset must include alleles representative of the population and cumulatively their frequencies must cover 85-95% of the population. **Re: D.5.2.11.8** See guidance for **D.5.2.11.5.**
D.5.2.12 Laboratories performing ABO/Rh typing must: **D.5.2.12.1** If using serological methods:	**Re: D.5.2.12.1.4 –** Subtyping must: • Be tested using pre-red blood cell transfusion samples

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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 or non-US equivalent criteria. A and B cells may be prepared by the laboratory provided there is documentation that they are satisfactory for the intended use. **D.5.2.12.1.3** Determine the ABO group on red cells using anti-A and anti-B sera, and test the serum or plasma for expected antibodies with A_1 and B cells. Cord cells and blood from newborns must be tested for red cell antigens only, not for antibodies. **D.5.2.12.1.4** If testing for the A_1 subgroup of ABO group A, use a reagent and a technique documented not to agglutinate non-A1 group A cells. **D.5.2.12.1.5** 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.5.2.12.1.6** Document reagent performance, with appropriate cell controls, for applicable antisera on each day of use. **D.5.2.12.1.7** 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.5.2.12.1.8** Have a policy with supporting documentation for verifying that each transplant patient has been ABO typed on two separate occasions by serological methods prior to the addition of the patient to the UNet deceased donor waitlist or any solid organ donor registry. "Two separate occasions" is defined as two samples, taken at different times, sent to the same or different laboratories. If a laboratory does not perform ABO typing for solid organ transplants or to list patients in UNet, then it is not obligated to follow this standard. **D.5.2.12.1.9** Have a policy with supporting documentation for verifying that each potential living donor has been ABO typed on two separate occasions by serological methods prior to donation. "Two separate occasions" is defined as two samples, taken at different times, sent to the same or different laboratories. If a laboratory does not perform ABO typing for solid organ transplants or to list patients in UNet, then it is not obligated to follow this standard. **D.5.2.12.1.10** Have a policy with supporting documentation for verifying that each deceased donor has been ABO typed on two separate occasions by serological methods prior to performing matchruns for allocation and procurement. "Two separate occasions" is defined as two samples taken at different times, sent to the same or different laboratories. If a laboratory does not perform ABO typing for solid organ transplants or to list deceased donors in UNet, then it is not obligated to follow this standard.	• Be drawn on two separate occasions • Have different collection times • Be submitted as separate samples **Re**: **D.5.2.12.1.8 –D.5.2.12.1.10:** The responsibility of verification and the supportive evidence that two ABO types are performed prior to the listing of a candidate in UNet, prior to living donation or prior to performing a matchrun, needs to be documented. ABO typing includes appropriate A group subtyping. The laboratory may not be the party responsible for listing but documentation must be available upon request.

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D.5.2.12.2 If performed using molecular techniques, exclusively for donor registry screening: **D.5.2.12.2.1** ABO/RhD typing by molecular methods must only be used as predicted phenotype and may not be used for patient transfusion and transplant compatibility evaluations. **D.5.2.12.2.2** If donor is selected for transplantation, two independent samples must be used for ABO/RhD typing by an FDA approved serological methods. **D.5.2.12.2.3** If the laboratory performs ABO/RhD typing by molecular and serological methods, the report must clearly differentiate these methods. **D.5.2.12.2.4** Agreements between laboratories and donor registries must state the intended use of ABO/RhD molecular typing results and the need for two independent ABO/RhD results performed by an FDA approved serological methods for selected donors. **D.5.2.12.2.5** Laboratory policy must state that ABO/RhD typing by molecular method is not used for clinical purposes. If reported, the following statement must be included in the report: ABO/RhD typing by molecular method is not to be used for clinical purposes.	**Re: D.5.2.12.2** Transplant donor registries often collect samples from potential donors using buccal swabs or saliva. These samples cannot be used for traditional serological ABO/RhD blood group typing because fresh intact red blood cells (RBCs) are not available. Molecular ABO/RhD typing may be performed to predict the ABO/RhD phenotype to aid in finding an appropriate donor. The use of molecular based screening assays is not acceptable for ABO and RhD blood type assignment for the purposes of transfusion or transplantation. ABO and RhD typing by FDA approved serological methods must be used for the purpose of transfusion or donor and recipient ABO and RhD typing for transplantation. **Re D.5.2.12.2.4** Laboratories performing typing for donor registries must have an agreement with donor registries to prove compliance with these standards.
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).	**Re: D.5.2.13.4** - Does not apply to the specific situations of CD34 and CD4 enumeration by 3 or 4-color fluorescence staining methods.

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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 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 their 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 patient for antibody to HLA antigens, including the frequency of screening serum samples.	

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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 living donor and transplant patient for HLA-A, -B, -Bw4/Bw6 and –DRB1. It is highly recommended that laboratories also type for HLA-C, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPA1, and -DPB1 loci. **D.5.3.2.1.2** Screen transplant patient 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 patient receives a blood transfusion, has an allograft that is rejected or removed, or experiences any other potentially sensitizing event.	**Re D.5.3.2.1.1** -Typing for HLA-DPA1 (and screening for DPB1 antibodies) may also be considered since antibodies to DP antigens have been reported to cause positive crossmatches and to have effects on graft outcomes.
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 transplant patient for HLA-Bw4/Bw6. **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 patient 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, whichever is the longer, and must be available for audit by UNOS.	**Re: D.5.3.2.2.4** –There must be documentation of review by the laboratory of UNOS HLA data within one month of entry. **Re: D.5.3.2.2.6 -** Verify prospective molecular typing of donors for HLA-A,-B,-C,-DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, and -DPB1. **Re: D.5.3.2.2.7-** There are many null alleles on the lists in the ASHI Ad Hoc Committee's report on Common and Well Documented Alleles (Mack et al, *Tissue Antigens* 81: 2013). Laboratories must be able to distinguish the following alleles: *A*24:09N* (associated with *B*40* or *B*27), B*51:11N* (associated with *A*02:01*,

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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 patients. A solid phase method must be used if unacceptable antigens based on antibody screening are listed. **D.5.3.2.2.6** All UNOS histocompatibility laboratories prospectively typing deceased donors for OPTN matchruns must use a molecular method to assign HLA-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPB1 and -Bw4/Bw6 at the antigen level of resolution needed to meet the most recent OPTN/UNOS tables of antigen and unacceptable antigen equivalencies**.** **D.5.3.2.2.7** All UNOS histocompatibility laboratories prospectively typing deceased donors for OPTN matchruns must distinguish common null alleles from expressed genes (antigens) as appropriate for solid organ allocation and transplantation.	*C*15:02/15:13* and *DRB1*04:02*), *C*04:09N* **(**associated with *B*44:03***);** *DRB4*01:03N (*associated with *DRB1*07* and *DQB1*03:03 (DQ9*) and *DRB5*01:08N (*associated with *DRB1*15:02).*
D.5.3.3 Blood, Bone Marrow and Hematopoietic Cell Transplantation (HCT) **D.5.3.3.1** Laboratories performing testing for blood, bone marrow and hematopoietic 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 cell donor registry and/or the Transplant Program.	**Re: D.5.3.3.1.1 -** Laboratories must type the specified HLA loci at the specified level of resolution as defined in their HLA testing agreements with the transplant center(s). Testing agreements may require more stringent HLA typing resolution based on the standards of the specific accrediting organization(s) /registries that dictate transplant center practices. For example, high resolution typing of one or more loci may be required even if HLA identity at low resolution between donor and recipient is confirmed via descent in family studies.
D.5.3.3.1.2 Repeat HLA typing of transplant patient using a new sample such that the individual's HLA typing is verified prior to final donor selection for both related and unrelated donor transplants. **D.5.3.3.1.3** Repeat HLA typing of a related or unrelated hematopoietic cell donor using a new sample such that the individual's HLA typing is verified prior to hematopoietic cell collection. **D.5.3.3.1.3.1** For unrelated donors, HLA registry data is acceptable as the first of these two samples. **D.5.3.3.1.3.2** For unrelated donors, high resolution verification typing must be performed by the laboratory having a written agreement with the transplant center.	**Re: D.5.3.3.1.2 -** The laboratory must have a policy that defines how this is met. For initial or verification testing, it is acceptable for a lower resolution typing to be performed on one of the samples as long as documentation exists that the results of both samples correlate. For patients and related donors, a typing result reported by another laboratory using a different sample is acceptable as the first of these two samples with documentation that the two results correlate.

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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 or from the unit itself. **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 verify the HLA type. **D.5.3.3.1.4.2** When the laboratory performs the 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.5.3.3.1.6** Have a policy for HLA antibody testing for mismatched donors and recipients.	**Re: D.5.3.3.1.5 -** This assessment may be achieved by such additional testing as: 1. Testing enough relatives to determine genotypes for patient and donor 2. High resolution molecular typing 3. Functional assays to assess HLA identity/differences 4. Other means as deemed appropriate to assess HLA identity.
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), 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.	

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D.5.3.4.1.8 Document the purity obtained if processing involves isolation of cell subsets. If purity is not assessed, document 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.	**Re: D.5.3.4.1.8** - Laboratories that separate subpopulations of cells post-transplant for chimerism analysis (e.g., T-cells, myeloid cells or NK cells) must either document the purity of each preparation tested (e.g., by flow phenotyping) or indicate on the report the approximate purity based on method validation with previous samples. For systems with discrete alleles, it is not necessary to run pre-transplant patient or donor samples in each post-transplant run as long as appropriate test system controls are used (i.e., internal lane standards, positive and negative controls).
D.5.3.5 Transplantation of Other Organs and Tissues **D.5.3.5.1** 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 patients for HLA antibody screening and crossmatching. **D.5.3.5.2** All UNOS histocompatibility laboratories prospectively typing deceased donors for non-renal transplantation use a molecular method to assign HLA-A, -B, -Bw4/Bw6, -C,- DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, and -DPB1 at the level of resolution needed to meet the most recent OPTN/UNOS tables of antigen and unacceptable antigen equivalencies**.** **D.5.3.5.3** All UNOS histocompatibility laboratories typing deceased donors for non-renal transplantation must distinguish common null alleles from expressed genes (antigens) as appropriate for solid organ allocation and transplantation. **D.5.3.5.4** All UNOS histocompatibility laboratories typing deceased donors for Heart, Heart-Lung or Lung must report HLA typing to the OPO prior to final acceptance, if required by the transplant program. **D.5.3.5.5** All UNOS histocompatibility laboratories typing deceased donors for Liver must report HLA typing to the OPO within the period specified by the transplant program.	**Re: D.5.3.5.2 -** Laboratories must have and follow joint-agreement policies for each organ type transplanted including policies that require no testing for specific organ types.
D.5.3.6 Transfusion Support **D.5.3.6.1** Laboratories performing testing for platelet and granulocyte transfusion support must:	**Re: D.5.3.6 -** When investigations of Transfusion Related Acute Lung Injury

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D.5.3.6.1.1 Type the patient 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 collected 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.	(TRALI) are conducted, the laboratory's protocol would be expected to include screening donor blood products for both recipient specific Class I and Class II antibodies.
D.5.3.7 Disease Risk, Drug Hypersensitivity Reaction Risk and Vaccine Eligibility Assessment **D.5.3.7.1** Laboratories performing HLA typing for disease risk/drug hypersensitivity reaction risk and/or vaccine eligibility assessment must perform HLA typing at the appropriate level of resolution for HLA antigens or alleles.	**Re: D.5.3.7.1 -** As an example, laboratories testing for narcolepsy risk would be expected to type for *DQB1*06:02* not -*DRB1*15*; if laboratories receive orders to test for -*DRB1** for risk for narcolepsy, they would be expected to use that as an opportunity for physician education.
D.5.3.8 Virtual Crossmatch **D.5.3.8.1** The laboratory must have a written policy to document criteria for and procedures to use in assessing prospective compatibility (i.e., physical versus virtual crossmatch) for each transplant program it serves. **D.5.3.8.1.1** The written agreement between the HLA laboratory and the transplant program shall include: **D.5.3.8.1.1.1** Patient eligibility criteria, to include evaluation of historic and potentially sensitizing events. **D.5.3.8.1.1.2** Schedule for sample collection and solid phase methods for antibody testing to be used for virtual crossmatch. **D.5.3.8.1.1.3** How the antibody data interpretation is used for virtual crossmatch (cutoffs and thresholds). **D.5.3.8.1.1.4** When additional donor typing (such as -DQA1, -DPA1 allele-level typing) is required for any locus/allele against which the patient has documented antibody reactivity.	**Re: D.5.3.8** These standards apply only when a crossmatch is required but a physical crossmatch is not performed (prospectively) **Re: D.5.3.8.1** This agreement may be included in the existing agreement with the transplant program or as a separate document. The UNOS match system does not fulfill the requirement for performing a pre-transplant final crossmatch.
D.5.3.8.1.1.5 Circumstances under which a physical crossmatch is required.	

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D.5.3.8.2 All testing used for a virtual crossmatch assessment shall be performed in an ASHI or CAP accredited laboratory.	
D.5.3.8.3 Patients and donors must have sufficient histocompatibility typing by molecular methods to permit accurate virtual crossmatch assessment.	**Re: D.5.3.8.3** For broadly sensitized patients this may include typing at all major HLA loci (-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPA1, and -DPB1). Patients without HLA antibodies may be assessed differently per transplant agreement.
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.	**Re: D.6.1.1 -** Patients or their authorized representatives are now entitled to receive laboratory results directly from the laboratory. Laboratories must develop policies for how they will document verification that the individuals who request such results are the patients or their authorized representatives. CLIA requires that reports requested by patients be issued within 30 days of request.
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.	**Re: D.6.1.4 -** Proficiency testing samples must never be referred for any testing including supplementary testing even though similar patient samples would be so referred.
D.6.1.5 When errors in any reported test results are detected, the laboratory must do the following:	

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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 pertinent to the interpretation of the test. **D.6.2.2.3** The Laboratory / Institution's name, director's name, address and CLIA number or ASHI accreditation number for laboratories not subject to CLIA. **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 WHO approved nomenclature. **D.6.2.2.12** A list of unresolved alleles appropriate to the clinical use of the results as defined in each transplant program or OPO agreement, and as required by regulatory agencies governing solid organ or hematopoetic cell transplantation (HCT). **D.6.2.2.12.**1 Results reported using G or P group codes do not need to list unresolved alleles that are within the G or P group listed.	**Re: D.6.2.2.3 –** If required by the state where the laboratory is located, the director's name on the report must be the director listed on the CLIA certificate. **Re: D.6.2.2.5** – The date of the report cannot change if exactly the same report is resent, for example, because the original report was lost. **Re: D.6.2.2.9** – When no recipient or no donor DNA is detected, it is appropriate to indicate the level of sensitivity for detection. It is recommended that this be established by analyzing known mixtures of donor and pre-transplant recipient concurrently with patient samples collected post-transplant. Reports are expected to state something like "2% recipient DNA would have been detected, if present". **Re: D.6.2.2.11** – The WHO recognized serological specificities can be found in the last published HLA Dictionary. Additional information can be found at http://hla.alleles.org/nomenclature/index.html. **Re: D.6.2.2.12 –** All approved WHO nomenclature codes and/or multiple allele (NMDP) codes must be fully defined by

Standard	Guidance
D.6.2.2.12.2 Results reported do not need to list non-CWD alleles if stated in the report, transplant agreement, or client written request. **D.6.2.2.12.3** Typing results reported using NMDP codes must define all unresolved alleles represented by the allele code.	either listing the ambiguity string or providing a link to a published reference to the clinicians.
D.6.2.2.13 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 [laboratory name]. It has not been cleared or approved by the U.S. FDA."	**Re: D.6.2.2.13 -** Non-U.S. laboratories do not need to have this on reports
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.	**Re: D.6.2.3 -** Password protected electronic signatures are acceptable documentation of review.
D.6.3 Post-Analytical Systems Assessment **D.6.3.1 Analytical 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. The evaluation must be documented.	**Re: D.6.3.1.1 -** Examples of tests performed using more than one method or instrument include typing by cytotoxicity and DNA, antibody tests by cytotoxicity and solid phase, and crossmatching using two different flow cytometers. Relationships can be evaluated by using the same proficiency test samples
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 testing subject to CLIA regulation is referred, the subcontracting laboratory must be certified by CLIA to perform the referred testing. All testing subject to CLIA regulation may only be referred to a laboratory that is CLIA certified; ASHI accreditation alone is not sufficient. **D.6.3.1.4.** Laboratories outside of the U.S. may refer samples for immunogenetics, histocompatibility and/or transplantation immunology testing to ARB approved (e.g., EFI accredited) laboratories that	

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meet ASHI accreditation requirements, but are not following CMS regulations because they are not testing samples from U.S. patients. **D.6.3.1.5.** Referring laboratories must keep on file the following: **D.6.3.1.5.1.** A copy of the subcontracting laboratory's accreditation documentation for the testing performed; and **D.6.3.1.5.2.** 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.	**Re: D.6.3.2 -** Laboratories must determine if patient test results may have been adversely affected if a corrective action has been required for any reason.
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.	

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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.	**Re: D.6.3.3.1.1 -** Testing registry donors or relationship testing requires consent but not a test requisition.
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.	**Re: D.6.3.3.1.7:** The laboratory must be able to produce a copy of all preliminary and final reports as released from a physical or electronic archival system.
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**.	**Re: E.1.1, E.1.2, E.1.3 -** A single individual may serve in more than one of these roles (e.g., director, technical supervisor, and clinical consultant) if qualified.

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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.	**Re: E.2.1 -** Appropriate documentation that individuals filling all technical positions meet ASHI personnel qualification standards (e.g., diploma or college/university transcripts) must be readily available to an inspector if requested. **Re: E.2.1** - Directors who also serve as clinical consultants need to meet board certification requirements, if applicable, even if they were serving as directors prior to February 24, 2003.
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:	

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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 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.2.1.4.2** If a candidate has relevant pre-doctoral experience supervising 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.	**Re: E.2.2 -** CMS considers that if a laboratory testing US specimens is cited for any "serious" deficiency (i.e., a deficiency that did or could cause harm to a patient or staff member), the ARB must consider also citing a deficiency for lack of involvement of the laboratory director (Standard **E.2.2.1**).
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.	**Re: E.2.2.2 -** If any one of more than 2 laboratories with a single director is located in New York State, a NYS waiver is required.

Standard	Guidance
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.	

Standard	Guidance
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 pre-analytical, analytical, and post-analytical 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-analytical, analytical, and post-analytical 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 in histocompatibility and/or immunogenetics testing at least to the level of the minimum requirements outlined by the ASHI Accreditation Review Board.	**Re: E.2.2.17 to E.2.2.20 -** If technologists in ASHI accredited laboratories are not involved in histocompatibility testing per se, then the director must ensure that such staff is participating in continuing education relevant to their work areas.
<u>E.3 Technical Supervisor Qualifications and Responsibilities</u> **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.	

Standard	Guidance
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 and/or anatomic, 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, antibody identification, 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.3.2 Responsibilities	

Standard	Guidance
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 analytical 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:	

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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.A. **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 Qualifications and Responsibilities **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**	**Re: E.5.1.1 -** Persons who would otherwise qualify to be a general supervisor, but lack experience in ASHI-approved laboratories must have their experience as laboratory supervisors count as if they worked in an ASHI-accredited laboratory if their original laboratory has alternative accreditation (e.g., EFI accreditation). Appropriate documentation that individuals filling all technical positions meet ASHI personnel qualification standards (e.g., diploma or college/university transcripts)

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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. **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. **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	must be readily available to an inspector if requested. **Re: E.5.1.2.3 & E.5.1.2.4**: Credentialing by the American Board of Histocompatibility and Immunogenetics as a Certified Histocompatibility Technologist (CHT) or Certified Histocompatibility Specialist (CHS) certification is recommended.
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 analytical performance are maintained.	

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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.	**Re: E.5.2.3 -** Written documentation must indicate which responsibilities are delegated.
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.	**Re: E.6.1 -** Appropriate documentation that individuals filling all technical positions meet ASHI personnel qualification standards (e.g., copies of diploma or college/university transcripts) must be readily available to an inspector if requested.
E.6.1.2 Have laboratory training that includes any of the following:	**Re: E.6.1.2.1 -** Such training could also take place in an ARB approved laboratory such as an EFI laboratory.

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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.	

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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** Possess a current license as a laboratory technologist issued by the state, if such licensing exists; and: **E.6.1.2.6.2** Have earned a bachelor's degree in medical technology from an accredited university; or **E.6.1.2.6.2.1** 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 of HHS, and has successfully completed a course of training of at least 12 months in such a school; or **E.6.1.2.6.2.2** Have earned a bachelor's degree in one of the chemical, physical, or biological sciences and, have 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.6.1.2.6.2.3** Have successfully completed three years (90 semester hours or equivalent) in an accredited college or university with the following distribution of courses: **E.6.1.2.6.2.3.1** For those whose training was completed before September 15, 1963, at least 24 semester hours in chemistry and biology courses of which: **E.6.1.2.6.2.3.1.1** At least 6 semester hours were in inorganic chemistry and at least 3 semester hours were in other chemistry courses; and **E.6.1.2.6.2.3.1.2** At least 12 semester hours in biology courses pertinent to the medical sciences; or **E.6.1.2.6.2.3.2** For those whose training was completed after September 14, 1963: **E.6.1.2.6.2.3.2.1** 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.6.1.2.6.2.3.2.2** Sixteen semester hours in biology courses that are pertinent to the medical sciences and are acceptable toward a major in the biological sciences. **E.6.1.2.6.2.3.2.3** Three semester hours of mathematics.	
E.6.1.2.6.2.3.2.4 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	

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education and training in medical technology equivalent to that described in paragraphs 42 CFR 493.1491 (b)(1) and (2); or **E.6.1.2.6.2.3.3** With respect to individuals first qualifying before July 1, 1971, the technologist: **E.6.1.2.6.2.3.3.1** Was performing the duties of a laboratory technologist at any time between July 1, 1961, and January 1, 1968; and **E.6.1.2.6.2.3.3.2** 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.6.1.2.6.2.3.3.3** 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.	

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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.	**Re: E.6.2.4 –** If the laboratory is accredited for solid organ transplantation: deceased donor and provides final crossmatch and/or deceased donor typing, 24/7 coverage is required.
E.7 Continuing Education The director, technical supervisor, clinical consultant, director in training, general supervisor, 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.	