Standards for Accredited Laboratories

American Society for Histocompatibility and Immunogenetics

2025 Revised Standards approved by the ASHI Board of Directors

Approved by CMS: January 27, 2026

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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 **ACHI** American College of Histocompatibility and Immunogenetics **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	

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MLC Mixed leukocyte culture **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. **Calibration verification**: A process of confirming that the current calibration settings remain valid. **Category:** The type of testing performed in an accredited laboratory. **CLIA certificate:** A certificate issued by CMS:	**Re: A.3 Analyte** - used in relation to Proficiency Testing refers to all Class I or Class II loci tests for a single sample for any method or combination of methods or any level of resolution that is reported and graded separately.

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(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. **Qualitative test:** An assay that detects the presence or absence of a specific analyte, but does not determine the specific amount of the analyte. **Semi-Quantitative test:** An assay that does not measure the precise amount of a substance, but provides an estimate of how much of a detected substance is present. Results for semi-quantitative tests may be expressed in units, median fluorescence intensity (MFI), titers, etc. **Quantitative test:** An assay that measures the amount of an analyte in a specimen and reports the results in units traceable to a recognized standard. **Common, Intermediate, and Well-Documented (CIWD)**: Version 3.0.0 of the CIWD catalog categorizes HLA alleles according to population frequencies, based on data from several donor registries (Hurley CK, et al. 2020. HLA. 1-16. Doi. 10.1111/tan.13811). Common alleles have a frequency of $\geq$ 1 in 10,000, intermediate alleles $\geq$ 1 in 100,000, and well-documented alleles have $\geq$ 5 occurrences in at least one population studied. **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. **Continuing education (CE) credit hours:** Continuing medical education (CME) or continuing education units (CEUs). Prior to qualifying as a laboratory director, the obtained CE credit hours must cover the applicable laboratory director responsibilities. **Control material:** a reagent or biological sample that tests the accuracy, precision or functionality of a test system. **Control procedure:** a process to monitor and ensure the reliability of a test system. **Confirmatory Testing**: A second analytical procedure performed to substantiate or bring into question the results of an initial laboratory test.	**Re: A.3 Semi-Quantitative test** - Even though a semi-quantitative test provides a numerical result, the number must not be interpreted as a quantitative measurement.

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Coverage (in regards to NGS): The percentage of bases called at a 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. **dd-cfDNA:** donor-derived cell-free DNA **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. **Director-in-Training (DIT):** A person who meets CLIA education requirements for a high complexity testing laboratory director and gaining practical experience and knowledge in laboratory operations and management. Must be registered with ASHI DTRC. **Director Training, Review and Credentialing Committee (DTRC):** A committee within the ASHI Professional Standards Division. The committee is responsible for the review of DIT candidate portfolio and recommending candidates for training, evaluation and credentialing. **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. **Doctoral degree:** An earned post-baccalaureate degree with at least 3 years of graduate level study that includes research related to clinical laboratory testing or advanced study in clinical laboratory science, medical laboratory science, or medical technology. For purposes of this part, doctoral degrees do not include doctors of medicine (MD), doctors of osteopathy (DO), doctors of podiatric medicine (DPM), doctors of veterinary medicine (DVM) degrees, or honorary degrees. **Digital PCR (dPCR):** Methods that use limiting dilutions and/or sample partitioning to generate large numbers of sub-microliter reactions containing either a few or no target sequences. **Droplet Digital PCR (ddPCR)** is a highly sensitive and precise method for nucleic acid quantification that partitions a PCR reaction into thousands of individual droplets, allowing for absolute quantification of target DNA without relying on standard curves.	

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Established: Validated in the laboratory and based upon documented local data and/or published peer reviewed data. **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. **Flow cell (in regards to Nanopore sequencing):** A slide made of proprietary synthetic amphiphilic polymer containing protein subunits that self-assemble forming a tiny nanopore. These proteins are embedded in an electro resistant membrane. Each nanopore corresponds to an electrode connected to a channel and a sensor chip. The electrode measures the electric current flowing through the nanopore. Nanopore flow cells may be reusable after washing to remove DNA from previous sequencing reactions. **High resolution typing:** A high-resolution HLA genotype is reported in molecular HLA nomenclature to at least the second field, and may include P or G group designations. A high resolution HLA genotype may contain either a) only one unambiguously assigned genotype or b) multiple alternative genotypes if only one combination includes common and/or intermediate alleles (CIWD version 3.0.0), with the exception of null alleles; alternative genotype combinations containing a null allele listed as either common, intermediate, or well-documented must be resolved. **IMGT/HLA Sequence Database:** Specialized 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 next generation and/or third generation sequencing):** The computational work flow through which raw sequencing reads, obtained from a particular sequencing 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 sequencing data in a scalable manner. **Kit:** All components of a test that are packaged together.	**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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Laboratory Information System (LIS): A software application designed to manage and streamline operations within a laboratory environment. **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 genotype result is defined as sufficient to assign genotypes to the level of serological splits. Some alleles may require 2-field genotype results to satisfy this requirement. A list of serological splits can be accessed at http://hla.alleles.org/nomenclature/index.html **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. **Nanopore sequencing:** A technology that uses changes in electrical conductivity as a strand of nucleic acid passes through a protein nanopore to determine the sequence of a specific nucleic acid polypeptide. Nanopore 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. **Next Generation Sequencing (NGS):** Technologies that utilize 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.	**Re: A.3 Test method -** Because CMS allows a laboratory to utilize a specific test method once it has been validated and

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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's 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. **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 the 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.	approved by the laboratory director, ASHI 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, NGS and/or third generation sequencing, and 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, and complement dependent cytotoxicity. **Re: A.3 Unsatisfactory** - Is defined by CMS for serologic ABO as <100% concordance and <80% for all other tests. **Re: A.3 Unsuccessful** - The same definition of "Unsuccessful" applies to all analytes, including ABO typing. Unsuccessful performance requires enhanced PT. **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.

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Replacement certificate: An active CLIA certificate that is reissued with no changes made. **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. **Reportable range:** The span of test result values over which the laboratory can establish and verify the accuracy of the instrument or test system. **Revised certificate:** An active CLIA certificate that is reissued with changes to one or more fields displayed on the certificate, such as the laboratory's name, address, laboratory director, or approved specialties/subspecialties. For purposes of this part, revised certificates do not include the issuance, renewal, change in certificate type, or reinstatement of a terminated certificate with a gap in service. **RT-PCR typing assay:** a PCR-based assay performed on a quantitative or "real-time" thermocycler. These assays may employ melt-curve analysis or hydrolysis probes to detect amplicons. **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 the 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. **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. **Third Generation Sequencing:** Technologies that offer the capability for single molecule real-time sequencing of longer reads. **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.	

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Unsatisfactory proficiency testing performance: Failure to attain the acceptable response for an analyte or test or a testing event. **Unsuccessful participation in proficiency testing:** Means any of the following: (1) Unsatisfactory performance for the same analyte in two consecutive or two out of three testing events. (2) Repeated unsatisfactory overall testing event scores for two consecutive or two out of three testing events for the same analyte. (3) An unsatisfactory testing event score for those subspecialties not graded by analyte (blood compatibility, immunohematology) for the same subspecialty for two consecutive or two out of three testing events. **Validated:** 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 the 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.	

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A.4 Applicability These Standards apply to ASHI-accredited laboratories that perform testing of human specimens for purposes of reporting specific results relevant to the diagnosis, prevention, or treatment of any disease or impairment, or the assessment of the health of individuals. In addition, these Standards apply to 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.	**Re: A.4 Applicability -** Per CMS, any (ASHI purview) test results for U.S. patients that are reported to physicians with patient 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. **B.1.2.4** Permit the ASHI Accreditation Program and HHS to monitor the correction of any deficiencies found through the inspection process. **B.1.2.5** For laboratories using ASHI accreditation for compliance with CLIA regulations or other organizations for which it has "deemed status", authorize ASHI to release to HHS or other organizations, as applicable, the laboratory's inspection findings whenever HHS conducts random sample or complaint inspections. **B.1.2.6** For laboratories using ASHI accreditation for compliance with CLIA regulations or other organizations for which ASHI has "deemed status", authorize ASHI to submit to HHS or other organizations, as applicable, the results of the laboratory's proficiency testing. **B.1.2.7** For laboratories using ASHI accreditation for compliance with CLIA regulations, have a mechanism to provide laboratory workers with information about how to file anonymous complaints.	**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. **Re: B.1.2.4** - Previous deficiencies will be reviewed during the next inspection. **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.1.3 A certificate of accreditation is valid for no more than 2 years. In the event of a non-compliance determination as a result of a random sample validation or complaint inspection, a laboratory will be subject to a full review by the ASHI Accreditation Program and/ or (for US laboratories) CMS. **B.1.4** A laboratory seeking to renew its certificate of accreditation must complete and return the renewal application to the ASHI Accreditation Program by the deadline specified by the ASHI Accreditation Program, meet the requirements of ASHI Standards, submit appropriate accreditation fees, and submit its CLIA certificate if applicable. **B.1.5** An ASHI-accredited laboratory failing to meet the requirements in B.1.2 may be subject to suspension, revocation, or limitation of the laboratory's certificate of accreditation or certain alternative sanctions. The ASHI Accreditation Program must provide the laboratory with a written statement of the grounds on which the determination of noncompliance is based. The ASHI Accreditation Program must offer an opportunity for appeal, re-accreditation, or limited accreditation. **B.1.6** If the ASHI Accreditation Program determines that an application for accreditation is to be denied or limited, the ASHI Accreditation Program must notify the laboratory in writing of the basis for denial or limitation of the application. The ASHI Accreditation Program must offer an opportunity for appeal or limited accreditation. **B.1.7** If the laboratory submits an appeal within 30 days of notification of the ASHI Accreditation Program's action to suspend, revoke, limit, or deny the certificate of accreditation, the laboratory will retain its certificate of accreditation until a decision is made by the ASHI Accreditation Program unless the ASHI Accreditation Program finds that conditions at the laboratory pose an imminent and serious risk to human health.	
B.2 Notification Requirements **B.2.1** Laboratories issued ASHI accreditation must notify the ASHI Accreditation Program 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. **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.	**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."
C. Proficiency Testing **C.1 Enrollment, Testing and Evaluation of Samples**	**Re: C.1.1 -** For each analyte tested, the lab must identify a primary PT provider and must test the PT

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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. **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**.** **C.1.2** Laboratories performing proficiency testing must not engage in any inter-laboratory communications pertaining to the results of proficiency testing sample(s) until after the reporting deadline has passed. This includes situations in which one Director oversees multiple laboratories. **C.1.3** Laboratories must not send their proficiency testing results or their proficiency testing samples to another laboratory for analysis. **C.1.4** Proficiency test samples must be: **C.1.4.1** incorporated into the regular workload. **C.1.4.2** tested in a manner comparable to, and not more extensively than, routine clinical samples. **C.1.4.3** rotated among all testing personnel. **C.1.5** The laboratory must document the handling, preparation, processing, examination, and each step in the testing and reporting of results for all proficiency testing samples. A copy of all records related to proficiency testing must be retained by the laboratory for a minimum of two years. This includes the following: **C.1.5.1** A copy of the proficiency testing program report forms used by the laboratory to record proficiency testing results.	samples according to a patient testing algorithm in use at the time of the PT challenge. As labs may have multiple patient testing algorithms (e.g. the testing algorithm used for solid organ transplant candidates and the testing algorithm used for hematopoietic cell transplant donors), they must choose one to follow for testing PT samples, giving preference to the highest resolution, most complex or highest volume algorithm. As the identified patient testing algorithm may make use of multiple methods, labs are allowed to follow the patient testing algorithm and test the PT samples by multiple methods. This patient testing algorithm, and the methods used, must be documented in a lab policy, procedure, or testing agreement. Overall, labs must test PT samples as they would patient samples in their identified patient testing algorithm. Labs may, but are not required to, subscribe to other PT challenges to test methods not included in their primary PT challenge; the results of these non-primary PT results do not need to be included in the lab's accreditation application. Regardless of the methods used to test PT samples, all methods used in the lab must be correlated according to ASHI standard D.6.3.1. **Re: C.1.2 -** There can be no participation of another laboratory in reported PT results even though that is routinely done for

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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.	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. **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 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 password 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.	**Re: C.2.1 -** Note: For any CMS Regulated Analyte, e.g., serologic ABO Typing, CLIA-certified laboratories must have a mechanism for reporting each PT survey's results to CMS at the time the results are

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C.2.1.1 For all clinical testing except serologic ABO 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 typing, satisfactory performance is 100% concordance. **C.2.2** Unsuccessful participation in a PT program is defined as unsatisfactory performance on 2 consecutive assessments; or on 2 out of 3 assessments. If a laboratory's performance in an external proficiency testing program is unsuccessful: **C.2.2.1** The laboratory must determine and document the cause for each unsatisfactory proficiency test result and take appropriate measures to prevent the recurrence of the problem. **C.2.2.2** The laboratory must take immediate corrective action to ensure that the problem identified through proficiency testing has not resulted and will not result in the 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.	available. ABO by DNA methods may be performed to predict the ABO phenotype. The independent use of molecular DNA based assays is not acceptable for ABO assignment for the purposes of transfusion.. **Re: C.2.2 -** If a CLIA certified laboratory's ABO typing is unsatisfactory in 2 consecutive or 2 of 3 assessments, testing 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, including a defined organizational structure and defined process for maintaining patient confidentiality. **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 a review of the effectiveness of corrective actions taken to resolve problems, revision of policies and procedures necessary to prevent recurrence of problems, and documented discussion of assessment review results with appropriate staff.	**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. **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	**Re: D.2.1.1 -** There must be evidence that policies and procedures are revised to

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D.2.1.1 Each laboratory that performs testing must meet the applicable general laboratory systems requirements. The laboratory must monitor and evaluate the overall quality of the general laboratory systems and correct identified problems for each type of test performed. **D.2.1.2** The laboratory must be in compliance with all applicable federal, state and local laws including but not limited to, 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. **D.2.1.3** The laboratory must establish and follow written procedures for standard precautions as defined by the CDC or if applicable, non-US equivalent during collection, transport, storage and handling of blood and tissue specimens. **D.2.1.4** All records must be retained for a minimum of 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. **D.2.1.6** The laboratory must establish written policies to implement data security and document incidents when security is breached. These policies must describe processes that maintain patient confidentiality, including access control and/or user management, to ensure donors' and patients' records can only be accessed by authorized personnel.	prevent the recurrence of problems. Follow-up procedures must assess the effectiveness of corrective actions. Discussions with the staff of problems must be documented. **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 requirements for "blood-borne pathogens" including the 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. **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:	**Re: D.2.2.3 -** Uninterruptible or emergency power supplies must be available at least for equipment essential for 24 hour deceased

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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 in the laboratory. Archived records may be stored in an offsite location, but must be easily retrievable within 48 hours or the time period specified by local, state and federal regulations. **D.2.2.1.3** Adequate facilities for refrigerator and freezer storage of reagents and specimens are immediately available to the laboratory. **D.2.2.2** Lighting and ventilation must be adequate. **D.2.2.3** Uninterruptible or emergency power supplies must be used for essential equipment. **D.2.2.4** Laboratories performing amplification of nucleic acids must: **D.2.2.4.1** Use physical and/or biochemical barriers to prevent nucleic acid contamination (carry-over). **D.2.2.4.2** Perform pre-amplification procedures in a work area that excludes amplified nucleic acid that has the potential to serve as a template in any other amplification assays performed in the laboratory (e.g., PCR product, plasmids containing HLA genes or relevant STR/VNTR sequences). Restricted traffic flow is recommended. **D.2.2.4.3** Use dedicated 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.	donor testing and preservation of essential specimens and reagents, as applicable. **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 the confidentiality of protected health information throughout all phases of the testing process, including prevention of unauthorized access of data, limiting data access based on the user role, control of software installation, and maintaining subject confidentiality and security during internal and external storage and transfer of data. 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. Examples as follows from DHHS include but are not limited to: 1. Name 2. Address (including subdivisions smaller than state such as street address, city, county, or zip code) 3. Any dates (except years) that are directly related to an individual, including birthday, date of admission or discharge, date of

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	death, or the exact age of individuals older than 89 4. Telephone number 5. Fax number 6. Email address 7. Social Security number 8. Medical record number 9. Health plan beneficiary number 10. Account number 11. Certificate/license number 12. Vehicle identifiers, serial numbers, or license plate numbers 13. Device identifiers or serial numbers 14. Web URLs 15. IP address 16. Biometric identifiers such as fingerprints or voice prints 17. Full-face photos 18. Any other unique identifying numbers, characteristics, or codes
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 must be 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 it as necessary.	**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

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D.2.5.2 The laboratory must have a system in place to document problems and relevant corrective actions that result from a breakdown in communication between the laboratory and authorized individuals who order tests or receive results. **D.2.5.3** The laboratory must, upon request, make available to clients a list of test methods employed by the laboratory, a list of performance specifications for each method (including normal ranges, if applicable) and a list of interfering factors that could affect the test results or interpretation of test results. Pertinent updates of testing information must be provided to clients whenever changes occur that affect the test results or the interpretation of test results. **D.2.5.4** 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 test results, and the laboratory that initially requested the test.	changes are made that require the program's review and approval. **Re: D.2.5.1-** For UNOS laboratories **Transplant Program Affiliation** 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.

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	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. 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.

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	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. 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 all testing personnel. **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.	**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.2.6.1.2.1 - D.2.6.1.2.3 -** The timeline for semiannual and annual competency evaluation starts when training is completed, and patient specimen testing begins. This applies independently to each testing system for which competency evaluation is conducted. **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

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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. **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.	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).
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.	**Re: D.2.7.3 -** Documentation of PT performance review by technologists can be in the minutes of laboratory staff meetings.

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D.2.7.2 Every individual who participates in a proficiency test must be informed of the results of his/her performance in that proficiency test. **D.2.7.3** All proficiency testing evaluation and verification activities must be documented.	
D.2.8 Laboratory systems assessment **D.2.8.1** The laboratory must establish and follow written policies and procedures for an ongoing mechanism to monitor, assess, and, when indicated, correct problems identified in the general, preanalytic, analytic, and postanalytic laboratory systems.	
D.2.9 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.2.9.2.5.1** Procedures, including appropriate diluent, for performing dilutions on samples with results falling above the reportable range. **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.	**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** **Re: D.2.9.2.10** - Laboratories are expected to define their own criteria for "alert values". Examples are an extremely low Immune

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D.2.9.2.8 Limitations in the test methodology, including interfering substances and sample limitations. **D.2.9.2.9** Reference intervals and acceptable values. **D.2.9.2.10** Entering results in the patient record and reporting patient results including, when appropriate, the protocol for defining and reporting imminent life-threatening results or alert values. **D.2.9.2.11** Pertinent literature references. **D.2.9.2.12** Description of the course of action if a test system becomes inoperable. **D.2.9.2.13** 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 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.2.9.2.14** Management of notifications from vendors that may affect clinical care. **D.2.9.3** The Laboratory must have a document control system. 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 ASHI Laboratory Director and relevant Technical Supervisor. Written or electronic evidence of this review must be readily available.	function test result or a positive crossmatch for a heart transplant patient who has already been transplanted. **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** - The CLIA lab director must review and sign any new and revised procedures. This initial review cannot be delegated. Ongoing biennial review can be performed by the ASHI Director or Technical Supervisor.
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.	**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.

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D.3.1.2.3 Date of specimen collection **D.3.1.2.4** Time of specimen collection, when pertinent to testing **D.3.1.2.5** The test(s) ordered. **D.3.1.2.6** The source of the specimen when pertinent to testing. **D.3.1.2.7** Any relevant information, (e.g., transfusions, sensitization, primary or secondary graft, immunosuppressive therapy) to facilitate accurate and timely testing, interpretation, and reporting of results. **D.3.1.3** The laboratory must ensure the accuracy of all test request information transcribed into a record system or a laboratory information system.	**Re: D.3.1.2.3** Date of specimen collection can be provided either on the test requisition form or on the specimen container. **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). Re: **D.3.1.2.7 -** Laboratories are expected to solicit information about patient treatment 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.	**Re: D.3.2.1.2.2** Date of specimen collection can be provided either on the test requisition form or on the specimen container.
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:	**Re: D.4.1.1.1.4 -** Archived samples must be retrievable when requested.

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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** Retention of specimens based on the specific type of specimen. **D.4.1.1.1.5** A system to retrieve specimens for further testing in a timely manner.	
D.4.1.2 Testing Environment The following conditions must be monitored and documented as applicable: **D.4.1.2.1** Temperature of the following must be recorded each working day, or in case of continuous use each shift: **D.4.1.2.1.1** Incubators and water baths. **D.4.1.2.1.2** Ambient temperature of laboratory space. **D.4.1.2.1.3** Refrigerators and freezers must also: **D.4.1.2.1.3.1** Be monitored continuously. **D.4.1.2.1.3.2** Use an audible or centrally monitored temperature alarm system for critical reagents and relevant transplant 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	**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**

Standard	Guidance
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** Federal, State, or non-USA approved codes. **D.4.1.3.1.5** Other pertinent information required for proper use. **D.4.1.3.2** Reagents, water, solutions, culture media, control materials, calibration materials, and other supplies whether commercially purchased or prepared in-house must not be used when they have exceeded their expiration date, have deteriorated, or are of substandard quality. **D.4.1.3.3** There must be a documented system in place for identifying which lots and shipments of reagents were used for each assay. **D.4.1.3.4** Reagents received from the manufacturer without a specified expiration date must be subject to quality control protocols to determine an appropriate expiration date that ensures optimum performance. **D.4.1.3.5** Prior to reporting results obtained with new lots or shipments of reagents, satisfactory performance must be verified and documented. **D.4.1.3.6** Components of reagent kits of different lot numbers must not be interchanged unless otherwise specified by the manufacturer. **D.4.1.3.7** If commercial kits are used, the manufacturer's instructions must be followed unless the laboratory has performed and documented validation testing to support a deviation in technique or analysis. **D.4.1.3.8** 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.4.1.3.9** The laboratory must validate the specificity of locally procured human reagent sera and monoclonal antibodies using appropriate control cells. Subsequent quality control may consist of testing in parallel with previous lots. **D.4.1.3.10** The laboratory must verify that media: **D.4.1.3.10.1** Are sterile, if sterility is required.	**Re: D.4.1.3.1.4** - Examples: OSHA, NFPA, and/or GHS. **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. **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.

Standard	Guidance
D.4.1.3.10.2 Supports growth, if used for cell culture. **D.4.1.3.11** 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.4.1.3.11** - 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, including LIS components, 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, including analyses assisted by an LIS system, 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 analyses. **Re: D.4.1.4.2** - The laboratory must perform and document all system maintenance as specified by the LIS manufacturer or as established and validated by the laboratory, ensuring that environmental and operating conditions are maintained to preserve data integrity. This can be accomplished by running LIS audits, reconciliation reports (instrument vs LIS, LIS vs EHR), downtime drills and/or regression tests. **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:	**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

Standard	Guidance
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 a quantitative test system or values for a qualitative or semi-quantitative test system. **D.4.1.5.2.2** Verify that the manufacturer's reference values are appropriate for the laboratory's patient population.	accredited for that test by another organization).
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 characteristics required for test performance. **D.4.1.5.4** The laboratory must determine the test system's calibration procedures and control procedures based on 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, including computer systems used for analyses, 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 .	**Re: D.4.1.6.1.1 -** There needs to be a policy that indicates maintenance is performed by appropriate personnel to ensure accurate, clear and interference-free performance and

Standard	Guidance
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.4.1.6.1.3** For conventional thermal cycling and real-time PCR instruments: the accuracy of the achieving target temperatures and optical and fluorescent detection, as applicable, must be checked in accordance with D.4.1.6.1 and D.4.1.6.2 **D.4.1.6.1.4** For volumetric dispensers such as Hamilton syringes which cannot be calibrated, volume dispensed must be verified and documented every six months. **D.4.1.6.2** When using equipment and instruments developed in-house, commercial equipment modified by the laboratory, or equipment for which maintenance and function check protocols are not provided by the manufacturer, the laboratory must do the following: **D.4.1.6.2.1** Establish, perform and document maintenance and function check protocols that ensure equipment and instrument performance necessary for accurate and reliable test results. **D.4.1.6.2.2** Function checks must be within the laboratory's established limits before test results are reported.	transmission and this maintenance must be documented. **Re: D.4.1.6.1.3** For thermal instruments, actual temperature checks in at least selected individual wells must be performed as defined by the manufacturer or lab policy. Not every well needs to be tested.

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.

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.

Standard	Guidance
D.4.1.7.5 For flow cytometry and flow analysis using equipment designed for beads only (fluoroanalyzer), instrument standardization and calibration for the laboratory must, as applicable: **D.4.1.7.5.1** Include an optical standard, consisting of latex beads or other uniform particles, to ensure proper focusing and alignment of all lenses in the path for both the exciting light source and signal (e.g., light scatter, fluorescence) detectors. **D.4.1.7.5.2** Include a fluorescent standard for each fluorochrome to be used to ensure adequate detection of the fluorescent signal. These fluorescent standards may be incorporated in the beads or other particles used for optical standardization or may be a separate bead or fixed cell preparation. **D.4.1.7.5.3** Run both the optical and fluorescent standards each time the instrument is turned on and any time maintenance, adjustments or problems have occurred during operation that could potentially affect instrument function. **D.4.1.7.5.4** Record and monitor the results of optical focusing/alignment each day of use or each time the instrument is turned on. **D.4.1.7.5.5** Establish threshold values for acceptable optical and fluorescent standardization results for all relevant signals on each instrument used. **D.4.1.7.5.6** In the event a particular threshold value cannot be attained, have a written protocol detailing the corrective action. **D.4.1.7.5.7** If performing analyses that require the simultaneous use of two or more fluorochromes, use an appropriate procedure to compensate for overlap in their emission spectra. **D.4.1.7.5.8** 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.	**Re: D.4.1.7.3 -** Functional checks must be performed every six months or more frequently if recommended by manufacturer.

Standard	Guidance
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 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 controls are used to determine the value of a test or a patient test result, additional external 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. **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 and semi-quantitative 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. **D.4.1.8.6.3** Electrophoretic procedures of clinical specimens must include 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.	**Re: D.4.1.8.2 -** 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. **Re: D.4.1.8.6.2.2** - For assays other than molecular assays, even when the manufacturer's instructions for use of a qualitative procedure do not specify that a positive and negative control be included in the test run, the laboratory is required to include an external positive and negative control at least once each day that the test is run. The manufacturer's instructions do not obviate this minimum requirement. For molecular assays, laboratories must define and review quality parameters that ensure test accuracy for each test run. **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.

Standard	Guidance
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. **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. **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.	**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.5 Application and Test Systems	

Standard	Guidance
D.5.1 General Standards **D.5.1.1 Test systems** **D.5.1.1.1 Test systems** selected by the laboratory must be performed: **D.5.1.1.1.1** Following the manufacturer's instructions or as modified and validated by the laboratory and/or **D.5.1.1.1.2** As developed and validated by the laboratory and **D.5.1.1.1.3** In a manner that provides test results that are within the laboratory's stated performance specifications for each test system **D.5.1.2 Evaluation of test systems** **D.5.1.2.1** The laboratory must have a system to identify, assess, and document patient test results that appear inconsistent with the following relevant criteria, when available: **D.5.1.2.1.1** Patient age. **D.5.1.2.1.2** Sex. **D.5.1.2.1.3** Diagnosis or pertinent clinical data. **D.5.1.2.1.4** Distribution of patient test results. **D.5.1.2.1.5** Relationship with other test results.	
D.5.2 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.	**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.

Standard	Guidance
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. **D.5.2.2 Laboratories performing amplification-based nucleic acid testing must:** **D.5.2.2.1** Use a method to prepare DNA that provides sufficient quality (e.g., purity, concentration) and quantity to ensure reliable test results. Written protocols must specify the minimal acceptable sample in terms of volume or numbers of nucleated cells. If tests are performed without prior purification of nucleic acids, the method must be documented and validated in the laboratory. **D.5.2.2.2** Ensure that samples are stored under conditions that preserve the integrity of the nucleic acids that will be tested. **D.5.2.2.3** Ensure that template quantity and quality are sufficient to provide interpretable data for a locus (or loci) or allele(s). **D.5.2.2.4** Ensure that the amount of amplification template in each amplification reaction is in an acceptable range. **D.5.2.2.5** Ensure that aliquots of all batches of reagents (solutions containing one or multiple components) utilized in the amplification assay are demonstrated to be free of contamination. **D.5.2.2.6** Ensure that reagents used for primary amplification are not exposed to post- amplification work areas. **D.5.2.2.7** Ensure that reagents used for secondary amplification are stored in a contamination-free area. **D.5.2.2.8** Define criteria and perform quality control testing to confirm specificity for each lot and shipment of primers and probes. **D.5.2.2.9** Ensure that each lot and shipment of primers or probes is monitored to confirm stability and performance of the primers or probes.	

Standard	Guidance
D.5.2.2.10 Ensure that oligonucleotide probes and primers are stored under conditions that maintain specificity and sensitivity. **D.5.2.2.14** Monitor the quantity of specific amplification products (e.g., gel electrophoresis, hybridization). **D.5.2.2.15** Recognize and document ambiguous combination(s) of alleles for each template/primer or template/probe combination and have procedures available to resolve these as appropriate for the clinical use of the test results. **D.5.2.2.16** Define and document the genetic designation (e.g., locus) of the target amplified by each set of primers or hybridized with probes. **D.5.2.2.17** Define the specificity and sequence of each primer 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 retesting, 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. **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.5.2.2.15** - Resolution of ambiguous allele combinations must follow the written agreement(s) with the transplant programs, and the definitions of low resolution and high resolution typing in Standard A.3. **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.

Standard	Guidance
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.5.2.2.27 For laboratories utilizing laboratory-developed molecular reagents :** **D.5.2.2.27.1** 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.5.2.2.27.2** 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.5.2.2.27.3** 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.5.2.3 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.	**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.

Standard	Guidance
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 rehybridization assays and include controls to ensure that the sensitivity and specificity of the assay are unaltered.	
D.5.2.4 Laboratories performing SSP and RT-PCR methods must:	
D.5.2.4.1 Ensure that an internal control is included for each primer mixture that will detect technical failures and that produces a product distinguishable from the specific typing product.	
D.5.2.4.2 Ensure that the amplification conditions are acceptable for the primers used.	
D.5.2.4.3 Include a negative (no nucleic acid) or contamination control in each assay.	
D.5.2.4.4 Ensure that primers used produce adequate amounts of amplification products to be visualized.	
D.5.2.4.5 Have a written procedure for resolving indeterminate calls.	
D.5.2.5 Laboratories performing Sanger sequencing must:	
D.5.2.5.1 Ensure that the method for preparing sequencing templates reliably generates appropriate length templates that are free of inhibitors and contaminants capable of causing sequencing artifacts (e.g., residual primers).	
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.	**Re: D.5.2.4.5** Establish criteria for resolving indeterminate calls whether to call them negative, positive or no call.
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.	

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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 Laboratories must determine the sequences of both sense and anti-sense DNA strands if a sequence suggests a novel allele.	
D.5.2.6 Laboratories performing Next Generation Sequencing and/or Third Generation Sequencing methods must:	
D.5.2.6.1 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.5.2.6.2 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.	**Re: D.5.2.6.1 -** 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.
D.5.2.6.3 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.5.2.6.4 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**.**	Software that can detect the presence of a minor allele due to potentially biased amplification in sequencing data must be used.
D.5.2.6.5 For laboratories that reuse nanopore flow cells for sequencing assays, have a process in place to ensure that residual DNA is excluded from analysis.	
D.5.2.6.5.1 During validation, for each type of flow cell used, the laboratory must establish how many times the flow cell can be reused.	For validation and quality assurance, systematic co-amplification of closely related genomic sequences must be excluded or taken into consideration for genotype calling by the software analysis program.
D.5.2.6.6 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 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	PCR artifacts, such as in vitro cross-over events, must be quantified due to their

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the informatics process must be documented. Any scripts or configurations that deviate from standard vendor installations must be identified, versioned and validated. **D.5.2.6.7** Create a policy for the storage and transmission of primary, intermediate, and final sequencing datafiles. Retained datasets must support reanalysis of the sequencing data at a later date if indicated. **D.5.2.6.8** 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 reanalyzing previously sequenced datasets. **D.5.2.6.9** 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. **D.5.2.6.10** Have a protocol for monitoring any element of NGS testing referred to another laboratory. Standards for NGS-based testing cover the entire testing process including sample handling, target DNA enrichment, library generation, DNA sequencing, bioinformatic analysis, and reporting. Laboratories that refer any part of the NGS process to a referral laboratory are responsible for ensuring the referral laboratory is CLIA certified and /or accredited by an approved HHS Accreditation Organization (AO) to perform the relevant steps of testing in accordance with the following criteria: **D.5.2.6.10.1** Have a protocol for tracking specimens and data when any part of NGS testing is referred to another laboratory. This protocol must include methods and records for confirming sample and data identity. **D.5.2.6.10.2** Participate in PT relevant to components of NGS testing performed in the laboratory. **D.5.2.7 Laboratories performing solid phase techniques must:** **D.5.2.7.1** Validate all calculations. Determine the positive or negative cutoffs specific for each method. **D.5.2.7.2** Establish, verify and follow criteria to ensure a sufficient number of beads or other substrates of each specificity are analyzed in each assay. **D.5.2.7.3** 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.	potentially adverse effect on genotyping results. Quantification of the cross-over events can be performed computationally. **Re: D.5.2.6.2 -** 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.5.2.6.4** -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 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

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	performance of the sequencing instrument over time. **Re: D.5.2.6.6** - 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.5.2.6.7** - Laboratories must establish policies for storing unaligned, processed sequencing files (e.g., FASTQ) based on the requirements. **Re: D.5.2.6.8** - 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.5.2.6.9** - See guidance for D.5.2.6.6. **Re: D.5.2.6. 10** - If part of testing is performed by a lab with a different CLIA #, and this lab is not ASHI accredited, then this lab must provide documentation of personnel qualifications, competency,

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	instrument PM, QA/QC, etc. that ASHI requires. If part of testing is performed by a lab section not covered by ASHI but under the same CLIA#, this section would also be required to provide documentation of personnel qualifications, competency, instrument PM, QA/QC, etc. Laboratories must also adhere to local government regulations first, as some locations restrict referring portions of patient testing. **Re: D.5.2.7.1** - 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 cut-offs based on clinical consideration.
D.5.2.8 Laboratories performing flow cytometry techniques must: **D.5.2.8.1** Establish the optimum serum-to-target ratio. **D.5.2.8.2** 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.5.2.8.3** Define acceptable time periods between processing, labeling and data acquisition. Control samples must be treated in the same manner. **D.5.2.8.4** Laboratories must use the dilution and/or volume of reagents that have been locally validated prior to use. **D.5.2.8.5** 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.	**Re: D.5.2.8** Flow cytometry techniques include assays performed using either conventional flow cytometry instruments or Luminex-based platforms. **Re: D.5.2.8.1- D.5.2.8.**3 and D.5.2.8.7 These standards apply to the Laboratory-Developed and FDA-modified tests. Any laboratory using flow cytometry assay (including FDA-approved tests) must meet Standard D.5.2.8.6 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 (this is only an example), or other documented method. This

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D.5.2.8.6 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.5.2.8.7** Use anti-human immunoglobulin reagents according to manufacturer's protocol or tittered 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.5.2.8.8** Laboratories performing cell-based antibody screening and/or crossmatching by flow cytometry must: **D.5.2.8.8.1** 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.5.2.8.8.2** 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.5.2.8.8.3** For internal labeling, document that the method used to allow fluorochrome-labeled antibodies to penetrate the cell membrane is effective.	can be done via testing positive internal or external controls.
D.5.2.9 Immune function tests **D.5.2.9.1** Laboratories performing cell culture must: **D.5.2.9.1.1** Use a laminar flow hood or other appropriately aseptic work area for preparation of cultures incubating for >18 hours. **D.5.2.9.1.2** Monitor incubators for appropriate temperature, CO_2 concentration and humidity. **D.5.2.9.1.3** Document that lymphocyte viability is sufficient at the start of culture to maintain cell proliferation to ensure accurate test results if applicable. **D.5.2.9.1.4** Incubate cell cultures for the length of time shown to give appropriate cellular proliferation, if applicable. **D.5.2.9.2** Laboratories performing MLC or other cellular assays must also, as applicable: **D.5.2.9.2.1** Use a negative control for each responder cell that consists of responder cells stimulated with autologous cells. **D.5.2.9.2.2** Ensure that each assay includes HLA class II-disparate stimulator cells as positive controls for responder cell proliferation. **D.5.2.9.2.3** Show that stimulator cells are capable of stimulating unrelated HLA class II-disparate cells.	

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D.5.2.9.2.4 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.5.3 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 written 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 C**riteria and procedures for assessing prospective compatibility using crossmatch for each transplant program it serves. **D.5.3.1.1.1.3** Protocols for high risk recipients, versus unsensitized recipients. **D.5.3.1.1.1.4** 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** The laboratory must make a reasonable effort to have available monthly serum specimens for all potential transplant recipients for periodic antibody screening, identification, and crossmatch. **D.5.3.1.1.5** Have a policy to attempt to obtain and store serum samples after known sensitizing events. **D.5.3.1.1.6** Have a policy to periodically screen serum samples from each transplant patient for antibody to HLA antigens, including the frequency of screening serum samples. **D.5.3.1.1.7** Have a process for obtaining a recipient specimen on the day of the transplant (collected within 24 hours before transplantation), to be used for prospective or retrospective crossmatch. **D.5.3.1.1.7.1** Have a process for documenting efforts to obtain a recipient specimen on the day of the transplant, if the laboratory is unable to obtain such a sample.	**Re: D.5.3.1.1.6 and D.5.3.1.1.7** Specimen Collection Criteria: The specific criteria for specimen collection must be clearly defined and incorporated into all transplant agreements with participating programs. A variance report is not required in instances where specimens are not received in accordance with the established transplant agreement.

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D.5.3.2 Solid organ transplantation	
D.5.3.2.1 Laboratories performing testing for solid organ transplantation must:	
D.5.3.2.1.1 Type donors for HLA-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPA1, -DPB1 and -Bw4/Bw6.	
D.5.3.2.1.1.1 For kidney, pancreas, or islet donors, or multi-organ transplants including a renal organ, typing must be completed prior to organ offers.	
D.5.3.2.1.1.2 For heart or lung donors, or multi-organ transplants including a thoracic organ, typing must be completed prior to final organ acceptance.	
D.5.3.2.1.1.3 For donors of other organs or vascularized composite allografts, typing must be completed within the period specified in the transplant agreement.	
D.5.3.2.1.2 Prospectively type recipients of kidney, pancreas, or islet transplants, or multi-organ transplants including a renal organ, for HLA-A, -B, -DRB1, and -Bw4/Bw6.	
D.5.3.2.1.3 All UNOS histocompatibility laboratories prospectively typing deceased donors for OPTN matchruns must use a molecular method to type donors at the antigen level of resolution needed to meet the most recent OPTN/UNOS tables of antigen and unacceptable antigen equivalencies. Reporting of HLA typing must be sent to the OPTN contractor and OPO within the period specified by the written agreement with the OPO.	
D.5.3.2.1.4 All UNOS histocompatibility laboratories prospectively typing deceased donors for OPTN matchruns must distinguish the following null alleles: A*24:09N (when associated with B*40 or B*27), B*51:11N (when associated with A*02:01, C*15:02/15:13 and DRB1*04:02), C*04:09N (when associated with B*44:03); DRB4*01:03:01:02N (when associated with DRB1*07 and DQB1*03:03 (DQ9)), and DRB5*01:08:01N/01:08:02N (when associated with DRB1*15:02).	
D.5.3.2.1.5 Follow policies and procedures established by a joint agreement with the transplant program to test transplant patients for the presence of anti-HLA antibodies at initial evaluation, at intervals consistent with established clinical transplant protocols, and following sensitizing events.	
D.5.3.2.1.6 Perform crossmatching using samples and crossmatch type, as established with the transplant center.	
D.5.3.2.1.6.1 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 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.	

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D.5.3.2.1.7 Have a policy for selection of sera for crossmatching of allosensitized patients that addresses the impact of historic and current sensitizing events. **D.5.3.2.1.8** All UNOS histocompatibility laboratories must review and verify the data they completed and entered into the UNet Waitlist. There must be documentation of review by the laboratory of UNOS HLA data within one month of entry. Documentation of such review must be kept for at least three years or the interval required by local, state and federal regulations, whichever is longer, and must be available for audit by UNOS. **D.5.3.2.1.9** All UNOS histocompatibility laboratories must use a solid phase method for antibody identification that can identify HLA antibody specificities even in very highly sensitized transplant patients.	
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. **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. **D.5.3.3.1.4** In the case of cord blood units, ensure verification typing is performed prior to shipment. **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.	**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, two or more field typing of one or more loci may be required even if HLA identity at one field typing between donor and recipient is confirmed via family studies. **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.5.3.3.1.5 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: 5.3.3.1.4 -** It is recommended that the laboratory serving the HCT program perform an additional verification typing on the shipped unit to verify the correct unit was received. **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 Transplantation of Other Organs and Tissues **D.5.3.4.1** 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.	**Re: D.5.3.4.1 -** Laboratories must have and follow joint-agreement policies for each organ type transplanted including policies that require no testing for specific organ types.
D.5.3.5 Transfusion Support **D.5.3.5.1** Laboratories performing testing for platelet and granulocyte transfusion support must: **D.5.3.5.1.1** Type the patient and potential transfusion donor, if applicable, for HLA-A and -B antigens. **D.5.3.5.1.2** 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.5.3.5.1.3** Follow applicable Standards when performing crossmatch and antibody analysis tests to detect and differentiate HLA class I, platelet-and/or granulocyte-specific antibodies. **D.5.3.5.1.4** If applicable, provide recommendation regarding compatibility requirements for future transfusion support.	**Re: D.5.3.5 -** When investigations of Transfusion Related Acute Lung Injury (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.

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D.5.3.6 Disease Risk, Drug Hypersensitivity Reaction Risk and Vaccine Eligibility Assessment **D.5.3.6.1** Laboratories performing HLA typing for disease risk, drug hypersensitivity reaction risk, and/or vaccine eligibility assessment must establish a written policy specifying: **D.5.3.6.1.1** The clinically relevant HLA loci/antigens/alleles for each indication for **D.5.3.6.1.1.1** disease association **D.5.3.6.1.1.2** drug hypersensitivity **D.5.3.6.1.1.3** Other indications, including vaccine eligibility, HLA loss etc. **D.5.3.6.1.2** Typing resolution requirements and methods used for testing. **D.5.3.6.1.3** When high resolution is required, criteria for reporting rare or novel alleles to indicate association with the risk allele.	**Re: D.5.3.6.1** No additional proficiency testing (PT) is required when the laboratory performs HLA typing for non-transplant purposes using the same molecular methods that are used for transplant-related testing. However, PT is required when the laboratory uses different methods or kits for non-transplant testing. For example, if HLA-B27 testing is performed using flow cytometry or a specific SSP PCR kit (rather than the molecular methods used for transplant typing), the laboratory must participate in an appropriate PT program for those methods. **Re: D.5.3.6.1.1** Unlike transplant-related testing, there is no requirement for joint agreements between clinical programs and laboratories for non-transplant HLA testing. In these cases, the laboratory is expected to perform testing based on the physician's order. However, if a laboratory receives a test order that does not align with their written policy, this may serve as an opportunity for physician education. For example, in the context of narcolepsy risk assessment, the appropriate target is **HLA-DQB1*06:02**. If a physician instead orders **HLA-DRB1*15** typing, the laboratory could use this as a chance to clarify the most relevant marker and provide educational support. **Re: D.5.3.6.1.1.2** Identification of HLA alleles (or antigens) associated with drug hypersensitivity must be consistent with

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	Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines: https://cpicpgx.org/guidelines/ Re: **D.5.3.6.1.3** Rare or novel alleles detected may be reported at the level of P/G groups when highresolution is required (B*57: 173 can be reported as B*57:01P when testing for abacavir hypersensitivity).
D.5.3.7 Laboratories performing HLA typing must: **D.5.3.7.1** Ensure that the level of resolution of HLA typing is appropriate for the clinical application and is based on established criteria. **D.5.3.7.2** Have written criteria or protocols for: **D.5.3.7.2.1** Preparation of cells or cellular component isolations (for example, solubilized antigens and nucleic acids), as applicable to the HLA typing technique(s) performed. **D.5.3.7.2.2** Selection, quality control, and usage of all typing reagents and components. **D.5.3.7.2.3** The assignment of HLA antigens and alleles and for distinguishing common null alleles as appropriate for the clinical use of the test results. **D.5.3.7.2.4** Determining when antigen or allele redefinition and retyping are required. **D.5.3.7.2.5** Assignment of haplotypes, if reported: **D.5.3.7.2.5.1** 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.5.3.7.2.5.2** Reports must include an explanation of recombination when this occurs. **D.5.3.7.3** 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.5.3.7.4** Use HLA typing terminology that conforms to the latest report of the World Health Organization (WHO) 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 WHO terminology. **D.5.3.8 Laboratories performing antibody screening and/or identification must:** **D.5.3.8.1** Use HLA antibody screening and/or identification methods to characterize.	**Re: D.5.3.7.1** - 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.5.3.7.2.3** - 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). 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: https://pmc.ncbi.nlm.nih.gov/articles/PMC7317522/ **Re: D.5.3.7.2.5.1** - Genotypic identity can only be proven if both parents are available or if the segregation of the four haplotypes is clearly defined.

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HLA class I and class II antibodies. **D.5.3.8.2** The laboratory must have a written protocol/policy to identify and assess patient test results that appear inconsistent. **D.5.3.8.2.1** The laboratory must document comparison activities for inconsistent test results. **D.5.3.8.4** For antibody identification, use a documented panel sufficient in number and phenotypic distribution with respect to individual antigens and/or alleles for the intended use of the test results and for the population served. **D.5.3.8.6** For antibody screening, document that the pooled cells or antigens used for antibody screening include the major antigen specificities or CREGs. **D.5.3.8.7** When CREG/epitope/eplet nomenclature is reported, maintain documentation of antigens defined by each CREG/epitope/eplet. **D.5.3.8.11** Laboratories performing antibody analysis for non-HLA antigens must: **D.5.3.8.11.1** Define the target antigens in the assay. **D.5.3.8.11.2** Ensure that the antigens targeted by the assay are appropriate for the clinical application. **D.5.3.8.11.3** For multiplexed assays, ensure the specificity of antibodies to individual target proteins.	**Re: D.5.3.8.2** Results may be correlated with the relevant criteria described in D.5.1.2
D.5.3.9 Laboratories performing crossmatch testing must: **D.5.3.9.2** Establish and follow written policies and procedures for performing each type of crossmatch a laboratory performs. **D.5.3.9.3** For each crossmatch test, use patient's 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.	**Re: D.5.3.9.4.1 -** Protocols must define cutoffs for sample age, as well as when samples other than the most recent sample are to be used for crossmatch. For example, to test reactivity of historical peak antibody levels or after a sensitizing event.

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D.5.3.9.4 Have written criteria or protocols for: **D.5.3.9.4.1** Selecting appropriate patient serum samples for crossmatch. **D.5.3.9.4.2** Preparation of donor cells or cellular component isolations (for example, solubilized antigens) as applicable to the technique(s) performed. **D.5.3.9.4.3** Situations for which additional donor typing is required to determine patient antibody reactivity. **D.5.3.9.4.4** Circumstances under which each type of crossmatch will be performed. **D.5.3.9.5** When physical crossmatch is performed, **D.5.3.9.5.1** Use a technique(s) that detects HLA-specific antibody with a sensitivity superior to that of the basic complement-dependent microlymphocytotoxicity assay. **D.5.3.9.5.2** Include appropriate controls and calibrators on every run: **D.5.3.9.5.2.1** a negative control of human serum documented to be non-reactive against the antigenic target, and if needed, a separate calibrator **(refer to D.4.1.8.3 and D.4.1.8.4).** **D.5.3.9.5.2.2** A positive control of an appropriate isotype and specificity, known to react with the specific cell types or antigens being tested. The positive control must be tested at a dilution appropriate for the assay (i.e., a titer at which moderate changes in assay sensitivity are likely to be detected). **D.5.3.9.5.5** When applicable, use a method that detects antibodies to HLA class II antigens and distinguishes them from antibodies to HLA class I antigens binding to T and/or B cells. **D.5.3.9.5.6** Ensure that there is a procedure to monitor and address non-specific binding of antibody. **D.5.3.9.5.7** Use appropriate methods and/or controls to assess the impact of xenogeneic, chimeric, monoclonal, or other therapeutic antibodies in the assay. **D.5.3.9.5.8** If a donor has been transfused within the previous seven days, accept the results only if there is no evidence of potential interference from cells derived from transfusion products. **D.5.3.9.6** For virtual crossmatch, the laboratory must define criteria for: **D.5.3.9.6.1** HLA typing by molecular methods of donor and recipient of at appropriate loci and sufficient resolution to permit virtual crossmatch assessment. **D.5.3.9.6.2** Frequency of antibody screening and identification for high risk recipients versus unsensitized recipients. **D.5.3.9.6.3** Patient eligibility.	**Re: D.5.3.9.4.3** - For assessing compatibility for broadly sensitized patients, typing must include 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 the transplant agreement. **Re: D.5.3.9.5.2.2** Several dilutions may be needed to detect moderate changes in the assay sensitivity

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D.5.3.9.6.4 How the antibody data interpretation is used for virtual crossmatch, including: **D.5.3.9.6.4.1** Use of serum treatment to mitigate complement-mediated interference. **D.5.3.9.6.4.2** Use of pattern analysis (CREGS, epitopes/eplets, etc.). **D.5.3.9.6.4.3** Use of multiple serum samples.	**Re: D.5.3.9.6.3** - Eligibility criteria must include under what circumstances a patient is not eligible for virtual crossmatch. **Re: D.5.3.9.6.4 –** The pattern analysis can be used to adjust cut-offs, identify non-specific reactivity, and/or extrapolate reactivity to antigens/alleles not covered by antibody assays used for virtual crossmatch.
D.5.3.10 Laboratories performing chimerism testing must: **D.5.3.10.1** Have written protocols for test performance and analysis, including **D.5.3.10.1.1** determining and selecting informative markers for distinguishing DNA of two or more individuals in each test. **D.5.3.10.1.2** Evaluating the amounts of recipient and donor in a mixed chimeric sample if results are reported as a percentage of donor and/or recipient. **D.5.3.10.1.3** The minimal number of informative markers used for analysis to assure the accuracy and precision established during validation. **D.5.3.10.1.4** Requirements for informative markers on more than one chromosome to prevent errors in chimerism results reported for patients at risk for chromosomal deletions (e.g., malignancy, chromosomal abnormalities) **D.5.3.10.1.5** Method for determining presence of two or more genotypes when 100% donor and 100% recipient specimens are not available **D.5.3.10.1.6** Criteria for accepting or rejecting the amplification of a particular genetic locus or of an individual sample. **D.5.3.10.1.7** Ensuring that the level of sensitivity is appropriate for the clinical application and is based on criteria established during validation. **D.5.3.10.1.8** A method/analysis for recognizing non-specific signal and/or background signal.	**Re: D.5.3.10.1.2** - For systems with discrete alleles (e.g. STR, NGS, dPCR), 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).

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D.5.3.10.2 Detection limits must be provided in the reports. "Less than" or "Greater than" can be used for reporting test results that are below the laboratory's detection limits for an analyte. **D.5.3.10.3** The lab should establish the accuracy of chimerism detection across the reportable range using controlled mixtures during validation and perform accuracy assessments at least annually. **D.5.3.10.4** If chimerism testing is performed using cell subsets, have a written policy for evaluation of subset purity. **D.5.3.10.5** For conventional PCR-based methods, including STR, VNTR, NGS, and qPCR: **D.5.3.10.5.1** Have a method/analysis to adjust for preferential amplification in the data analysis when using amplification-based methods. **D.5.3.10.5.2** Assess and consider the stoichiometry of the reaction when more than one locus is amplified in a single amplification reaction mixture (multiplex). **D.5.3.10.6** For dPCR-based methods: **D.5.3.10.6.1** Have a policy to address factors (technical and interpretation) that can cause over or under-estimation of informative markers. **D.5.3.10.7** Establish minimum and maximum DNA requirements for optimal sensitivity and specificity. **D.5.3.10.8** The lab should have procedures to minimize carryover and/or contamination and have a policy for ongoing monitoring of contamination.	
D.5.3.11 Laboratories performing immunophenotyping and/or single antigen typing by flow cytometry must: **D.5.3.11.1** Use specificity controls consisting of appropriate cell types known to be positive for selected standard antibodies for each lot or shipment, where applicable. **D.5.3.11.2** Use a negative reagent control(s) for each test cell population. It is recommended that this control consist of monoclonal antibody(ies) of the same species and subclass and be prepared/purified in the same way as the monoclonal(s). **D.5.3.11.3** 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.5.3.11.4** 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.	**Re: D.5.3.11.4 -** 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.5.3.11.5 Employ gating strategies to assure that the population of interest is being selected without significant contamination. **D.5.3.11.6** Ensure the appropriate definition and purity of cell populations by the use of either a multi-color technique or other documented method. **D.5.3.11.7** 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.5.3.12 Laboratories performing ABO group typing must: **D.5.3.12.1** If using serological methods: **D.5.3.12.1.1** Use established procedures and criteria when performing titration of anti-ABO antibodies. **D.5.3.12.1.2** 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.5.3.12.1.3** Determine the ABO group on red cells using anti-A and anti-B sera (forward typing), and test the serum or plasma for expected antibodies with A_1 and B cells (reverse typing). Cord cells and blood from newborns must be tested for red cell antigens only, not for antibodies. **D.5.3.12.1.4** 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.5.3.12.1.7** Compare the current ABO 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.5.3.12.1.8** 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.5.3.12.1.9** 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.	**Re: D.5.3.12.1.4 –** Subtyping must: • Be tested using pre-red blood cell transfusion samples • Be drawn on two separate occasions • Have different collection times • Be submitted as separate samples **Re**: **D.5.3.12.1.8** –**D.5.3.12.1.10** 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, must 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.5.3.12.1.10 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.	
D.5.3.12.2 If genotyping of glycosyltransferases for prediction of ABO antigen typing:	
D.5.3.12.2.1 ABO typing by molecular methods must be used only as predicted phenotype.	
D.5.3.12.2.2 If the laboratory performs ABO typing by molecular and serological methods, the report must clearly differentiate these methods and specify that it is a predicted phenotype when molecular methods are used.	**Re: D.5.3.12.2** Transplant donor registries often collect samples from potential donors using buccal swabs or saliva. These samples cannot be used for traditional serological ABO/ blood group typing because fresh intact red blood cells (RBCs) are not available. Molecular ABO/ typing may be performed to predict the ABO/ phenotype to aid in finding an appropriate donor. The independent use of molecular based assays is not acceptable for ABO blood type assignment for the purposes of transfusion. ABO and typing by FDA approved serological methods must be used for the purpose of transfusion or donor and recipient ABO typing for transplantation.
D.5.3.12.2.3 Ensure that genotyping reagents are capable of clearly defining all ABO groups and common subgroups to provide a predicted phenotype accurately.	
D.5.3.12.2.4 The laboratories performing ABO typing by both molecular and serological methods, should establish and follow a written policy on:	
D.5.3.12.2.4.1 resolving discrepancies between serology and molecular results. Agreements between laboratories and donor registries must state the intended use of ABO molecular typing results and the need for two independent ABO results performed by FDA approved serological methods for selected donors.	
D.5.3.12.2.4.2 when molecular typing is used to resolve discrepancies between the forward and reverse typing or mixed field typing.	
D.5.3.12.2.4.3 how to report results for a deceased donor in the presence of a discrepancy. If a laboratory does not perform ABO group genotyping for deceased donors, then it is not obligated to follow this standard.	
D.5.3.13 Laboratories performing genotyping of loci other than classical HLA and ABO antigen genes (e.g., non-classical HLA loci, KIR loci) must:	**Re: D.5.3.12.2.4** The policy should specify how ABO serology and molecular results should be correlated and interpreted in the instances of leuko-reduced red blood cell transfusion, granulocyte transfusion, whole blood transfusion, or stem cell infusion. The lab can follow the OPTN guidance: **https://optn.transplant.hrsa.gov/professionals/by-topic/guidance/guidance-for-addressing-blood-type-determination/**
D.5.3.13.1 Define the target loci genotyped by the assay.	
D.5.3.13.2 Ensure that the level of resolution (gene presence, SNP and/or allele genotype, etc.) is appropriate for the clinical application.	
D.5.3.13.3 Have written criteria and protocols for:	
D.5.3.13.3.1 Isolation, preparation, and quality control of nucleic acids for analysis.	
D.5.3.13.3.2 Selection, use, and quality control of all reagents, data handling, and analyses.	

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D.5.3.13.4 Use standardized terminology when available.	The policy also has to delineate steps on how to adjudicate the ABO group typing discrepancy depending on circumstances (e.g. recollection, allocation as AB, buccal swabs etc.).
D.5.3.14 Laboratories performing dd-cfDNA testing must: **D.5.3.14.1** Use a blood collection tube that stabilizes cell-free DNA. **D.5.3.14.2** Establish that cfDNA is free from gDNA. **D.5.3.14.3** Have a policy on which specimens and/or analysis should be in place to distinguish DNA from two or more individuals (e.g. for re-transplant patients or recipients of different organs from different donors). **D.5.3.14.4** Have a policy for analyzing and reporting when the specimens for establishing identity are missing or such specimens are not required based on assay specifications. **D.5.3.14.5** Establish criteria for quantitating amounts of recipient- and donor-derived cfDNA, including: **D.5.3.14.5.1** The minimal number of informative markers for the analysis. **D.5.3.14.5.2** The criteria for accepting or rejecting the amplification of a particular genetic locus or of an individual sample. **D.5.3.14.5.3** The criteria for recognizing non-specific signal and/or background. **D.5.3.14.5.4** For conventional PCR-based methods, the criteria for adjusting for preferential amplification of informative markers. **D.5.3.14.5.5** For digital PCR-based methods, the criteria to address factors (technical and interpretation) that can cause over or under-estimation of informative markers. **D.5.3.14.6** Ensure sufficient input of cfDNA. **D.5.3.14.7** Ensure that the level of sensitivity is appropriate and can accurately and reproducibly quantify/measure dd-cfDNA levels appropriate for the clinical application based on established criteria. **D.5.3.14.8** Provide a comment on the report about the significance of detected dd-cfDNA levels.	
D.6 Post-Analytical Systems **D.6.1 Introduction**	**Re: D.6.1.1 -** Patients or their authorized representatives are now entitled to receive laboratory results directly from the laboratory. Laboratories must develop

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D.6.1.1 Test results must be released only to authorized persons and, if applicable, the individual responsible for using the test results and the laboratory that initially requested the test. **D.6.1.2** The laboratory must immediately alert the individual or entity requesting the test, and, if applicable, the individual responsible for using the test results when any test result indicates an imminent life-threatening condition, or panic, or alert values. **D.6.1.3** When the laboratory cannot report patient test results within its established time frames, the laboratory must determine, based on the urgency of the patient test(s) requested, the need to notify the appropriate individual(s) of the delayed testing. **D.6.1.4** If a laboratory refers patient specimens for testing: **D.6.1.4.1** The referring laboratory must not revise results or information directly related to the interpretation of results provided by the testing laboratory. **D.6.1.4.2** The referring laboratory may permit each testing laboratory to send the test result directly to the authorized person who initially requested the test. The referring laboratory must retain or be able to produce an exact duplicate of each testing laboratory's report. **D.6.1.4.3** The authorized person who orders a test must be notified by the referring laboratory of the name and address of each laboratory location where the test was performed. **D.6.1.5** When errors in any reported test results are detected, the laboratory must do the following: **D.6.1.5.1** Promptly notify the authorized person ordering the test and, if applicable, the individual using the test results of reporting errors. **D.6.1.5.2** Issue corrected reports promptly to the authorized person ordering the test and, if applicable, the individual using the test results. **D.6.1.5.3** Maintain a copy of the original report, as well as the corrected report.	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. **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.6.2 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.	**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.

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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, including the type of crossmatch performed 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** Document the purity of cell subsets for chimerism analysis. If purity is not assessed, document on the test report. **D.6.2.2.11** 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.6.2.2.12** All phenotype terminology using WHO approved nomenclature where it exists. **D.6.2.2.12.1** The laboratory must have a written policy for assignment of serologic equivalents outside of WHO terminology. This applies to the assignment of serologic equivalents for alleles and loci that do not have WHO approved serologic nomenclature, and/or assignment of serologic epitopes. **D.6.2.2.13** HLA antibody specificities when clinically relevant: **D.6.2.2.13.1** The laboratory must report antibody specificities corresponding to WHO-recognized serologic antigens when available and have a written policy for reporting anti-HLA antibody specificities that are not WHO-recognized serologic antigens. **D.6.2.2.14** 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 hematopoietic cell transplantation (HCT). **D.6.2.2.14.1** Results reported using G or P group codes do not need to list unresolved alleles that are within the G or P group listed. **D.6.2.2.14.2** Results reported may exclude non-common, intermediate, or well-documented (CIWD 3.0.0) alleles found in alternative genotypes if stated in the report, transplant agreement, or client written request.	**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.6.2.2.10 -** 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. **Re: D.6.2.2.12** - 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.6.2.2.12.1 -** When assigning serological equivalents for which WHO terminology is not available, laboratories must indicate on their report when they are not using WHO nomenclature. **Re: D.6.2.2.13.1** - Labs wishing to report allele-specific antibodies should use allelic designations (e.g. DQB1*03:19). The lab's written policy should state how they will report antibodies to epitopes or HLA heterodimers, etc.

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D.6.2.2.14.3 Typing results reported using NMDP codes must define all unresolved alleles represented by the allele code. **D.6.2.2.15** The virtual crossmatch analysis report used for final organ allocation must be documented, and must contain: **D.6.2.2.15.1** The recipient identifier. **D.6.2.2.15.2** The donor identifier. **D.6.2.2.15.3** The sample date for the patient antibody testing results used, and the source of donor typing information**.** **D.6.2.2.16** 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." **D.6.2.2.17** Limitations of the method(s) used, specific to the reported results. **D.6.2.3** Prior to release, final reports must be reviewed and approved by the director, technical supervisor or by a designee who must meet the requirements for general supervisor under **E.5.1.1** or a person who: **D.6.2.3.1** Meets educational requirements under E.2.1.1.2.1 **D.6.2.3.2** Have at least 1 year of laboratory training in directing histocompatibility testing; and **D.6.2.3.3** Be registered as a DIT with DTRC. **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.6.2.2.14 –** All approved WHO nomenclature codes and/or multiple allele (NMDP) codes must be fully defined by either listing the ambiguity string or providing a link to a published reference to the clinicians. Low resolution typing does not require consultation of the CIWD catalog. **Re: D.6.2.2.16 -** Non-U.S. laboratories do not need to have this on reports. **Re: D.6.2.3.3** – The mentor needs to provide attestation that the candidate is qualified by both education and experience and competency must be performed every 6 months for each test the director in training signs out until they meet the 3 years "general supervisor" qualification.

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	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 testing for the same analyte 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. **D.6.3.1.2** The laboratory must establish and employ policies and procedures, and document actions taken for the following: **D.6.3.1.2.1** Test systems do not meet the laboratory's established criteria including quality control results that are outside of acceptable limits. **D.6.3.1.2.2** Errors are detected in the reported clinical results. In these situations, the laboratory must promptly: **D.6.3.1.2.2.1** Notify the authorized person ordering or individual utilizing the test results of reporting errors. **D.6.3.1.2.2.2** Issue corrected reports **D.6.3.1.2.2.3** 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 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.	**Re: D.6.3.1.1** Examples of analytes tested using more than one method or instrument include typing, antibody tests by cytotoxicity and solid phase, or crossmatching using two different flow cytometers. Relationships must be evaluated by using test specimens that allow assessment of test accuracy and other pertinent test performance characteristics. Proficiency test samples can be used for this purpose provided the requirements for PT testing are met, including that correlation studies should be done after submitting PT results.

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D.6.3.2 Corrective Actions **D.6.3.2.1** Laboratories must have a mechanism in place for addressing any testing discrepancies that occur within or between different laboratories. **D.6.3.2.2** Corrective action policies and procedures must be available and followed as necessary to maintain the laboratory's operation for testing patient specimens in a manner that ensures accurate and reliable patient test results and reports. **D.6.3.2.3** The laboratory must document all corrective actions taken when test systems do not meet the laboratory's verified or established performance specifications which include, but are not limited to: **D.6.3.2.3.1** Equipment or methodologies that perform outside of established operating parameters or performance specifications. **D.6.3.2.3.2** Patient test values that are outside of the laboratory's reportable range of test results for the test system. **D.6.3.2.3.3** The reference intervals (normal values) for a test procedure that the laboratory determines are inappropriate for the laboratory's patient population. **D.6.3.2.3.4** Results of control and/or calibration materials fail to meet the laboratory's established criteria for acceptability. All patient test results obtained since the last acceptable test run must be evaluated to determine if patient test results have been adversely affected. The laboratory must take the corrective action necessary to ensure the reporting of accurate and reliable patient test results. **D.6.3.2.3.5** The criteria for proper storage of reagents and specimens are not met. **D.6.3.2.4** Any errors detected in patient or proficiency testing results must be documented, investigated, and corrective action taken as needed to prevent recurrence. **D.6.3.2.5** Any accidents determined to be attributable to inadequate laboratory space or to staff safety conditions must be documented, investigated, and corrective action taken, as needed, to prevent recurrence. **D.6.3.2.6** The laboratory must have a policy to address sentinel events/immediate jeopardy situations that includes immediate reporting to the ASHI Accreditation Program, with appropriate and complete documentation and investigation of the event.	**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.6.3.3 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:	**Re: D.6.3.3.1.1** - Testing registry donors or relationship testing requires consent but not a test requisition.

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D.6.3.3.1.1 The test requisition, if applicable. **D.6.3.3.1.2** The positive and/or unique identification of the specimen. **D.6.3.3.1.3** The tissue source of the specimen. **D.6.3.3.1.4** The date and time of specimen receipt into the laboratory. **D.6.3.3.1.5** The condition and disposition of specimens that do not meet the laboratory's criteria for specimen acceptability. **D.6.3.3.1.6** The records, test data, results, and dates of all specimen testing, including the identity of the personnel who performed the test(s). **D.6.3.3.1.7** Legally-reproduced copies of all preliminary and final reports. **D.6.3.3.1.8** Records of instrument printouts, if applicable. **D.6.3.3.1.9** Documented review of final test reports by a director, technical supervisor, or designee who must meet the requirements for general supervisor under **E.5.1.1** or. **D.6.3.3.1.9.1** Meet educational requirements under **E.2.1.1.2.1** and **D.6.3.3.1.9.2** Have at least 1 year of laboratory training in directing histocompatibility testing; and **D.6.3.3.1.9.3** Be registered as a DIT with the DTRC. **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**.	**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.
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** Be qualified by education, training, and experience in each area of accreditation 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 DTRC Committee. **E.2.1.1 Qualifications for US laboratories and international laboratories certified by CLIA -** The laboratory director must: **E.2.1.1.1** Possess a current license as a laboratory director issued by the State in which the laboratory is located, if such licensing is required; and **E.2.1.1.2** Meet the educational, certification and training requirements under E.2.1.1.2.1, E.2.1.1.2.2 or E.2.1.1.2.3: **E.2.1.1.2.1** **E.2.1.1.2.1.1** Be a doctor of medicine or doctor of osteopathy licensed to practice medicine or osteopathy in the State in which the laboratory is located; and	**Re: E.2.1 -** Appropriate documentation that individuals filling all technical positions meet ASHI personnel qualification standards (diploma, US college/university transcripts, or international US equivalency evaluation by an NACES accredited agency) must be readily available to an inspector if requested. **Re: E.2.1.1** applies to CLIA laboratory director

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E.2.1.1.2.1.2 Be certified in anatomic or clinical pathology, or both, by the American Board of Pathology or the American Osteopathic Board of Pathology; Or **E.2.1.1.2.2** **E.2.1.1.2.2.1** Be a doctor of medicine, doctor of osteopathy, or doctor of podiatric medicine licensed to practice medicine, osteopathy, or podiatry in the State in which the laboratory is located; and **E.2.1.1.2.2.2** Have had laboratory training or experience consisting of: **E.2.1.1.2.2.2.1** Have at least 2 years of experience directing or supervising high complexity testing; and **E.2.1.1.2.2.2.2** Have at least 20 CE credit hours in laboratory practice that cover the director responsibilities defined in **E.2.2 (**CFR §493.1445) **E.2.1.1.2.3** **E.2.1.1.2.3.1** **E.2.1.1.2.3.1.1** Hold an earned doctoral degree in a chemical, biological, clinical or medical laboratory science or medical technology from an accredited institution; or **E.2.1.1.2.3.1.2** Hold an earned doctoral degree; and **E.2.1.1.2.3.1.2.1** Have at least 16 semester hours of doctoral level coursework in biology, chemistry, medical technology (MT), clinical laboratory science (CLS), or medical laboratory science (MLS); or **E.2.1.1.2.3.1.2.2** An approved thesis or research project in biology/chemistry/MT/CLS/MLS related to laboratory testing for the diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of, human beings; and	**Re: E.2.1.1.1.2** - This applies to pathologists directing core laboratories with multiple sections. To become an ASHI accredited laboratory director pathologists must meet section E.2.1.1.1.2. Re: E.2.1.1.2.2.2.2 and E.2.1.1.2.3.4 Per CMS memo QSO-25-21-CLIA, ASHI will not require the additional 20 CE credit hours previously mandated under E.2.1.1.2.2.2.2 and E.2.1.1.2.3.4 as long as CMS is exercising enforcement discretion in these circumstances, allowing individuals to qualify without the extra CE credits. **Re: E.2.1.1.2.3.1.2.2** The thesis project should incorporate laboratory methodologies and/or analytical approaches in basic, clinical, or translational science projects with the aim of improving clinical diagnosis, disease prevention, health assessment, or treatment of human beings. Acceptable research includes projects involving assay development or interpretation of data from molecular diagnostics, immunologic assays, clinical chemistry, hematology, microbiology, or genetic testing.

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E.2.1.1.2.3.2 Be certified and continue to be certified by a board approved by HHS and **E.2.1.1.2.3.3** Have at least 2 years of: **E.2.1.1.2.3.3.1** Laboratory training or experience, or both: and **E.2.1.1.2.3.3.2** Laboratory experience directing or supervising high complexity testing; and **E.2.1.1.2.3.4** Have at least 20 CE credit hours in laboratory practice that cover the director responsibilities defined in **E.2.2 (**CFR §493.1445) **E.2.1.1.3** Notwithstanding any other provision of this section, an individual is considered qualified as a laboratory director of high complexity testing under this section if they were qualified and serving as a laboratory director of high complexity testing in a CLIA-certified laboratory as of December 28, 2024, and have done so continuously since December 28, 2024. **E.2.1.2 Qualifications for non-CLIA international laboratories -** The laboratory director must: **E.2.1.2.1** Meet at least one of the following educational, certification and training requirements: **E.2.1.2.1.1** Hold an earned doctoral degree in a chemical, biological, medical technology, or clinical laboratory science from an accredited institution. **E.2.1.2.1.2** Be a doctor of medicine, osteopathy, or podiatric medicine licensed to practice medicine, osteopathy, or podiatry in the jurisdiction in which the laboratory is located, if such licensing is required by law. **E.2.1.2.2** 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.2.2 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	Re: E.2.1.1.2.3.3: The requirements for high complexity laboratory directors require a total of two years of laboratory training or experience and laboratory experience directing or supervising high complexity testing. E.2.1.1.2.3.3.1 and E.2.1.1.2.3.3.2 can be performed concurrently. **Re: E.2.1.1.2.3.3.1** To meet qualifications for laboratory director for high-complexity testing in U.S. laboratories or in laboratories outside the U.S. that test specimens from U.S. patients, both training and experience must be obtained in a CLIA-certified laboratory.

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responsibilities to personnel meeting the qualifications under sections **E.3, E.4, E.5**, and **E.6**, respectively. **E.2.2.1.2** If the laboratory director reapportions performance of his or her responsibilities, he or she remains responsible for ensuring that all duties are properly performed. **E.2.2.1.3** Each director may direct no more than five laboratories. **E.2.2.2** The laboratory director must: **E.2.2.2.1** Be onsite at least once every 6 months, with at least 4 months between the minimum two on-site visits. Laboratory directors may elect to be on-site more frequently and must continue to be accessible to the laboratory to provide telephone or electronic consultation as needed; and **E.2.2.2.2** Provide documentation of these visits, including evidence of performing activities that are part of the laboratory director responsibilities. **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.	**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). **Re: E.2.2.1** - If any one of more than 2 laboratories with a single director is located in New York State, a NYS waiver is required.

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E.2.2.8.3 All proficiency testing reports are received and reviewed by the appropriate staff to evaluate the laboratory's performance and to identify any problems that require corrective action. **E.2.2.8.4** An approved corrective action plan is followed when any proficiency testing result is found to be unsuccessful or unsatisfactory. **E.2.2.9** Ensure that the quality control and quality assessment programs are established and maintained to assure the quality of laboratory services provided and to identify failures in quality as they occur. **E.2.2.10** Ensure the establishment and maintenance of acceptable levels of analytical performance for each test system. **E.2.2.11** Ensure that all necessary remedial actions are taken and documented whenever significant deviations from the laboratory's established performance characteristics are identified, and that patient test results are reported only when the system is functioning properly. **E.2.2.12** Ensure that reports of test results include pertinent information required for interpretation, according to section **D.6.2** of the standards. **E.2.2.13** Ensure that consultation is available to the laboratory's clients on matters relating to the quality of the test results reported and their interpretation concerning specific patient conditions. **E.2.2.14** Ensure that a general supervisor provides on-site supervision of high complexity test performance in accordance with ASHI Standards. **E.2.2.15** Provide appropriate consultation and supervision to ensure accurate testing and reporting of test results for all aspects of services provided by the laboratory. Ensure that the laboratory employs a sufficient number of laboratory personnel with the appropriate qualifications as described in sections **E.5** and **E.6** of this document. **E.2.2.16** Ensure that prior to testing patient specimens there is documentation that all personnel have the appropriate education and experience, receive the appropriate training for the type and complexity of the services offered, and have demonstrated that they can perform all testing operations reliably to provide and report accurate results. **E.2.2.17** Ensure that policies and procedures are established for monitoring individuals who conduct 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	**Re: E.2.2.17 to E.2.2.20** - If technologists in ASHI accredited laboratories are not involved in histocompatibility testing per se,

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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.	then the director must ensure that such staff are participating in continuing education relevant to their work areas.
E.3 Technical Supervisor Qualifications and Responsibilities **E.3.1 Qualifications -** The technical supervisor must: **E.3.1.1** Be qualified by education, training, and experience for each area of technology, analyte, test, or procedure to provide adequate technical supervision of the laboratory personnel and activities for which the laboratory is ASHI-accredited. Assessment of qualifications for each area of accreditation will be the responsibility of the ASHI Director Training Review Committee. Assessment of qualifications for each technology will be the responsibility of the Accreditation Review Board. **E.3.1.2** Qualifications for US laboratories and international laboratories certified by CLIA- The laboratory technical supervisor must: **E.3.1.2.1** Possess a current license issued by the State in which the laboratory is located, if such licensing is required; and **E.3.1.2.2** Meet the educational, certification and training requirements: **E.3.1.2.2.1** **E.3.1.2.2.1.1** Be a doctor of medicine, doctor of osteopathy, or doctor of podiatric medicine licensed to practice medicine, osteopathy, or podiatry in the State in which the laboratory is located; or **E.3.1.2.2.1.2.**Have an earned doctoral degree in a biological, clinical or medical laboratory science, or medical technology from an accredited institution; or meet the education requirement at **E.2.1.1.2.3.1.2 and** **E.3.1.2.2.2** Have training or experience that meets one of the following requirements: **E.3.1.2.2.2.1** Have 4 years of laboratory training or experience, or both, within the specialty of histocompatibility; or	

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E.3.1.2.2.2.2 **E.3.1.2.2.2.2.1** Have 2 years of laboratory training or experience, or both, in the specialty of general immunology; and **E.3.1.2.2.2.2.2** Have 2 years of laboratory training or experience, or both, in the specialty of histocompatibility. **E.3.1.2.2** Notwithstanding any other provision of this section, an individual is considered qualified as a technical supervisor of high complexity testing under this section if they were qualified and serving as a technical supervisor of high complexity testing in a CLIA-certified laboratory as of December 28, 2024, and have done so continuously since December 28, 2024. **E.3.1.3** Qualifications for non-CLIA international laboratories- The laboratory technical supervisor must: **E.3.1.3.1** Meet at least one of the following educational, certification, and training requirements: **E.3.1.3.1.1** Hold an earned doctoral degree in a chemical, biological, medical technology, or clinical laboratory science from an accredited institution. **E.3.1.3.1.2** Be a doctor of medicine, osteopathy, or podiatric medicine licensed to practice medicine, osteopathy, or podiatry in the jurisdiction in which the laboratory is located, if such licensing is required by law. **E.3.1.3.2** Have training or experience that meets one of the following requirements: **E.3.1.3.2.1** 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 **E.3.1.3.2.2** 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** For laboratories performing ABO testing, the technical supervisor must meet the CMS requirements in immunohematology or histocompatibility as equivalent for the limited immunohematology (ABO testing) performed by facilities using ASHI accreditation to meet CLIA requirements.	**Re: E.3.1.2.2.2** To meet qualifications for technical supervisor for high-complexity testing in U.S. laboratories or in laboratories outside the U.S. that test specimens from U.S. patients, both training and experience must be obtained in a CLIA-certified laboratory. Two years of laboratory training experience in histocompatibility may be pre-doctoral**.**

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E.3.1.5 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** **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, 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	

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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 the educational and training requirements for laboratory director under E.2.1. **E.4.1.2.1** For CLIA-certified laboratories performing ABO testing, meet the CMS requirements for clinical consultant in immunohematology or histocompatibility as equivalent for the limited immunohematology (ABO 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.	
<u>E.5 General Supervisor Qualifications and Responsibilities</u> **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.5.1.1.3** Possess a current license issued by the State in which the laboratory is located, if such licensing is required **E.5.1.2** Each general supervisor(s) must meet at least one of the following requirements:	**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.5.1.2.1 Laboratory director under section **E.1** **E.5.1.2.2** Technical supervisor under **E.2** **E.5.1.2.3** Be a doctor of medicine, osteopathy, or podiatric medicine licensed to practice medicine, osteopathy, or podiatry in the state in which the laboratory is located and have at least three years of laboratory training or experience in human immunogenetics, human histocompatibility, and/or human transplantation immunology testing under the supervision of a director and/or technical supervisor in an ASHI-accredited laboratory. **E.5.1.2.4** Have earned a doctoral, master's, or bachelor's degree in a chemical, biological, clinical or medical 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 must be serving as a general supervisor in an ASHI-accredited laboratory as of December 28, 2024, and having done so continuously since December 28, 2024.	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. **Re: E.5.1.2.5** If there is a break in service after December 28, 2024, the individual must meet the general supervisor requirements of at least one of the following: E.5.1.2.1- E.5.1.2.4
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 Laboratory Director. **E.5.2.2.4** Be responsible for monitoring test analyses and specimen examinations to ensure that acceptable levels of analytical performance are maintained. **E.5.2.3** The director or technical supervisor may delegate to the general supervisor the responsibility for:	**Re: E.5.2.3 -** Written documentation must indicate which responsibilities are delegated.

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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** Evaluating and documenting the competency of all testing personnel.	
E.6 Testing Personnel Qualifications and Responsibilities **E.6.1 Qualifications** Each individual performing high complexity testing must: **E.6.1.1** Possess a current license as a laboratory technologist issued by the state, if such licensing exists; and **E.6.1.2** Meet one of the following requirements: **E.6.1.2.1** Be a doctor of medicine or a doctor of osteopathy, or doctor of podiatric medicine licensed to practice medicine, osteopathy, or podiatry in the state in which the laboratory is located or have earned a doctoral, master's, or bachelor's degree in a chemical, biological, clinical or medical laboratory science, or medical technology from an accredited institution or **E.6.1.2.2** Have earned an associate degree in a laboratory science or medical laboratory technology from an accredited institution. **or** **E.6.1.2.3** 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.6.1.2.3.1** Twenty four semester hours of medical laboratory technology courses; or **E.6.1.2.3.2** Twenty four semester hours of science courses that include: **E.6.1.2.3.2.1** Six semester hours of chemistry. **E.6.1.2.3.2.2** Six semester hours of biology. **E.6.1.2.3.2.3** Twelve semester hours of chemistry, biology, or medical laboratory technology in any combination; and **E.6.1.2.3.3** Have laboratory training that includes any of the following:	**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. **Re: E.6.1.2.3.1.3.2** – For US laboratories, such training must take place in an CLIA

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E.6.1.2.3.3.1 Completion of a clinical laboratory training program approved or accredited by Accrediting Bureau of Health Education Schools (ABHES) and Commission on Accreditation of Allied Health Education Programs (CAAHEP). This training may be included in the 60 semester hours listed in paragraph **E.6.1.1.2** or **E.6.1.2.3.3.2** At least three months documented laboratory training in each specialty in which the individual performs high complexity testing. **E.6.1.2.4** 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** An individual is considered qualified as a high complexity testing personnel under this section if they were qualified and serving as a high complexity testing personnel in a CLIA-certified laboratory as of December 28, 2024, and have done so continuously since December 28, 2024.	certified laboratory. For international laboratories such training could take place in an ASHI or EFI accredited laboratories, or other ARB approved laboratories.
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.	

Standard	Guidance
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, DIT, 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.	