Summary of Proposed Revisions to the 2018 Standards
November 22, 2019

The following revisions are proposed to the 2018 ASHI Standards.

The following changes were made to the Table of Contents:

D.2.6 Personnel ~~technical~~ competency assessment

The following changes were made to A.3 Definitions:

Low resolution typing: A low-resolution HLA genotype result is defined as sufficient to assign genotype 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

High Resolution Typing: A high-resolution HLA genotype is defined as an allele or set of alleles that encode the same protein sequence for the region of the HLA molecule called the antigen binding site. The high resolution HLA genotype results must contain only one unambiguously assigned genotype or may contain multiple alternative genotypes if only one includes two common and well-documented (CWD) alleles. https://protect2.fireeye.com/url?k=48d5e2bd-14958feb-48d5c5a0-0cc47adb57f0-bf6293e266775110&u=http://igdawg.org/cwd.html

Unresolved alleles: alleles or genotypes that have not been excluded.

Re: C.1.4.2 – If the laboratory patient specimen testing procedures normally require reflex, distributive, or confirmatory testing at another location, the laboratory ~~should~~ 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).

Renumbered ~~**D.2.3.2**~~ to **D.2.5.4** The test results (including electronic records access and electronic record distribution) must be released only to the: tested patient; authorized persons; ordering physician and/or provider; the individual responsible for using the tests results, and the laboratory that initially requested the test.

Renumbered ~~**Re: D.2.3.2**~~ to **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.2.6.3.2 Competency assessment for the Technical Supervisor ~~should~~ must include the responsibilities listed in E.3.2

D.2.6.3.3 Competency assessment for the Clinical Consultant ~~should~~ must include the responsibilities listed in E.4.2

D.2.6.3.4 Competency assessment for the General Supervisor ~~should~~ must include the responsibilities listed in E.5.2

Re: D.2.7.3 - Documentation of PT performance review by technologists can~~, e.g.,~~ be in the minutes of laboratory staff meetings.

D.2.9.2.13 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 ~~should~~ must be documented at the time of procedure review.

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

Re: D.4.1.3.1.4 – Examples: OSHA, NFPA, and/or GHS.

D.4.1.7.5.8 ~~Record laser power output and current input each day of use. Acceptable thresholds and corrective action protocols must be documented. If the instrument cannot report these data, the laboratory must obtain documentation from the manufacturer that the laser cannot operate under substandard conditions.~~ 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.5.2.1.7 Record the results of each cell-serum combination in a manner that indicates the approximate percentage of cells killed. ~~Use of the numerical scores in the latest edition of the ASHI Laboratory Manual is recommended.~~

Re: D.5.2.2.25 – Inspectors ~~should~~ 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.

Re: D.5.2.11.1

- Assay validation ~~should~~ 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 ~~should~~ must include sufficient representation of all pertinent allelic specificities of the locus tested in order to evaluate possible allele drop-outs. Alleles with consistently poor representation in sequencing data (drop-out) must be addressed by alternative methods for detection
- Software that can detect the presence of a minority allele due to potentially biased amplification in sequencing data must be used.
- For validation and quality assurance, systematic co-amplification of closely related genomic sequences ~~should~~ must be excluded or taken into consideration for genotype calling by the software analysis program.
- PCR artifacts, such as *in vitro* cross-over events, ~~should~~ must be quantified due to their potentially adverse effect on genotyping results. Quantification of the cross-over events can be performed computationally.

Re: D.5.2.11.2

- During validation, laboratories ~~should~~ 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 ~~should~~ must establish procedures to identify potential allele dropouts and preferential amplifications, and if necessary adjust the software program to detect preferential amplification levels.

D.5.2.11.3 Document the sequencing chemistry, reagents, chips, and flow cells used for each sequencing run, including run parameters and read configuration (e.g., bidirectional, paired-end/single-end or mate-pair). The anticipated read length, depending on the particular chip or the selected size of DNA fragments during size selection of the library, ~~should~~ must be ~~disclosed and~~ documented.

Re: D.5.2.11.3

- Laboratories wishing to run HLA and non-HLA genotyping ~~should~~ must validate and document depths of coverage for each of the assays run simultaneously.
- Laboratories ~~should~~ 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 performance of the sequencing instrument over time.

Re: D.5.2.11.5 - Laboratories ~~should~~ must establish procedures to identify the limitations of the software analysis program. The genotyping software ~~should~~ 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 ~~should~~ must determine the acceptable values for each quality metric in order to assure an accurate result.

Re: D.5.2.11.6 - Laboratories ~~should~~ must establish policies for storing unaligned, processed sequencing files (*e.g.*, FASTQ) based on the requirements.

Re: D.5.2.11.7 - Validation of software for NGS analysis can be performed using existing sequencing datasets. For HLA genotyping, the validation dataset ~~should~~ must include alleles representative of the population and cumulatively their frequencies ~~should~~ must cover 85-95% of the population.

D.5.3.5.2 All UNOS histocompatibility laboratories prospectively typing deceased donors for non-renal transplantation must use a molecular method to assign HLA-A, -B, -Bw4/Bw6, -C, - DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, and -DPB1 at the level of resolution needed to meet the most recent OPTN/UNOS tables of antigen and unacceptable antigen equivalencies**.**

Re: D.6.2.2.3 – If required by the state where the laboratory is located, the director's name on the report ~~should~~ must be the director listed on the CLIA certificate.

D.6.2.2.12 A list of ~~all ambiguous alleles when 2 field (or more) typing is reported~~ 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.6.2.2.12.1 Results reported using G or P group codes do not need to list unresolved alleles that are within the G or P group listed.

D.6.2.2.12.2 Results reported do not need to list non-CWD alleles if stated in the report, transplant agreement, or client written request.

Renumbered ~~D.6.2.2.14~~ to D.6.2.2.12.3 ~~If NMDP allele codes are reported:~~ Typing results reported using NMDP codes must define all unresolved alleles represented by the allele code.

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

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

Renumbered ~~Re:D.6.2.2.14.2~~ to **Re: D.6.2.2.12 –** 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.

Renumbered ~~D.6.2.2.15~~ to **D.6.2.2.13** For U.S. laboratories using a test method and reagents that are not FDA-approved, a statement to the effect that "This test was developed in and its performance characteristics determined by [laboratory name]. It has not been cleared or approved by the U.S. FDA."

Renumbered ~~Re:D.6.2.2.15~~ to **Re: D.6.2.2.13** - Non-U.S. laboratories do not need to have this on reports

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 ~~should~~ must consider also citing a deficiency for lack of involvement of the laboratory director (Standard **E.2.2.1**).

Re: E.2.2.17 to E.2.2.20 - If technologists in ASHI accredited laboratories are not involved in histocompatibility testing per se, then the director ~~should~~ must ensure that such staff is participating in continuing education relevant to their work areas.