ASHI QAS Committee
Responses to Public Comment regarding revisions to the 2024 ASHI Standards

Standard	Comment	QAS Response
Real-time PCR method	The real-time PCR method lacks standards for monitoring the method and instruments, despite sharing principle with SSP. Notably qPCR employs different instruments compared to SSP.	The QAS committee agrees and will work on drafting standards in the next cycle.
D.4.1.8.6.7	For calibration materials, my understanding is that initially, these were used for SSO assays due to some laboratories not including positive and negative samples in batches, assuming NC and PC beads from kits suffice as controls. CMS's explanation suggests controls included in kit determine the cutoff and are considered as calibrator. Now, this extends to controls not included in kits, complicating matters further. Obtaining adequate negative controls remains a challenge for many laboratories. Additionally, most HLA assays are qualitative rather quantitative, and they are different from chemistry assays. I would like to suggest NC/PC included in kits are considered calibrator, external controls are not considered as calibrators.	Calibration materials that are used to calculate the cutoff value of a test or a patient test result cannot also be used as external controls (positive control or negative control), regardless of their source. Positive and negative controls are required for all assays, and if the same sample type (for example, normal human serum) is used as a calibrator, then a different source of that same sample type (for example, from a different manufacturer, or a different lot from the same manufacturer) must be used as the control. This is the CLIA standard, so no changes will be made.
D.5.3.1.1.1.2 and D.5.3.1.1.1.1	I recommend removing D.5.3.1.1.1.2, and editing D.5.3.1.1.1.1 as follows: Individual protocols for each type of transplant differentiated by type of donor, organ or transplanted tissue, and recipient risk factors, as applicable. D.5.3.1.1.1.2 is awkwardly written, and some programs may reasonably choose to use the same protocol for all of their patients. The CLIA regulation 493.1278 (f)(1) states "The laboratory's policies must include, as applicable-" so there is no federal requirement to specifically have different protocols for high risk patients.	The CLIA regulations were updated to include the following: (1) Testing protocols that address: (i) Transplant type (organ, tissue, cell); (ii) Donor (living, deceased, or paired): and (iii) Recipient (high risk vs. unsensitized); In particular, there is an emphasis on high risk vs. unsensitized patients in the context of crossmatching. Having a separate standard will help the laboratories to make corresponding changes to their current transplant agreements. Therefore, there will be no changes to this standard at this time.
D.5.3.1.1.4	I appreciate the committee's work on the proposed revised standards to meet the new CMS regulations on virtual and physical crossmatches. However, regarding standard D.5.3.1.1.4, the term "reasonable effort" makes this standard ambiguous. Providing additional guidance would be beneficial. Additionally, expecting laboratories to obtain monthly samples from all patients may be unrealistic and could pose extra challenges, especially for labs serving large transplant centers that already face storage space issues.	The CLIA regulations were updated to include: (c) Antibody screening and identification. 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. The QAS committee does not want to include more specifics for this standard to avoid imposing a more stringent requirement than required by the CLIA regulations. The way this standard is phrased now allows laboratories to determine what may consitute "reasonable" effort for their center.
D.5.3.1.1.4	evidence in the literature to suggest that storage of monthly pre-transplant sera improves transplant outcomes or patient safety. Collection and storage of monthly sera is costly, requires significant effort on the part of laboratory personnel, and is a burden on patients. Stored serum samples are not useful at the time of a virtual crossmatch as they are untested. Laboratories should be able to determine the frequency of longitudinal testing or sample storage with transplant programs according to local risk tolerances for high and low risk patients. Of note, quarterly testing has been documented to be beneficial in identification of new sensitization in pre-transplant patients after transfusion (Balasubramaniam et al, Transplantation, 93(4):418, 2012). In a cohort of patients with chronic kidney disease, quarterly testing identified significant changes in HLA antibodies in 30% of patients with known sensitizing events, and in 5% of patients in the absence of a known sensitizing event (Valenzuela, et al, 2024, manuscript in preparation). Operationally, the National Kidney Registry for living paired donation has found that quarterly antibody testing to identify new sensitization resulted in a significant reduction in crossmatch failures and consequently fewer broken chains of donation. It would be helpful for ASHI to provide guidance on the meaning of the term Reasonable Effort. As written the word 'must' modifies the phrase 'make reasonable effort' - a legal term used when a party is unable to control or guarantee an outcome and is obligated to consider and do what is reasonable. In this context, laboratories in collaboration with transplant programs may be obligated to discuss published and center data during annual review of clinical pathways to agree on appropriate frequency of sample storage and testing. Sound judgement may dictate that increased frequency of serum storage makes reasonable sense for patients in high risk categories- such as those undergoing immunosuppression withdrawal, desensitization or those with known sensitizing events. Collaborative drafting and signing of written clinical pathways is sufficient to establish that reasonable effort was made in the design and execution of program management for patient safety, especially in the context of an entire medical system with infrastructure supporting continuous learning and quality improvement. This is especially so given that medical laboratory professionals are required by licensure to be effective stewards in optimizing value-based testing- ensuring that samples are tested according to timing agreed upon in the clinical pathway and that financial responsibility is maintained.	These are all excellent points. Since the ASHI's standards have to be at least as stringent as CLIA regulations, the standard must be incoprated into the revised version. However, the standard gives laboratories sufficient flexibility to define criteria for "reasonable effort".

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D.5.3.1.1.6	This standard is unnecessary as it is covered in the transplant program agreements (D.2.5.1 #8 and D.5.3.2.1.5). If kept, I recommend editing it to: Have a policy for screening transplant patient serum samples for antibodies to HLA antigens on an ongoing basis. Then, in the guidance mention that this may be covered in the transplant program agreement. The CLIA regulation which this appears to try to match, 493.1278(d)(5), does not require periodic screening. In addition, 493.1278(d)(5) was removed with the 12/28/2023 rule. The new CLIA requirement, "Assessing recipient antibody presence or absence on an ongoing basis," is intentionally more flexible and does not require screening at set periodic intervals.	This standard is needed to cover the requirements for all types of transplants, not just solid organ transplantation.
D.5.3.1.1.7	This standard is obsolete as transplant programs move toward virtual crossmatching and reduce utilization of physical crossmatching. Only rare events require virtual or physical crossmatch confirmation with a new serum in the immediate transplant period- in the context of an unexplained early rejection or primary non-function. At that time, a new serum could be drawn from the patient for solid phase antibody testing which is more sensitive and specific than a lymphocytotoxicity or flow crossmatch.	Starting from January 2024, CMS does not make a distinction between physical and virtual crossmatch. ANY crossmatch type can be used as established by transplant center policy. Therefore, no further guidance is required. Furthermore, the standard only requires collection of the specimen for crossmatch. Whether it is tested would be determined by the transplant center policy.
D.5.3.1.1.7	I recommend adding guidance to this standard recognizing that virtual crossmatch is acceptable.	
D.5.3.1.1.7	Requiring the recipient specimens to be collected within 24 hours before transplant for retrospective crossmatches is reasonable. In regards to prospective crossmatches, while it is certainly necessary to have a recent sample to ensure the results of the crossmatch remain valid and applicable at the time of transplant, the requirement for the recipient specimen to be collected within 24 hours before transplant is impractical and unjustifiable. Attempts to comply with this proposed updated standard will certainly delay transplant and adversely affect the transplant process and patient outcomes. Currently, we often perform prospective crossmatches for both living and deceased kidney offers. In cases of a deceased donor, we would perform the prospective crossmatch with a patient specimen that was collected within 30 days as long as there was no additional sensitization event since that specimen was drawn. Logistically, this is often performed and even resulted before the patient arrives at our hospital (when a new sample could be obtained). Obtaining a new patient sample will delay resulting of the prospective crossmatch, increase the duration that a donor needs to be maintained on life sport or cold ischemic time, and delay transplant. This will unnecessarily increase healthcare costs and potentially negatively impact graft function. Furthermore, as a pediatric transplant program, some of our patients can receive multiple offers within a week. Requiring a new recipient sample for each offer would create undue stress for the patients, their families, and the clinical transplant team. Having frequent repeat blood draws for small/critically ill pediatric patients is also not advisable. Even for living donor transplants, this requirement would unnecessarily delay transplant and prolong hospitalization for both donor and recipient. Requiring to "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" is not a reasonable alternative as D.5.3.1.1.7 does not serve the best interests of our transplant recipients when it comes to the requirement for prospective crossmatch.	
D.5.3.1.1.7 and D.5.3.1.1.7.1	This standard is practical for retrospective crossmatches but will be very difficult for some living donor (NKR) and prospective deceased donor kidney transplants. Our center still performs all deceased donor kidney crossmatches prospectively following VXM. We use a current serum sample within the past 30 days and sensitization history is documented. These patients are almost always outpatient up to their admission for surgery. In order to comply with D.5.3.1.1.7 the patient would need to come in to be collected for each potential deceased donor the program is considering, which may or may not result in transplantation. For NKR and paired exchange cases it would require an extra crossmatch to be completed immediately prior to transplant. This is possible but NKR would need to change their practices and it is another collection for the donor as well.	The standard only requires collection of the specimen for crossmatch. Whether it is tested would be determined by the transplant center policy.
D.5.3.10.4	There is a typo: "performed" was mistakenly typed as "perfumed."	Corrected.
D.5.3.9.4.1	The intended meaning of this edit is not entirely clear to me. What is a historic event in this context? Was this intended to refer to historic samples? If so, would these now be required for all crossmatches? That seems excessive. This concept is already well-expressed by D.5.3.2.1.7. Perhaps you could move that language to D.5.3.9.4.1 or remove 5.3.9.4.1 as it is redundant?	The QAS committee agrees and has revised this standard.

D.5.3.9.6 and D.5.3.9.6.1	I believe the proposed change in language from the current version (D.5.3.8.3 Patients and donors must have sufficient histocompatibility typing by molecular methods to permit accurate virtual crossmatch assessment) places directors, immunogenetics labs, transplant programs, as well as ASHI at risk from a medical-legal standpoint. The wording of “accurate virtual crossmatch assessment” is already semi-contradictory as accuracy is inherently not applicable to any assessments (test results and determinations can be accurate/inaccurate, assessments are inherently evaluations/estimations that cannot guarantee accuracy). This sentiment was also noted by others who commented to CMS/CLIA that virtual crossmatch is an immunologic assessment, and not a test. Technically, to guarantee accuracy, virtual crossmatches would require 2 field typing and every A/B/C/DR/DQ/DP donor allele to be represented in the solid-phase antibody detection testing. In all other cases, whether we have to infer allele level typing through population linkage data or choose surrogate beads through amino acid/eplet comparison, accuracy cannot be guaranteed. Transplant program labs typically have no control of the donor typing provided by the OPO (unless the lab also serves as the OPO typing lab). Currently, most OPO typing is at intermediate resolution or lower. In light of this, the combination of wording that combines “sufficient resolution” and “accurate virtual crossmatch assessment” in our standards puts everyone involved at risk from a medical-legal standpoint, regardless of how each lab ends up defining its criteria to satisfy D.5.3.9.6.1. If a lab defines their criteria to allows low or intermediate donor typing to be used for inference and a bad outcome results following a virtual crossmatch compatible assessment, and it is later determined that inferred 2-field donor-typing from the intermediate OPO typing does not match the actual allele level/2-field typing, then I can certainly foresee a legal challenge that argues that any inferred allele level donor typing cannot fulfill “sufficient resolution” needed for “accurate virtual crossmatch assessment”. Conversely, if the lab defines that at least 2-field molecular donor typing is required for accurate virtual crossmatching to avoid legal jeopardy, then no virtual crossmatch could be performed until OPOs can provide at least 2-field typing. To avoid this issue, I recommend D.5.3.9.6.1 to be rephrased as follows: Sufficient donor and recipient HLA typing by molecular methods at appropriate loci to permit consistent/reproducible virtual crossmatch assessment.	The word "accurate" was removed from the standard.
D.5.3.9.6 and D.5.3.9.6.1	ASHI should consider rewording “sufficient resolution” back to the original wording of “sufficient HLA Typing by molecular methods. The HLA lab is unable to control the level of resolution provided by the OPOs and therefore not up to them to define the level of resolution for an accurate VXM. The most accurate VXM requires 2 field typing at all loci for the donor and recipient. There are no OPOs offering this resolution at this time.	
Re: D.5.3.10.1.2	Digital PCR is referred as ddPCR while dPCR is mentioned elsewhere.	Corrected.