

testing (now at § 493.1278(a)) and specifying the requirements for HLA typing (now at § 493.1278(b)), disease associated studies (now at § 493.1278(c)), antibody screening (now at § 493.1278(d)), crossmatching (now at § 493.1278(e)) and transplantation (now at § 493.1278(f)). In addition, we believe this reorganization, along with other revisions, will greatly enhance the readability of this section and clarify the requirements that must be met for each type and level of histocompatibility testing performed by the laboratory.

*Comment:* One commenter pointed out the requirement at § 493.1265(a)(4) that addresses reagent typing sera inventories prepared in-house should also require that the specificity of the reagent be indicated. The commenter also requested clarification of the term "typing tray" used at § 493.1265(a)(9)(i) since the term can refer to any 96, 72, or 60 well microtiter tray used in the HLA laboratory. The commenter stated that without clarification, it is unclear whether the control requirements specified at this requirement refer only to trays used for HLA typing or if they include trays run in an attempt to identify the presence of circulating HLA antibodies.

*Response:* We agree that reagent specificity must be indicated on the laboratory's in-house prepared reagent typing sera inventory and are amending the requirement now at § 493.1278(a)(3) accordingly.

The commenter is correct to question the scope of the requirement formerly at § 493.1265(a)(9)(i) that addressed control requirements for typing trays. In addition, the term "typing tray" is somewhat restrictive in that testing performed with newer and emerging technologies may not necessarily use microtiter trays. Therefore, we are revising the requirement for clarification, and, with the reorganization of this section, § 493.1278(b)(6) now describes the controls a laboratory must use for each HLA typing, and § 493.1278(d)(6) addresses the controls a laboratory must use when performing antibody screening.

*Comment:* One commenter requested that the CLIA regulations mandate HLA antibody identification when panel screening studies indicate the presence of a lymphocyte-reactive antibody. In addition, the laboratory should determine if this is an autoantibody or alloantibody. The commenter also requested the CLIA rule require that the specific technique used in HLA antibody screening be at least as sensitive as the complement-dependent

lymphocytotoxicity technique used in the final donor crossmatch.

*Response:* Histocompatibility testing is a rapidly evolving, highly complex specialty. Its role in predicting long-term allograft survival is the subject of numerous research studies. Not all antibody reactions have a defined specificity, and the clinical relevancy of each antibody has not been established. Mandatory antibody identification may be impractical, if not impossible, and uninformative in these cases. However, we agree that antibody identification must be performed when appropriate to support clinical transplant protocols and § 493.1445(e)(3)(i) requires the laboratory director to select test methods that are capable of providing the quality of results required for patient care. It is the laboratory director's responsibility to institute more stringent testing protocols as necessary for quality patient care. Therefore, we are adding a requirement at § 493.1278(d)(7) for laboratories that perform antibody identification to have available and follow written criteria and procedures for antibody identification to the level appropriate to support clinical transplant protocol.

We agree with the commenter that the laboratory must use a technique that detects HLA-specific antibody with a specificity equivalent or superior to that of the basic complement-dependent microlymphocytotoxicity assay. In addition, to detect antibodies to HLA Class II antigens, the laboratory must use a method that distinguishes antibodies to HLA Class II antigens from antibodies to Class I antigens. We are adding these two new requirements at §§ 493.1278(d)(1) and 493.1278(d)(2).

To ensure quality laboratory practices and for consistency with the two new requirements, we are specifying that techniques used for crossmatching must be documented to have increased sensitivity in comparison with the basic complement-dependent microlymphocytotoxicity assay (now at § 493.1278(e)(1)). In addition, when performing HLA typing, the laboratory must use a technique that is established to optimally define, as applicable, HLA Class I and II specificities (now at § 493.1278(b)(1)).

*Comment:* A number of commenters were opposed to the elimination of mandatory monthly screening for HLA antibodies, since most, if not all, laboratories lack access to accurate information regarding each potential transplant recipient's exposure to sensitizing events. This is compounded by the probability that not all potentially sensitizing events have been identified. A few commenters

acknowledged that the cost of monthly screening can be prohibitive and suggested there may be some instances when monthly screening may not be necessary. However, most commenters agreed that studies need to be done to determine the optimum frequency of antibody screening.

*Response:* We agree with the commenters and recognize the importance of developing an accurate immunological history of the potential transplant recipient and the difficulty of identifying and obtaining information on all potential sensitizing events. We also appreciate the efforts to control healthcare costs by eliminating unnecessary and or redundant testing. To provide flexibility and allow responsiveness to emerging research data and information, we are revising the requirements formerly at §§ 493.1265(a)(2)(ii) and (a)(8)(i) (now at §§ 493.1278(d)(4) and (d)(5)) to require the laboratory to make a reasonable attempt to have available monthly serum specimens for all potential transplant recipients for periodic antibody screening and crossmatch. In this regard, the laboratory must have available and follow a policy, consistent with clinical transplant protocols for the frequency of screening potential transplant recipient sera for preformed HLA-specific antibodies.

*Comment:* Three commenters noted that DNA typing involves the genes rather than the expressed antigens; therefore, § 493.1265(a)(10) would be more accurate if changed to read, "Compatibility testing for HLA class II polymorphisms should utilize techniques, for example, mixed lymphocyte culture, homozygous typing cells, or DNA analysis."

*Response:* We agree with the commenters that the wording of the requirement formerly at § 493.1265(a)(10) is somewhat inaccurate and also believe that the requirement may be too restrictive for future methodologies, technologies, and transplantation protocols. Therefore, we are deleting this requirement for the laboratory to use specific techniques, for example, mixed lymphocyte cultures, to determine HLA Class II incompatibilities.

*Comment:* One commenter stated that the requirement at § 493.1265(a)(13) to have histocompatibility testing personnel evaluate unknowns on a monthly basis is excessive and should be reduced to once every 6 months.

*Response:* Histocompatibility testing is a highly complex specialty with great potential for harm to the patient if the testing is incorrectly performed. CLIA regulations specify formerly at