The laboratory has the capability to detect HLA antibodies with sufficient sensitivity and to distinguish HLA antibodies from IgM autoantibodies or non-HLA antibodies.

NOTE: Methods to detect HLA antibodies must be more sensitive than the basic/NIH technique. There must be written procedures to differentiate HLA antibodies from autoantibodies.

REFERENCES
1) Foundation for the Accreditation of Cellular Therapy (FACT) and Joint Accreditation Committee ISCT and EBMT (JACIE). FACT-JACIE International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration. 8th Edition, December 14, 2021.
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2023(Dec 28): [42CFR1278(d)(1)].
3) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2023(Dec 28): [42CFR1278(e)(1)].

HSC.32861 Antigenic Diversity and Targets — Phase II

There is sufficient antigenic diversity (individual antigens and/or crossreactive groups) for HLA Class I and II, and sufficient numbers of antigenic targets for optimal HLA antibody detection and specificity determination.

NOTE: There must be sufficient diversity for Class I and II HLA antigens and cross reactive groups, as well as sufficient numbers of well-characterized panel cells or HLA-purified protein targets for antibody detection and specificity determinations, and strength/avidity of the antibody when applicable.

Evidence of Compliance:
- ✓ Listing of antigenic targets for each panel cell

HSC.33048 Antibody Detection and Specificity QC — Phase II

For HLA antibody detection and specificity determinations, positive and negative controls are used, and sera tested undiluted and diluted when appropriate.

Evidence of Compliance:
- ✓ Records of QC for antibody detection and identification

HSC.33235 Target Source for Class I/II Antibody Determination — Phase II

There are records that the appropriate target sources are used for separate HLA Class I and II antibody determination including appropriate methods to distinguish antibody mixtures.

NOTE: There must be records that the appropriate target sources are used for HLA Class I and II antibody determination. The targets for HLA Class I antibody determination should be blood, spleen, lymph nodes, and cell lines. In addition, well-characterized purified HLA protein targets may also be used. Class II antibodies are best detected utilizing B-lymphocytes, B-lymphoblastoid cell lines, CLL cells or specific Class II purified HLA protein. Mixtures must be defined by methods shown to distinguish Class I from Class II reactivity.

Evidence of Compliance:
- ✓ Records of target sources used for HLA Class I/II antibody determination and to differentiate antibody mixtures

HSC.33422 Recipient Sera Screening — Phase II

All recipient sera are screened for HLA antibodies including, at minimum, an initial sample at the time of HLA typing, after sensitizing events and upon request.

Evidence of Compliance:

- ✓ Defined criteria for screening recipient sera **AND**
- ✓ Records showing initial screening of recipient sera and all subsequent screening results

REFERENCES

1) Organ Procurement and Transplantation Network (OPTN) Bylaws. Appendix C. Membership Requirements for Histocompatibility Laboratories. US Department of Health and Human Services. December 5, 2022.
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2023(Dec 28): [42CFR1278].

HSC.33475 Antibody Identification/Crossmatching **Phase I**

The laboratory performs antibody identification and crossmatching as defined by the transplantation programs supported by the laboratory (includes solid organ and hematopoietic progenitor cell transplantation).

MOLECULAR TESTING

If next generation sequencing (NGS) methods *are used for histocompatibility testing, the applicable requirements in the Molecular Pathology Checklist (eg, assay validation, quality control, specimen handling, NGS section) must be used in conjunction with the Histocompatibility Checklist for inspection.*

Inspector Instructions:

	• Sampling of molecular HLA typing policies and procedures • Specimen storage/handling procedure • Sampling of QC records • Sampling of HLA typing and hematopoietic progenitor cell engraftment reports for completeness • Sampling of the following records: molecular weight marker, in-house probe labeling validation, nucleic acid measurement, electrophoretic gel interpretation, and chimerism measurement
	• Raw data (eg, gel images, sequencer histograms, flow microbead fluorescence intensity histograms) • Current databases of known sequences for all alleles recognized by the World Health Organization (WHO) Nomenclature Committee for Factors of the HLA System (available) • Pre/post amplification areas (adequate physical separation)
	• What is your laboratory's process for assessing the quality (intactness) of high molecular weight DNA or RNA? • How does your laboratory avoid cross-contamination when performing amplification procedures? • How does the laboratory ensure the level of HLA typing resolution is adequate for each transplant service or registry supported (eg, allele-level resolution for hematopoietic progenitor cell transplants)?

GENERAL REQUIREMENTS FOR MOLECULAR TESTING

The requirements in this section are intended to apply to all molecular-based histocompatibility testing.