REFERENCES
1) Shapiro HA. Practical flow cytometry. New York, NY: Alan R. Liss, 1985

# FLOW CYTOMETRY CROSSMATCH

## Inspector Instructions:

| | |
|---|---|
|   | • Sampling of flow cytometry crossmatch policies and procedures<br>• Sampling of QC policies and procedures<br>• Sampling of QC records<br>• Sampling of positive cutoff validation records |
|   | • How has your laboratory established the cutoff for positive crossmatch results?<br>• Are cutoffs for crossmatches reviewed with the clinical transplant service?<br>• Have the cutoffs been correlated with signal strength or other measure of antibody concentration in the HLA antibody screen and detection methods used?<br>• How does your laboratory ensure separation of Class I & Class II antibodies? |

**HSC.30056 Crossmatch** **Phase II**

**The flow cytometry crossmatch identifies antibodies to T and B-cells.**

*NOTE: Two or multiple color techniques must be used to identify antibodies to T cells. Antibodies to B cells and other target cells must also be identified properly.*

**HSC.30243 IgG Antibody Identification** **Phase II**

**IgG antibodies are identified by appropriately labeled heavy chain-specific F(ab')2 reagents.**

**HSC.30430 Sensitivity** **Phase II**

**There is a record of the number of cells and volume of serum used for optimal sensitivity.**

**HSC.30617 Negative Control - Normal Human Serum** **Phase II**

**Normal human serum with demonstrated lack of reactivity against any potential target cell is used as a negative control.**

**Evidence of Compliance:**
✓ Records of control results

**HSC.30804 Positive Control - Diluted Human Serum** **Phase II**

**The positive control is an appropriately diluted human serum containing suitable HLA antibodies of appropriate immunoglobulin class known to react with lymphocytes from all donors.**

**Evidence of Compliance:**
✓ Records of control results

**HSC.30991 Antibody Reagents** **Phase II**

**The antibody reagents (anti IgG, IgM, IgA, etc.) are used at a selected dilution for optimal sensitivity and class specificity.**

**HSC.31178 Positive Crossmatch Results Cut-off** **Phase II**

**The cut-off for positive crossmatch results is determined by testing an appropriate number of sera from non-alloimmunized individuals and established for all pertinent target cells (T-cells, B-cells, etc.).**

**Evidence of Compliance:**

✓ Records for the validation of the positive cut-off

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.

**HSC.31552 HLA Class II Antibody Procedure** **Phase II**

**The procedure for HLA Class II antibodies readily separates Class I from Class II specificity.**

## HLA ANTIBODY SCREENING

## Inspector Instructions:

| | |
|---|---|
| READ | • Sampling of HLA antibody screening policies and procedures, including protocol for screening for each organ transplanted or hematopoietic progenitor cell recipient and the frequency of such screening<br>• Agreement for reflex testing using more sensitive screening method, if applicable<br>• Sampling of antibody identification QC records<br>• Sampling of initial and subsequent recipient sera screening records |
| ASK | • What is your laboratory's course of action for antibody identification/crossmatching for high risk patients?<br>• How does the laboratory determine cutoffs for identification of HLA antibody based on the clinical programs supported?<br>• How does the laboratory determine the assignment of unacceptable antigens for organ transplantation? |

**HSC.32487 Immunizing Event** **Phase II**

**There is a system to record any potential immunizing event that could cause sensitization in a patient.**

*NOTE: There must be a policy that encourages timely blood sample collection at 14 days after the potential immunizing event in a patient. This new sample should be available for use in antibody screening and crossmatch studies.*

REFERENCES

1) 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):[42CFR493.1278(c)].
2) Organ Procurement and Transplantation Network (OPTN) Bylaws. Appendix C. Membership Requirements for Histocompatibility Laboratories. US Department of Health and Human Services. December 5, 2022.

**HSC.32674 HLA Antibody Detection** **Phase II**