The laboratory defines when the percentage of viable cells in each test specimen is measured.

NOTE: Selective loss of cell subpopulations and/or the presence of dead cells may lead to spurious results. This does not mean that all specimens with low viability must be rejected. Finding an abnormal population in a specimen with poor viability may be valuable but the failure to find an abnormality should be interpreted with caution. If specimen viability is below the established laboratory minimum, test results may not be reliable and this should be noted in the test report. Routine viability testing may not be necessary. However, viability testing of specimens with a high risk of loss of viability, such as disaggregated lymph node specimens, is required.

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition*. CLSI document H43-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2007.

HSC.30021 Immunoglobulin Staining Phase II

The laboratory ensures that immunoglobulin binding is specific.

NOTE: Many cell types will bind serum immunoglobulin nonspecifically via Fc receptors, and steps may have to be taken to ensure that immunoglobulin staining detected by flow cytometry is specific rather than non-specific.

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition*. CLSI document H43-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2007.

HSC.30029 Cell Population Distinction Phase II

The laboratory distinguishes fluorescence-negative and fluorescence-positive cell populations.

NOTE: This does not imply that a separate negative control sample must be run. It is possible to coordinate panels of monoclonal antibodies to compare the binding of monoclonal antibodies of the same subclass that typically have mutually exclusive patterns of reactivity of subsets of hematopoietic cells. In this way, test antibodies may also double as control reagents.

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition*. CLSI document H43-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2007.

HSC.30037 Staining Methodology Phase II

The staining and analytical processes described in the procedure manual are based upon established methodology (reference cited).

NOTE: Many different variables need to be controlled to ensure proper stoichiometry of dye binding to DNA. Therefore, it is essential that procedures adopted by a laboratory are based on published work.

HSC.30045 Specimen Treatment Phase II

Specimen treatment with nucleic acid dye includes treatment with RNAse if the dye is not specific for DNA.

NOTE: Certain dyes used to stain fixed cells, (eg, ethidium and propidium iodide) bind to RNA. Prior treatment with RNAse eliminates artifactual broadening of the DNA content distributions that would result from fluorescence of complexes of the dye with RNA.

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 • Sampling of QC policies and procedures • Sampling of QC records • Sampling of positive cutoff validation records
	• How has your laboratory established the cutoff for positive crossmatch results? • Are cutoffs for crossmatches reviewed with the clinical transplant service? • Have the cutoffs been correlated with signal strength or other measure of antibody concentration in the HLA antibody screen and detection methods used? • 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**