Evidence of Compliance:

✓ Records of calibration results using appropriate fluorochrome standards

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline—Second Edition*. CLSI document H42-A2 (ISBN 1-56238-640-9). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007

HSC.29981 Color Compensation Settings — Phase II

The laboratory defines appropriate color compensation settings.

NOTE: For two or more color analysis there must be a procedure to ensure that cells co-labeled with more than one fluorescent reagent can be accurately distinguished from cells labeled only with one reagent. Cells stained with mutually exclusive antibodies bearing the relevant fluorochromes are the proper reference material for establishing appropriate compensation settings.

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.29989 Laser Performance — Phase I

The laboratory ensures acceptable and constant laser instrument performance.

NOTE: For some instruments, current is a better gauge of laser performance than is power output, which may be relatively constant.

HSC.29997 Gating Techniques — Phase II

Appropriate gating techniques are used to select the cell population for analysis.

NOTE: This may involve a combination of light scatter and/or fluorescence measurements. This is particularly important if the cell samples have a low lymphocyte count and/or a relatively high monocyte-granulocyte count. Lymphocyte gates may be validated using linear forward angle light scatter and 90-degree side scatter, and/or by using monoclonal antibodies to markers, such as CD45 and CD14.

REFERENCES

1) Clinical and Laboratory Standards Institute. *Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline.* 2nd ed. CLSI Document H42-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2007.

HSC.30005 Markers/Cursors — Phase II

The laboratory has defined criteria for setting markers (cursors) to distinguish fluorescence negative and fluorescence positive cell populations.

NOTE: Each laboratory must have a set of objective criteria to define the appropriate placement of markers (cursors) to delineate the population of interest. Isotypic controls may not be necessary in all cases, and cursor settings for the isotype control may not be appropriate for all markers. Cursor settings must be determined based on the fluorescence patterns from the negative and positive populations for CD3, CD4, and CD8.

REFERENCES

1) National Institute of Allergy and Infectious Diseases/Division of AIDS flow cytometry guidelines, sec 3.09B and 5.03A
2) Sreenan JJ, *et al*. The use of isotypic control antibodies in the analysis of CD3+ and CD3+, CD4+ lymphocyte subsets by flow cytometry. Are they really necessary? *Arch Pathol Lab Med.* 1997;121:118-121

HSC.30013 Cellular Viability — Phase II

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.