

**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**