Evidence of Compliance:
- ✓ Records of anti-D control results

FLOW CYTOMETRY

INSTRUMENTATION AND PHENOTYPING

Inspector Instructions:

READ	• Sampling of flow cytometry policies and procedures • Sampling of QC policies and procedures (includes acceptable control type/frequency for each flow cytometric application) • Sampling of QC records • Sampling of optical alignment/laser output checks
ASK	• How does your laboratory monitor instrument reproducibility? • How does your laboratory ensure each fluorochrome is appropriately calibrated? • How does your laboratory determine appropriate color compensation settings? • How does your laboratory ensure nucleic acid dye specificity?

HSC.29957 QC - Quantitative Assays **Phase II**

The laboratory analyzes at least two levels of positive cellular controls for quantitative assays (eg, CD4+, CD34+ cell concentrations) each day of patient testing or after an instrument restart to verify the performance of reagents, preparation methods, staining procedures, and the instrument.

NOTE: One of the levels of these controls should be at (or near) clinical decision levels (eg, low CD34).Control testing is not necessary on days when testing is not performed.

Evidence of Compliance:
- ✓ Records of QC results

HSC.29965 Optical Alignment **Phase II**

The laboratory monitors optical alignment (where applicable) and instrument reproducibility on each day of use or after each time the flow cytometer is started.

NOTE: Instrument performance must be monitored under the same conditions used to run test samples.

Evidence of Compliance:
- ✓ Records for monitoring optical alignment (where applicable) and instrument reproducibility

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.29973 Fluorochrome Standards **Phase II**

Appropriate standards for each fluorochrome (eg, fluorescent beads) are run each day that the instrument is used as part of the calibration.

NOTE: These steps are necessary to optimize the flow system and the optics of the instrument.

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