

FLO.30430 Specimen Integrity**Phase II****The laboratory has a defined process to evaluate specimen integrity.**

NOTE: The yield of lymphocytes from blood samples is affected by a number of factors. If specimens are not processed immediately after collection, the laboratory should verify that its anticoagulant, holding temperature and preparation method maintain specimen integrity. Selective loss of cell subpopulations and/or the presence of dead cells may lead to spurious results. Routine viability testing is not necessary on specimens of whole blood that are analyzed within 24 hours of drawing. Analyses on older samples are possible if the laboratory has verified the absence of statistical differences between the fresh and aged specimen phenotype fractions being evaluated.

Evidence of Compliance:

- ✓ Records of specimen evaluation (eg, viability results) as applicable

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

FLO.30450 Specimen Storage**Phase II****Specimens are stored appropriately after initial processing.**

NOTE: As one example, paraformaldehyde (0.5%) fixation of stained cells preserves cellular integrity and fluorescence for up to five days. Caution must be exercised in utilizing this procedure, as fluorescence may be diminished with some reagents and cytometers.

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.

FLO.30460 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 verified 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 (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.
- 2) Clinical and Laboratory Standards Institute (CLSI). *Validation of Assays Performed by Flow Cytometry - Approved Guideline-First Edition*. CLSI Document H62. Clinical and Laboratory Standards Institute, Wayne, PA, 2021.
- 3) World Health Organization. WHO Protocol for Performance Laboratory Evaluation of Lymphocyte Subsets Enumeration Technologies. 2017.

FLO.30470 Gate Purity**Phase II****Results of lymphocyte subset analysis are corrected for gate purity as appropriate.**

NOTE: When >5% non-lymphocyte events are included in a gate, results must be corrected for the proportion of contaminating cells. One method uses low side scatter and bright CD45 fluorescence for identification of lymphocytes, where an assumption is made that the only cells meeting these criteria are lymphocytes, and therefore the lymphocyte purity of the gate is close to 100%. Other methods may also be appropriate, and must be recorded.

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
- 2) Clinical and Laboratory Standards Institute (CLSI). *Validation of Assays Performed by Flow Cytometry - Approved Guideline-First Edition*. CLSI Document H62. Clinical and Laboratory Standards Institute, Wayne, PA, 2021.
- 3) World Health Organization. WHO Protocol for Performance Laboratory Evaluation of Lymphocyte Subsets Enumeration Technologies.2017.

FLO.30480 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) 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
- 2) Clinical and Laboratory Standards Institute (CLSI). *Validation of Assays Performed by Flow Cytometry - Approved Guideline-First Edition*. CLSI Document H62. Clinical and Laboratory Standards Institute, Wayne, PA, 2021.
- 3) World Health Organization. WHO Protocol for Performance Laboratory Evaluation of Lymphocyte Subsets Enumeration Technologies.2017.

CD34 STEM CELL ENUMERATION

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of CD34 analysis policies and procedures • Sampling of CD34 records (events counted)
	<ul style="list-style-type: none"> • How does your laboratory record CD34 cellular viability? • How does your laboratory gate to define the population of CD34+ cells? • What class of anti-CD34 monoclonal antibodies does your laboratory use, and how are they conjugated?

FLO.30564 CD34 Cellular Viability - Apheresis and Cord Blood Products**Phase II**

The laboratory measures the viability of CD34 positive cells in samples aliquoted during processing of hematopoietic progenitor cell products, apheresis products, and cord blood products.

NOTE: CD34 cell viability testing of cord blood products must be done on a sample aliquoted prior to the addition of cryoprotectant. For any hematopoietic progenitor cell product, the laboratory must define if and when additional testing for CD34 cell viability will be performed (eg, after storage). The viability dye 7-amino actinomycin-D (7-AAD) yields excellent results in this analysis. The viability assay must be performed using a flow cytometric method with the viability dye included in the same tube with the CD34 and CD45 monoclonal antibodies for the CD34+ viability determination. Estimates of total cellular viability (for example, trypan blue exclusion) may not be used as an alternative because the method can overestimate the viability of the CD34 stem cell population.