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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

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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:

READ	• Sampling of CD34 analysis policies and procedures • Sampling of CD34 records (events counted)
ASK	• 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.

REFERENCES
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5) Lee S., *et al.* Post thaw viable CD34+ cell count is a valuable predictor of haematopoietic stem cell engraftment in autologous peripheral blood stem cell transplantation. *Vox Sang* Feb: 2008: 94:46-152
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8) Kao GS, Kim HT, Daley H, et al. Validation of short-term handling and storage conditions for marrow and peripheral blood stem cell products. *Transfusion*. 2011; 51(1):137-47.

FLO.30578 Monoclonal Antibodies Reagent Class — Phase II

Appropriately conjugated Class II or Class III anti-CD34 monoclonal antibodies are used.

NOTE: Class I reagents are not recommended. Class II reagents conjugated to FITC are not recommended.

Evidence of Compliance:
- ✓ Reagent logs

FLO.30585 CD34 Events — Phase II

A statistically valid number of CD34+ events are collected to ensure clinically relevant precision and accuracy.

NOTE: The maximum coefficient of variation for CD34+ cell counts should be 10%. To achieve this precision, a minimum of 100 CD34+ events should be counted, as recommended by the ISHAGE guidelines and European Working Group on Clinical Cell Analysis. If the CD34+ cell count in a sample is 0.13%, for example, then 75,000 events must be collected to reach a count of 100 CD34+ events. This level of precision is not required for extremely low counts, provided they are below clinical decision points. Precision is most important at clinical decision thresholds and laboratories should verify their precision at such decision points.

Evidence of Compliance:
- ✓ Records of number of events counted

REFERENCES
1) Sutherland DR, Anderson L, Keeney M, et al. The ISHAGE Guidelines for CD34+ Cell Determination by Flow Cytometry. *J Hematotherapy.* 1996;3:213-226
2) Gratama JW, Orfao A, Barnett D, et al. Flow cytometric enumeration of CD34+ hematopoietic stem and progenitor cells. European Working Group on Clinical Cell Analysis. *Cytometry*. 1998;34:128-142

FLO.30592 Sequential Gating Techniques — Phase II

Sequential (Boolean) gating techniques are used to define the CD34+ stem cells.

NOTE: Negative reagent controls (isotypic/isoclonic) are of limited, if any, utility in the enumeration of rare events, such as CD34+ cells. Some isotype controls can stain more cells nonspecifically than are stained specifically by a CD34 conjugate. Studies of a large number of normal hematopoietic samples have shown that the sequential gating approach best delineates specific from nonspecific staining, and that traditional isotype controls provide no useful information regarding the levels of nonspecific staining in the flow cytometric analysis of rare events. For this reason, the use of isotypic/isoclonic controls is not recommended. In their place, sequential Boolean gating and cluster analysis should be used to define the population of interest (CD34+ cells).

REFERENCES