REFERENCES
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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
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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).

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*****NEW** 12/26/2024***

FLO.30595 Diluted Samples **Phase II**

Samples with high cellular concentrations are diluted to yield a result within the analytic measurement range (AMR) of the assay.

NOTE: High WBC count samples being processed for CD34 counts may require dilution prior to setting up the assay to obtain a result that falls within the AMR. A measured value that exceeds the AMR may be unreliable and should not be reported in routine practice. The measured result must be within the AMR before it is mathematically corrected by the dilution factor to obtain a reportable absolute CD34 count (CD34+cells/µl).

The composition of the diluent solution, the appropriate volumes of sample and diluent, and the maximum dilution that may be performed must be specified in the procedure manual. Specifying acceptable volumes is intended to ensure that the volumes pipetted are large enough to be accurate without introducing errors in the dilution ratio. The laboratory director is responsible for establishing the maximum allowable dilution of samples that will yield a credible laboratory result for clinical use.

Dilutions should be performed in a way that ensures that the diluted specimen reacts similarly to the original specimen in the assay system.

This checklist requirement does not apply if the CD34 cell count that exceeds the AMR is reported as "greater than" the upper limit of the AMR.

Evidence of Compliance:

✓ Patient results or worksheets

LEUKEMIA AND LYMPHOMA

Inspector Instructions:

READ	• Sampling of leukemia/lymphoma immunophenotyping policies and procedures • Sampling of patient reports and histograms (to include abnormal cell immunophenotype, interpretive comments, etc.)
ASK	• If flow leukemia/lymphoma immunophenotyping is done at an outside facility, how does your laboratory ensure that the testing is sufficiently comprehensive to facilitate accurate diagnosis, with appropriate gating and retention of records? • Under what circumstances does your laboratory measure the percentage of viable cells? • How does your laboratory distinguish neoplastic from non-neoplastic cells? • How does your laboratory distinguish between intrinsic and extrinsic immunoglobulin staining?

*****REVISED** 12/26/2024***

FLO.30610 Cellular Viability **Phase II**

The laboratory defines when the percentage of viable cells in each test specimen is measured.