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, PA 19087-1898 USA, 2007
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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.

NOTE: Selective loss of cell subpopulations and/or the presence of dead cells may lead to spurious results. This does not mean that all specimens with low viability must be rejected. Finding an abnormal population in a specimen with poor viability may be valuable but the failure to find an abnormality should be interpreted with caution. If specimen viability is below the established laboratory minimum, test results may not be reliable and this should be noted in the test report. Routine viability testing may not be necessary. However, viability testing of specimens with a high risk of loss of viability, such as disaggregated lymph node specimens, is required.

For those laboratories only performing the flow interpretation, the laboratory ensures that the percentage of viable cells in each test specimen is provided by the laboratory performing the flow technical component, as applicable.

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.30640 Appropriate Antibodies — Phase II

The panel of antibodies used is sufficiently comprehensive to address the clinical problem under consideration.

NOTE: Knowledge of the clinical situation and/or the morphologic appearance of the abnormal cells may help to guide antibody selection. Because antibodies vary in their degree of lineage specificity, and because many leukemias lack one or more antigens expected to be present on normal cells of a particular lineage, it is recommended that a certain degree of redundancy be built into a panel used for leukemia phenotyping.

Laboratories interpreting immunophenotyping data from an outside facility (ie, technical flow laboratory) must ensure that the antibody panels used for interpretation are appropriate. There must be a process by which individuals interpreting the results can provide feedback on the appropriateness of the antibody panels used. Records of such feedback and corrective action taken when problems are identified may be incorporated into the laboratory's quality management system.

Evidence of Compliance:

✓ Gated data plots, histograms, and patient reports

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.
2) Rimsza LM, *et al*. The presence of CD34+ cell clusters predicts impending relapse in children with acute lymphoblastic leukemia receiving maintenance chemotherapy. *Am J Clin Pathol.* 1998;110:313-320
3) Siebert JD, *et al*. Flow cytometry utility in subtyping components of composite and sequential lymphomas. *Am J Clin Pathol.* 1998;110:536
4) Kampalath B, *et al*. CD19 on T cells in follicular lymphocytic leukemia/small lymphocytic lymphoma, and T-cell-rich B-cell lymphoma: an enigma. *Am J Clin Pathol.* 1998;110:536
5) Krasinskas AM, *et al*. The usefulness of CD64, other monocyte-associated antigens, and CD45 gating in the subclassification of acute myeloid leukemias with monocytic differentiation. *Am J Clin Pathol.* 1998;110:797-805
6) Wood BL, *et al*. 2006 Bethesda International Consensus Recommendations on the Immunophenotypic Analysis of Hematolymphoid Neoplasia by Flow Cytometry: Optimal Reagents and Reporting for the Flow Cytometric Diagnosis of Hematopoietic Neoplasia. Cytometry Part B (Clinical Cytometry) 2007;72B:S12-S22
7) 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.

FLO.30670 Cell Concentrations — Phase II

Cell concentrations are adjusted for optimal antibody staining.

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.30720 Immunoglobulin Staining — Phase II