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

FLO.23737 QC - Flow Cytometry Reagents/Stains - Qualitative Assays Phase II

The laboratory evaluates negative and positive staining patterns of the residual normal cell population for qualitative assays (eg, leukemia/lymphoma analysis) each day of patient testing.

NOTE: An evaluation of the performance of each qualitative assay must be performed on each day of patient testing. If a component/tube of an assay or panel is not run on a particular day, the evaluation does not need to be performed on that component.

The assay procedure must include the control material to be used, criteria for acceptability of the assay, and remedial action to be taken when acceptability criteria are not met. A record of the evaluation and any remedial action must be made each day of patient testing.

Most labs will use normal populations present in patient samples as an appropriate control material; however, other control materials may be used at the discretion of the laboratory director. Other examples include commercial control materials, cryopreserved cells, or normal patient/volunteer samples. The evaluation can be run concurrently with active patient samples as long as the evaluation takes place prior to release of patient results. Multiple samples may be required to evaluate all components/tubes of a panel depending on the evaluation criteria. For example, if an add-on tube is run later in the day, the patient sample on which the add-on tube is run can be used to evaluate the performance of that individual tube prior to the release of patient results.

The criteria of acceptability and form of record are at the discretion of the laboratory director, but the criteria must include a positive and negative control for each antibody/stain used in the assay. Antibodies/stains do not need to be assessed singly if they are used in a cocktail; however, each component/tube must be evaluated. If antibodies are repeated in multiple tubes, they must be evaluated in each tube. Examples of acceptability criteria:

- *CD2: normal T-cells positive, normal B-cells negative*
- *CD19: normal T-cells negative, normal B-cells positive*
- *CD45 tube 1: positive in mature lymphocytes, negative in erythroid precursors*

When positive cellular control material is not readily available (eg, rare flow antigens such as CD1a, CD30, CD103), there must be a written procedure for an alternative mechanism to evaluate for positive staining that is performed at least every six months. The laboratory procedure must define the rare flow antigens and method of evaluation.

Evidence of Compliance:

✓ Records of QC results and any corrective actions taken

REFERENCES

1) Wood BL, Arroz M, Barnett D, 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 B Clin Cytom.* 2007;72 Suppl 1:S14-S22.
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) Wood B, Jevremovic D, Bene MC, et al. Validation of cell-based fluorescence assays: practice guidelines from the ICSH and ICCS - part V - assay performance criteria. *Cytometry B Clin Cytom.* 2013;84(5):315-323.

FLO.23800 QC - Flow Cytometry Reagents/Stains - Quantitative Assays Phase II

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

NOTE: Quantitative assays have an absolute numeric reference range and include lymphocyte subset enumeration and CD34+ cell enumeration. One of the levels of these controls should be at (or near) clinical decision levels. Examples include a low CD4+ lymph count of 200 cell/uL in a HIV+ individual, or a 5 – 20 CD34+ stem cells/uL concentration in the peripheral blood of an

individual being readied for peripheral stem cell pheresis. Control testing is not necessary on days when patient testing is not performed.

There must be written guidelines defining objective criteria for acceptable performance of control material and records of the evaluation of the actual performance.

If the laboratory performs quantitative test procedures for which control materials are not commercially available, there are written procedures for an alternative mechanism to detect immediate errors and monitor test system performance over time. The performance of alternative control procedures must be recorded. "Performance" includes elements of accuracy, precision, and clinical discriminating power. Examples of alternative procedures may include split sample testing with another method or with another laboratory, the testing of previously tested patient specimens in duplicate, testing of patient specimens in duplicate, or other defined processes approved by the laboratory director.

Evidence of Compliance:

✓ Records of QC results

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 1992(Feb 28):7146 [42CFR493.1256]

FLO.23925 Control Range Establishment or Verification — Phase II

The laboratory establishes or verifies an acceptable control range for each lot of control material.

NOTE: For unassayed control materials, the laboratory must establish an acceptable control range by repetitive analysis in runs that include previously tested control material. For assayed control materials, the laboratory must verify control ranges supplied by the manufacturer.

Control values supplied by the manufacturer may be used without verification for qualitative (eg, positive or negative) testing.

Evidence of Compliance:

✓ Records for control range establishment or verification of each lot

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Precision of Quantitative Measurement Procedures. Approved Guideline.* 3rd ed. CLSI document EP05-A3. Clinical and Laboratory Standards Institute, Wayne, PA; 2014.
2) Clinical and Laboratory Standards Institute. *Statistical Quality Control for Quantitative Measurement Procedures, Principles and Definitions.* 4th ed. CLSI guideline C24. Clinical and Laboratory Standards Institute, Wayne, PA, 2016.

FLO.24230 QC Corrective Action — Phase II

The laboratory performs and records corrective action when control results exceed defined acceptability limits.

NOTE: The actions taken must be consistent with the laboratory's quality control program (GEN.30000). Patient test results obtained in an analytically unacceptable test run or since the last acceptable test run must be re-evaluated to determine if there is a significant clinical difference in patient/client results. Re-evaluation may or may not include re-testing patient samples, depending on the circumstances.

Even if patient samples are no longer available, test results can be re-evaluated to search for evidence of an out-of-control condition that might have affected patient results. For example, evaluation could include comparison of patient means for the run in question to historical patient means, and/or review of selected patient results against previous results to see if there are consistent biases (all results higher or lower currently than previously) for the test(s) in question.

Evidence of Compliance:

✓ Records of corrective action for unacceptable control results

REFERENCES